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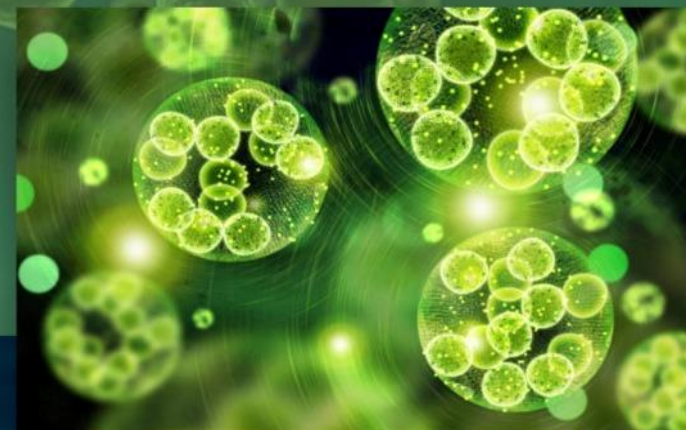
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ALGAL FUNGAL AND MICROBIAL RESEARCH

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**Algal, Fungal
and
Microbial
Research**

Algal, Fungal and Microbial Research

Editors:

**Mr. Mukul M. Barwant, Mr. Balwant Singh,
Ms. Shivangi Tripathi, Ms. Santvana Tyagi,
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Algal, Fungal
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Research

Preface

The book *Algal, Fungal and Microbial Research* covered entire current trends in phycology, mycology and microbiology. Simple plants known as algae can range in size from microscopic (microalgae) to enormous seaweeds (macroalgae), including massive kelp that can grow to a length of more than a hundred feet. The term "microalgae" refers to both green, brown, and red algae as well as cyanobacteria, a type of bacterium originally known as "blue-green algae." (Though there are other types of microalgae as well, these are the most common.) Topics like A Study on Microbial Bioconversion of Cellulose: A Promising Biomaterial were addressed. Part 1 of a Checklist of Fungi from Kalsubai, Maharashtra, An Updated Review of Botanical Fungicide, Ecology and Biotechnological Applications of Marine Cyanobacteria: A Review, New Antifungal Agents and Therapeutic Approaches to Overcome the Crisis in Antifungal Therapy, Top 5 Pathogenic Bacteria For Fungi, Fungicides and Plant Pathology an inclusive review of "Endophytes A Boon For Sustainable Crop Production," A Quantitative and Qualitative Analysis of Microalgae and Seaweed as Ruminant Feeds, Research on the Effects of *C. arietinum* Seed Borne Mycoflora Isolated from Jabalpur District (M.P.) on Post Harvest Losses, An Overview of the Potential and Challenges in the Production of Biofuels from Algae, Growing Interests in Nanotechnology and Genetic Engineering in Cyanobacteria.

The specialised field of fungi study is mycology. After insects, fungi are the second-largest biotic community on Earth and include the most diversified group of heterotrophic organisms. They are divided up into different kingdoms of fungi. Without cells arranged into tissues and organs, fungi have thalloid bodies. In nature, fungi can be parasitic, saprophytic, or symbiotic. They are essential to terrestrial ecosystems as well. The principal keepers of significant carbon storage in soil as well as the primary decomposers of lignocellulosic material are fungi. Topics like A Review on the Antioxidant, Anticoagulant, and Antiangiogenesis Potential of Fucoidan from Brown Seaweeds are also covered. The primary causes of biofouling, colouring, staining, and biological deterioration of cultural heritage are Algae Secondary Metabolites, Aquatic Fungal Diversity in Freshwater Habitat, In the Maharashtra's Khelna Reservoir, there are euglenoids. Health Effects of Airborne Algae, Distributional Records of A Few Notable Corticoid Fungi Order Boletales Members from Himachal Pradesh's District Chamba, An overview of Investigating Endophytic Fungi Connected to *Prunus* species Microorganisms'

Function in Ecosystem Restoration, Overview of Marine Algae: Unlocking The Developmental Potential, A Comprehensive Review of Incredible and Beneficial Lichen Applications, The Potential Medicines, Algal Polysaccharides Investigating Algae As a New Source for Sustainable Textiles, A Review on the Fabrication of Metal Nanoparticles Using Algae, The Role of *Aspergillus tubingens* is in Mycoremediation of Polyurethane, Agricultural Importance of Algae.

This book offers different aspects of diversity examples to show how group collaboration actually occurs in the workplace. Additionally, it will assist in making the distinction between best and effective practises as well as in assisting them in identifying the best practises they wish to implement in order to enhance their research interaction. This paradigm enables the authors and readers to situate the acquisition of new knowledge and skills within a more comprehensive innovation environment. The approach taken in this book's title enables both researchers and teachers to communicate in groups while building relationships for effectiveness. The writers and readers of this will learn the distinctive dynamics of communication of their own research work, the crucial abilities that ensure success, and the roles, activities, and processes that. It will helpful for the students, researchers, academicians in aspect of plant science.

The Editors

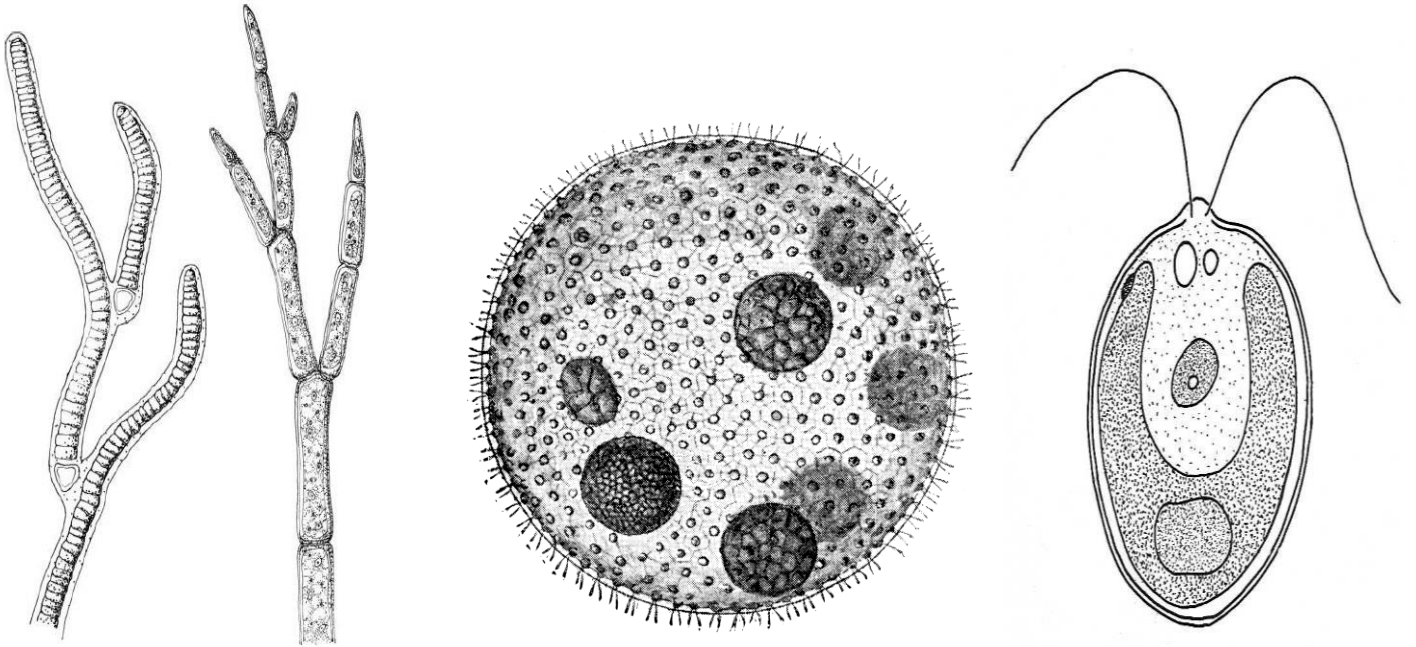
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Section-I

PHYCOLOGY



ALGAL RESEARCH

CHAPTER

01

AN OVERVIEW OF PRODUCTION OF BIOFUELS FROM ALGAE: POTENTIAL AND CHALLENGES

Abhishek Maurya*, Vishwas Gaur and
Gauri Saxena

A

bstract

The rapid growth in the world's population and the rising demand for energy sources are causing a rapid and ongoing depletion of fossil fuel sources. Since the use of fossil fuels can cause various ecological imbalances, air pollution, and global warming, as well as health hazards, it is important to use them responsibly. The present goal of this study may help to fulfill the future needs of the world. Both macroalgae and microalgae have enormous potential for a variety of industrial uses. We go into detail about the factors influencing algae growth, such as pH, temperature, light source, and nutrient availability. The advantages that these new generation biofuels offer is also explored. The creation of algae-based biofuel would transform not only how environmental pollution is controlled but also how producers advance economically and socially.

Keywords: *Air Pollution, Ecological Imbalance, Fossil, Global Warming, pH.*

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Introduction

Algae are aquatic organisms that produce biomass by utilizing sunlight as well as carbon dioxide (CO₂). Algae are divided into two groups: macroalgae and microalgae. Lipids are extracted from micro- and macroalgae in a variety of ways. Macroalgae have been used a lot in the past for extraction and optimization processes. Another interesting feature of microalgae is that they generate oil and lipids (Scott *et al.*, 2010). Although the synthesis of biodiesel from microalgae has enormous promise for use, the method is still far away from being carbon neutral or economically viable. When compared to terrestrial crops, microalgae grow much more quickly. Algal mass culture can be carried out on non-arable fields utilizing non-potable saline water and waste water. As a result, interest in using microalgae as a substitute for traditional biodiesel feedstock is growing among academics, businesspeople, as well as the general public (Rajvanshi *et al.*, 2012). There is a desire for an alternative fuel source due to the growth in the cost of commercial gasoline and the increase in greenhouse gas emissions. Due to its attributes such as being non-toxic, renewable, and biodegradable, biodiesel might be seen as a better and more suitable replacement for commercial fuel. Any fuel produced from biomass or plant, algae or animal waste is called biofuel. Because such raw materials can be easily replenished, biofuels are considered a renewable energy source, unlike fossil fuels such as oil, coal, and natural gas. In light of rising oil prices and growing concern about the role of fossil fuels in global warming, biofuels are often promoted as a viable and environmentally friendly alternative to oil and other fossil fuels (Scott *et al.*, 2010).

The biofuels have been classified as **first generation, second generation, third generation and fourth generation.**

The first generation of biofuels refers to the earliest types of biofuels that were produced from conventional food crops or agricultural residues. These biofuels include ethanol, diesel, vegetable oil, Biogas etc. The class of biofuels made from non-food sources such as algae, agricultural waste, and non-edible plant matter is treated as the second generation. These biofuels are seen as more environmentally friendly and have the potential to alleviate some of the drawbacks of first-generation biofuels. They include feedstock, conversion process etc. A sophisticated class of biofuels that are produced from non-food sources and make use of specific feedstocks and growth methods are referred to as the third generation of biofuels (Behera *et al.*, 2015). Comparing these biofuels to both first- and second-generation biofuels. They are thought to be even more efficient and sustainable. The use of microorganisms, such as algae, to produce biofuels from sunlight and carbon dioxide is frequently the focus of third-generation biofuels.

Scope and Need of Biofuels

A renewable, clean-burning alternative fuel, biodiesel is made from organic materials including recycled cooking oil, animal fats, and vegetable oils. Several environmental, economic, and energy security concerns determine its extent and need: **Environmental Benefits**, compared to traditional petroleum-based diesel, biodiesel is thought to be a more environmentally friendly fuel. It generates fewer toxic pollutants including carbon monoxide, sulfur oxides, and particulate matter, which improves air quality and lowers greenhouse gas emissions that can slow down climate change. **Renewable resource**, renewable resources, primarily plant-based oils, may be cultivated and collected continuously and are used to make

biodiesel. In contrast to the limited supply of fossil fuels, biodiesel offers a long-term, sustainable energy alternative. **Energy Security**, due to the domestic production of biodiesel, using it can lessen a country's reliance on foreign crude oil, improving energy security and lowering vulnerability to geopolitical risks connected with oil-rich countries. **Agricultural Benefits**, agricultural crops (such as soybeans, rapeseed, and palm oil) are frequently used in the manufacturing of biodiesel, offering farmers an additional market and possibly bolstering rural economies. **Compatibility with Existing Infrastructure**, A seamless switch from conventional diesel to biodiesel is possible thanks to the fact that biodiesel may be utilized in existing diesel engines and infrastructure with little to no modification. **Diversification of Fuel Sources**, incorporating biodiesel into the fuel mix diversifies energy sources, reducing the impact of price fluctuations in the global oil market. **Waste recycling**, recycled cooking oils and animal fats can be used to make biodiesel, which encourages waste reduction and provides a sustainable method of disposal.

Types and Sources of Biofuels

According to the biomass utilized in their production, biofuels are categorized in the following types: -

Solid Biofuel- This biofuel is made from solid, organic, non-residual biomass derived from living organisms. This biomass is widely used in the production of electricity, energy and heat. These biofuels are made from renewable industrial waste such as coal, fuel, wood chips, wood chips and animal waste. One of the most prominent examples is biochar.

Liquid Biofuel- These biofuels include all types of liquids made from natural biomass or biodegradable components. Due to their high energy density, liquid biofuels are significantly superior to solid and gaseous fuels, making them the best choice for transportation, storage and processing. Bioethanol, biodiesel and bio-oil are some of the most important examples of liquid biofuels. Other types of liquid biofuels include:

Triglycerides Based Biofuels- these include biomass such as vegetable oil, pyrolytic oil, biodiesel, bio-gasoline and hydrogenated oil.

Lignocellulosic Based Biofuels- these include biofuels feedstocks such as bio-oils, along with BTL diesel and drop-in biofuels.

Gaseous Biofuel- Low-density gaseous materials form gaseous fuels. Biogas, biohydrogen and biofuels are notable examples. Pyrolysis, or gasification, is used to convert biomass into gaseous fuel. This bio fuel is then connected to an electrical generator to produce heat or electricity in an Otto engine.

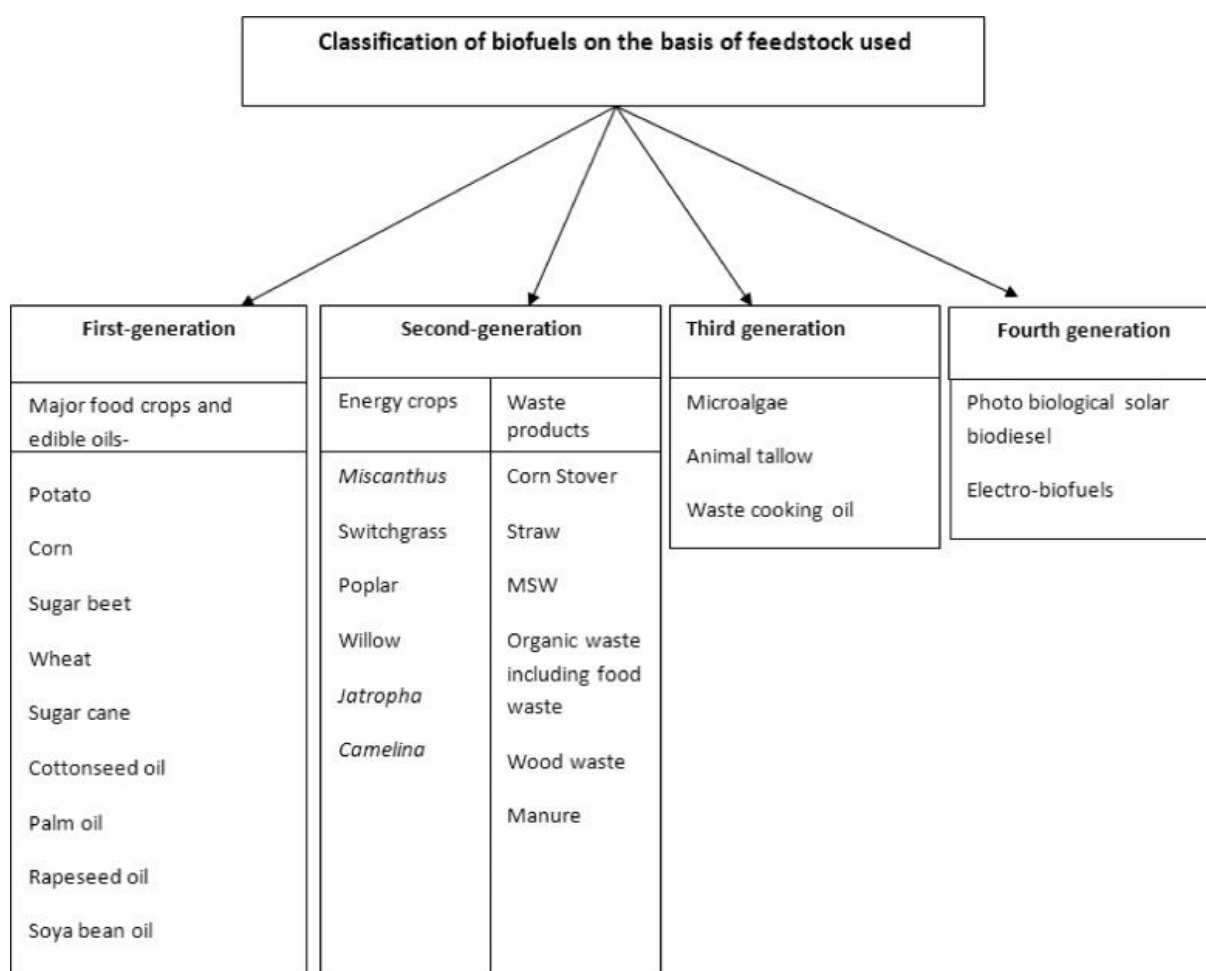
Algae: Source of Biofuels

Algae is a broad category of prokaryotic and eukaryotic organisms, ranging from unicellular species such as *Chlorella* and diatoms to multicellular species such as giant kelp, to giant brown algae that can reach 50 meters in length. (Lee *et al.*, 2008). Microalgae are small organisms that usually grow in ponds or bodies of water and measure in micrometers. In nature, microalgae grow fast and have a higher lipid content than macroalgae (Li *et al.*, 2014). There are more than 50,000 microalgal species available for research, of which only 30,000 species have been identified for research (Surendhiran and Vijay, 2012; Richmond

and Qiang, 2013; Rajkumar *et al.*, 2014). A short algae harvest cycle is the main advantage of its importance, better than other conventional crops with a harvest cycle once or twice a year (Chisti, 2007; Schenk *et al.*, 2008).

Algal biomass has several advantages for biofuel production: The productivity of algal oil is higher than conventional oilseed crops due to (a) its ability to grow throughout the year; (b) higher tolerance to high carbon dioxide content; (c) the rate of water consumption is too low; d) no need for herbicides or pesticides in algae cultivation; (e) higher growth potential than other species; (e) Various wastewater sources containing nutrients such as nitrogen and phosphorus can be used for algae cultivation in addition to providing additional nutrients; and (g) the ability to grow in harsh environments such as brackish, brackish, and coastal seawater that is not affected by conventional agriculture. (Spolaore *et al.*, 2006; Dismukes *et al.*, 2008; Dragone *et al.*, 2010). Algae can be converted into a variety of renewable biofuels such as bioethanol, biodiesel, biogas, photobiologically produced biohydrogen, and processing for bio-oil and syngas production through liquefaction and gasification, respectively (Kraan, 2013).

These algae species have the potential to produce various types of biofuels, including biodiesel, bioethanol, and biogas.



Graph.1: Type of Biofule (Mahapatra *et al.*, 2021)

Table.1: Algal species and their biofuel production.

Algae species	Biofuel potential	Key features
<i>Chlorella</i>	Biodiesel, Bioethanol	Fast growth, high lipid content
<i>Nannochloropsis</i>	Biodiesel, Biogas	High lipid content, adaptable
<i>Spirulina</i>	Biodiesel, Biogas	Rich in protein, grows in various conditions
<i>Haemaococcus</i>	Astaxanthin, Biodiesel	High value pigment, can accumulate lipids
<i>Botryococcus</i>	Hydrocarbons (Botryococcenes)	Produce hydrocarbon for biofuels
<i>Dunaliella</i>	Biodiesel, Biogas	High lipid content, thrives in high salinity
<i>Isochrysis</i>	Biodiesel, Biogas	Rich in fatty acid, suitable for biodiesel
<i>Scenedesmus</i>	Biodiesel, Biogas	Rapid growth, potential for wastewater treatment

Process of Biofuel Extraction

Processing of algae is necessary to convert algae into biofuel. The harvested algae must undergo several steps in order to produce consumer-grade products, such as biodiesel and bioethanol.

Oil Separation- Lipids, which are oils contained in algal cell walls, must be separated in order to be extracted. The three typical methods listed below extract various amounts of oil from the algae, albeit a higher extraction requires more equipment, which raises the cost.

Oil press or mechanical press- Algae can have their oil extracted using a mechanical press. Up to 70% of the algal oil can be extracted using this method, which drives the algal paste into a nozzle that extracts the oil and expel much drier biomass. However, this method typically needs a dry algae feedstock. Dewatering results in dry algae. This procedure is frequently used in conjunction with the hexane solvent technique.

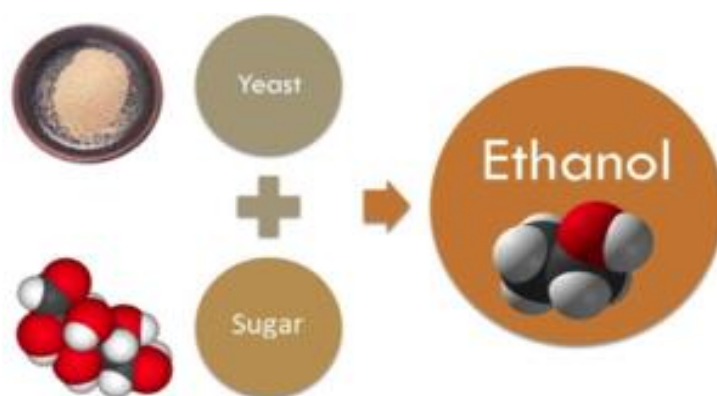
Hexane solvent method- Oil from the algae biomass dissolves in the solvent hexane, a molecule with six carbons. The solvent is then recycled through other procedures once the oil has been purified through distillation. Hexane solvents can be used to extract up to 95% of the oils at minimal cost and energy expenses, while there are some worries about the safety and health impacts of utilizing a caustic solvent repeatedly during the procedure.

Supercritical method- Supercritical fluids, as a whole, are fluids with extremely high temperatures and pressures that are neither obviously liquids nor gases. Algal cells can be

ruptured and oil released using superfluid carbon dioxide. It is possible to extract up to 100% of the oil, but the energy and equipment requirements are quite high, limiting the method's commercial viability and appeal.

Other methods-There are many other ways to extract oil from algae, but since algae biofuel is not currently produced commercially, most of these are in the research and testing stage. Consequently, the scale required for commercial biofuel production has yet to be scaled up.

Bioethanol Production- There is a large amount of solid material, known as biomass, left over after the oil is taken from the algae. Bioethanol, which is ethanol made from biomass, may be produced using this leftover biomass, which is an additional algae-based fuel product. The biomass's carbohydrate content can be converted into sugars. This can be done by fermenting yeast and releasing carbon dioxide, which can then be recycled back into the algae's growth process. The sugars continue to ferment after that to produce ethanol. Additionally, if the algae's biomass still retains any of the original oil, decomposition can be started to break down cell walls and release sugars, which can then be fermented.



Economic Viability- Economic viability is a critical factor in the success of any biofuel production method, including those based on algae. For algae-based biofuels to become competitive with traditional fossil fuels, several economic challenges must be addressed: Capital Costs: Establishing algae cultivation facilities, whether open ponds or closed photobioreactors, requires significant upfront investment. This includes infrastructure, equipment, land, and technology. Operating Costs: ongoing operational expenses include labor, energy for lighting and temperature control (in closed systems), nutrient supply, water management, and maintenance. Harvesting and Dewatering Costs: efficiently separating algae from the growth medium and concentrating them for further processing can be energy-intensive and expensive. Developing cost-effective harvesting methods is crucial. Lipid Extraction Costs: extracting lipids from algae cells while maintaining the integrity of both the cells and the extracted compounds is challenging and can be costly. Conversion Costs: converting extracted lipids into usable biofuels (biodiesel or bioethanol) involves additional processing steps, which come with their own costs. Yield and Productivity: the amount of biofuel that can be produced per unit of cultivated algae biomass directly impacts the overall economic feasibility. Higher lipid content and faster growth rates translate to better yields. Market Prices: the price of algae-based biofuels must be competitive with or lower than conventional fossil fuels for consumer adoption. Fluctuations in oil prices and the availability of other renewable energy sources can impact market dynamics. Byproduct Value: maximizing the utilization of byproducts generated during the biofuel production process (such as residual biomass, co-products, and nutrients) can help offset costs and enhance the

overall economic picture. **Policy Incentives:** government incentives, grants, tax credits, and subsidies for renewable energy production can significantly influence the economic viability of algae-based biofuels. **Scaling Challenges:** transitioning from small-scale laboratory experiments to large-scale commercial production presents challenges in terms of maintaining consistent quality, efficiency, and cost-effectiveness (Sudhakar et al,2012).

Efforts to improve the economic viability of algae-based biofuels include-

Research and Development: continued research to optimize algae strains for higher lipid content and faster growth, as well as developing more efficient cultivation methods. **Technological Innovations:** advances in harvesting, lipid extraction, and conversion technologies to reduce costs and increase yields. **Integrated Systems:** developing integrated systems that recycle nutrients, minimize waste, and make the most of co-products to enhance overall efficiency and profitability. **Economies of Scale:** as the industry grows, economies of scale can lead to reduced costs in production, equipment, and operations. **Collaboration:** partnerships between research institutions, industry players, and government agencies can foster innovation and knowledge sharing, driving down costs and accelerating progress. **Life Cycle Analysis:** comprehensive analysis of the entire biofuel production process, from cultivation to consumption, helps identify areas of high costs and environmental impact, leading to targeted improvements.

Challenges-- There are many challenges to produce biofuels from algae. Producing biofuels from algae holds significant promise due to the high lipid content of many algae strains and their rapid growth rates. However, there are several challenges that need to be addressed to make this process economically viable and environmentally sustainable-

Land Use and Food vs. Fuel Debate: concerns concerning resource and land rivalry between food and fuel production can arise from the manufacture of biodiesel from crops. To prevent detrimental effects on food security and prices, it is critical to strike a balance.

Energy Intensive Production: biodiesel production can be energy-intensive, particularly if it relies on energy sources from non-renewable origins. It's crucial to ensure that the overall energy balance remains positive.

Feedstock Selection: the social and environmental sustainability of biodiesel production can be strongly impacted by the feedstock choice. For instance, there are worry regarding the sustainability of palm oil because it has been linked to habitat damage and deforestation.

Limited Feedstock Availability: access to sufficient feedstock, which might not be available in enough numbers in some places, is necessary for scaling up biodiesel production.

Transesterification Process: transesterification, which produces fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE) and glycerin, is the most widely used method for producing biodiesel. Triglycerides in the feedstock react with an alcohol (typically methanol or ethanol) in the presence of a catalyst (such as sodium hydroxide or potassium hydroxide). Reaction temperatures, times, alcohol-to-oil ratios, and catalyst type can all have an impact on how effectively transesterification occurs.

Catalyst Type and Concentration: the transesterification reaction rate and biodiesel yield can be considerably influenced by the catalyst's selection and concentration. Different

catalysts have varying levels of activity and can need particular circumstances for a reaction to occur.

Alcohol Quality: the caliber of the methanol or ethanol used for transesterification is crucial. To reduce the risk of adverse reactions and to enable a more effective conversion of triglycerides to biodiesel, high-purity alcohol with less water content is desired.

Water Content: the transesterification process may be negatively impacted by water in the reaction mixture. It may result in the creation of soap and lower the output of biodiesel. Alcohol and feedstock must be properly dried to reduce water content.

Reaction Time: the amount of conversion is influenced by how long the transesterification process lasts. Higher biodiesel yields may result from longer reaction times, but too lengthy of a reaction time can raise the likelihood of adverse reactions.

Temperature: the reaction rate and quality of the biodiesel can be affected by the temperature at which the transesterification process is carried out. The ideal temperature is often set to strike a compromise between yield and reaction pace.

Free Fatty Acid (FFA) Content: the transesterification process can become more challenging when the feedstock contains a lot of free fatty acids. To lower the FFA concentration, the feedstock may need to undergo acid esterification or other pretreatment procedures.

Washing and Purification: The biodiesel is often cleaned to eliminate contaminants and catalyst residues after transesterification. To get high-quality biodiesel, it is essential to follow proper washing and purifying procedures.

Scale of Production: Small- or large-scale biodiesel production can have an impact on the process design, equipment selection, and overall efficiency.

Conclusion

As a third-generation feedstock for biodiesel, microalgae have much better yields than other crops. The generation of biodiesel from microalgae has attracted a lot of attention in recent years. For microalgae-based biodiesel production to be more affordable, the most effective method and ideal conditions for lipid extraction must be found. Since making biodiesel from microalgae oil is equivalent to making it from any other oilseed, it is possible to make biodiesel using the same conversion techniques. With the exception of NO_x emissions, biodiesel use in diesel engines results in a minor reduction in engine output. The operation state affects the engine output reaction. Finally, more funding and research are required to identify novel strategies that could reduce the cost of producing biodiesel from microalgae. Additionally, studies on the combustion of microalgae in internal combustion engines should be conducted to enhance engine efficiency and reduce emissions.

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CHAPTER

02

A REVIEW ON ANTIOXIDANT, ANTICOAGULANT AND ANTIANGIOGENESIS POTENTIAL OF FUCOIDAN FROM BROWN SEAWEEDS

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A bstract

Sulfated polysaccharide from seaweeds comprises a complex group of macromolecules (fucoidan, laminarin and alginate) with a wide range of important biological properties with low toxicity. Fucoidan represent 40% of the dry weight of isolated cell walls of brown seaweeds. Fucoidan and laminarin are known for their potential biological activities whereas alginate is mostly used as food ingredients. The general sulfated polysaccharide from Phaeophyta (brown algae) is called fucans, which compete with other families of polydisperse molecules by having sulfated L-fucose. Fucoidan from algae possess wide pharmacological activities with antioxidant potential. So, this chapter was focused on the extraction method, antioxidant, anticoagulant and antiangiogenesis potential of fucoidan from seaweeds.

Keywords: *Antioxidant, Bioactivity, Brown Seaweeds, Fucoidan, Parkinsons Disease.*

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Introduction

Globally 20,000 species of seaweeds were found to be distributed and in India 850 species were reported (Oza and Zaidi, 2001). Over the past few decades seaweeds and their compounds have been reported to possess biological activity with potential medicinal value because of their antioxidant property (Heo *et al.*, 2005). Moreover, synthetic antioxidants are used by the food industry to expand the shelf life of their products. These food additives prevent lipid peroxidation, although they have been reported to exert possible toxic and carcinogenic effects on health (Hu *et al.*, 2010). Algal polysaccharides were reported to be useful candidates in the search of effective non-toxic antioxidants. They play an important role as free radical scavengers *in vitro* and antioxidants for the prevention of oxidative damage in living organisms (Meenakshi *et al.*, 2011; 2014; 2016).

Polysaccharides are the main component of brown seaweed cell walls, which are not found in terrestrial plants. Sulfated polysaccharide from seaweeds comprises a complex group of macromolecules (fucoidan, laminarin and alginate) with a wide range of important biological properties with low toxicity. Fucoidan represent 40% of the dry weight of isolated cell walls of brown seaweeds (Berateau and Mulloy, 2003). Fucoidan and laminarin are known for their potential biological activities whereas alginate is mostly used as food ingredients. The general sulfated polysaccharide from Phaeophyta (brown algae) is called fucans, which compete with other families of polydisperse molecules by having sulfated L-fucose (Fonseca *et al.*, 2008). Fucoidan is composed of fucose, uronic acids, galactose, xylose and sulfated fucose. They are comprised of long chains of linked sugar molecules, along with sulfate groups which make them negatively charged (Ermakova *et al.*, 2011).

Fucoidan interfere in several cellular processes such as migration, adhesion, proliferation and apoptosis (Berateau and Mulloy, 2003). So, the search of new drugs with antioxidant properties has recently risen towards the sulfated fucans. Compared with other sulfated polysaccharides, fucoidans are widely available in cheap sources, so more and more fucoidans have been investigated in recent years to develop natural antioxidants in pharmaceutical and food industry. The present review highlights the various bioactive materials of fucoidan from seaweed origin and their potential biomedical applications.

Fucoidan from Seaweeds

Fucoidan is a uniquely structured sulfated polysaccharides found in the cell walls of several types of brown seaweed. They contain L-fucose as the main sugar constituent and sulfate ester. Their structure varies according to the season, age of population, species and geographic location. Ray (2006) isolated a water soluble sulfated heteroglycuronan from *Enteromorpha compressa* with molecular mass of 50 ± 20 kDA. Degradation of the xyloglucan rich extract (Ec1OH) by endo (1 $\rightarrow$ 4)- β -D-glucanase and analysis of the resulting fragments by MALDI-TOF-MS showed Glc_3Xyl_1 , Glc_4 , $\text{Glc}_3\text{Xyl}_2(\text{So}_3\text{Na})_2$, $\text{Glc}_4\text{Xyl}_2(\text{So}_3\text{Na})_2$ and $\text{Glc}_5\text{Xyl}_2(\text{So}_3\text{Na})_2$ as major oligomeric building sub-units.

Wang *et al.* (2008) studied the antioxidant activity of sulfated polysaccharide fractions from *L. japonica*. Three fractions were collected through anion-exchange column chromatography

and their antioxidant activities were investigated. From the chemical analysis F1 and F3 are heteropolysaccharide and F2 was a fucoidan which showed a high level of antioxidant capacity and the ratio of sulfate content/fucose was an effective indicator of antioxidant activity of the samples. Yang and Zhang (2009) studied the chemical structure of chain conformational characterization of some bioactive polysaccharides isolated from natural sources. The structures were analyzed by FTIR, liquid-state NMR, solid state NMR, GC, GC-MS, HPLC and Raman spectroscopy. Pereira *et al.* (2009) suggested that the combination of two spectroscopic techniques (FTIR-ATR and FT-Raman) is possible to identify the seaweed colloids.

Rioux *et al.* (2009) studied the structural features of laminarians and galactofucans extracted from the brown seaweed *Saccharina longicruris* were determined from four harvest periods (Mo5, Ao5, No5 and Jo6). Laminarians and galactofucans were fractionated using DEAE sepharose anion exchange chromatography with increasing levels of sodium chloride (0.5, 1 and 2M). Ordonez *et al.* (2010) applied a simple ion chromatography method for inorganic anion analysis in edible seaweeds and when characterized they yielded 33.7 – 36.9% of chloride content. The method developed was well applicable to mineral anion analysis in edible seaweeds and shows suitability and reliability of use in other food samples of nutritional importance. Ordonez and Ruperez, (2011) stated that FTIR-ATR spectroscopy as a tool for polysaccharide identification. Polysaccharide from 3 brown and 5 red seaweeds were studied. Alginate, agar, kappa and lambda carrageenan was used as standard. The specific absorption bands were observed at 808/787 cm⁻¹ for brown seaweeds and for the red seaweeds the band was 929.0, 844.7 and 803.2 cm⁻¹. Therefore FT-ATR spectroscopy is proposed as a useful tool for the food, pharmaceutical and cosmetic industry to check the phycocolloid quality of a raw seaweed material by a quick and non-destructive method. Wang *et al.* (2022) obtained 102.67 kDa fucoidan from *Hizikia fusiforme* using enzymes with antioxidant potential.

Antioxidant Activity of Fucoidan

Li *et al.* (2001) observed sulfated polysaccharides of *Laminaria japonica* have antioxidant abilities against lipid and low-density lipoprotein peroxidation induced by free radicals *in vitro*. Heo *et al.* (2005) identified potential antioxidative activities of enzymatic extract from seven species of brown seaweed using different reactive oxygen species (ROS) scavenging assays containing DPPH free radical, superoxide anion, hydroxyl radical and hydrogen peroxide scavenging assays. Kuda *et al.* (2006) evaluated the antioxidant properties of *Petalonia binghamiae* (brown algae) by ferrous-reducing power, ferrous ion chelation, DPPH radical-scavenging activity and superoxide anion radical scavenging activity. Xu *et al.* (2009) studied antioxidant activity of a 4, 58, 000 Da water-soluble polysaccharide from *Pteridium aquilinum*. It was fractionated with DEAE-Sepharose and further purified with sephacryl s-400 HR column chromatography. Regarding antioxidant activity it has strong reductive power moderate scavenging activities against DPPH radicals and superoxide radicals and moderate inhibiting power of self-oxidation of 1,2,3-phentriol.

Barahona *et al.* (2010) employed a kinetic approach in determining the antioxidant capacity of sulphated polysaccharide from seaweeds. They employed ABTS and hydroxyl radical scavenging assays. The studies showed a fast reaction followed by a slower reaction that leads polysaccharides derived radicals to inhibit the antioxidant activity. Chattopadhyay *et al.* (2010) revealed the structural features and antioxidant capacity of polysaccharides from *Turbinaria conoides*. The fucoidan is highly branched and has an average molecular mass of 50 kDa. The *in vitro* antioxidant capacity of the fractions was studied by FRAP and DPPH assays. Fucoidan showed the highest potential followed by alginate and laminaran. These findings demonstrate that these polysaccharides could be used as natural antioxidants by the food industry.

Meenakshi *et al.* (2014, 2016) studied the hepatoprotective and neuroprotective effect of water-soluble polysaccharide from *Turbinaria decurrens*. Fucoidan treatment increased the body weight, food intake and serum protein levels, it decreases the level of hepatic markers. Fucoidan improved the antioxidant status of animals, which is evaluated by the decreased levels of lipid peroxidation markers and increased level of enzymatic antioxidants were observed in liver and brain. Husni *et al.* (2022) revealed four different methods of extraction of fucoidan from *Sargassum hystrix* and obtained highest antioxidant activity in 85% ethanol extraction method.

Anticoagulant and Antiangiogenic Activity of Fucoidan

Athukorala *et al.* (2007) studied the anticoagulant activity for 22 brown and green seaweeds, in that *Codium fragile* and *Sargassum horneri* exhibited the most potent anticoagulant activity. The crude polysaccharide fraction (>30kDa) of *C. fragile* composed of 80% carbohydrate and 19% of protein; the crude polysaccharide fraction (>30 kDa) of *S. horneri* was composed of 97% of carbohydrate and 2% of protein. Therefore, they stated that the active compound or compounds of the algal species were related to high molecular weight polysaccharide, or a complex form with carbohydrate and protein.

Zoysa *et al.* (2008) studied the anticoagulant activity of sulfated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*. APTT, PT, and TT clotting assays showed that seaweed polysaccharide was able to inhibit both intrinsic and extrinsic blood coagulation pathways. Carvalho *et al.*, (2009) studied anticoagulant activity (APTT, PT) of low MW (15.2 kDa) fucoidan from *Fucus vesiculosus* and *in vivo* using male wistar rats. The APTT test showed high activity at 5 mg (≥ 240 s) and PT test showed high anticoagulant activity at 50 mg (≥ 120 s). They demonstrated a two-phase response to platelet aggregation at 50 mg/mL. However, at a concentration of 0.1 mg/mL, a hypoaggregate profile was observed and the results showed that fucoidans irreversibly induced platelet aggregation in high concentration.

Irhimeh *et al.* (2009) examined the anticoagulant activity of fucoidan from *U. pinnatifida*. The *in vitro* anticoagulant activity of fucoidan was found prominent. It increased activated partial thromboplastin time, thrombin time and prothrombin time, whereas antithrombin-III decreased. *In vivo* effect of fucoidan on hemostasis was not obvious. Silva *et al.* (2010) studied the APTT for kappa and iota carrageenans and the results shows 240s and 132s respectively, lamda carrageenans was the most potent anticoagulant at 240s (20µg). These

carrageenans demonstrated no anticoagulant action using the PT test *in vitro*. Thus, quantitative evaluation of inflammation demonstrated that iota and lamda carrageenans have higher inflammatory potential than the kappa carrageenans. Camara *et al.* (2011) studied the anticoagulant and antioxidant activities of the brown seaweed polysaccharide of *Canistrocarpus cervicornis*. Their results clearly indicate the beneficial effect of *C. cervicornis* polysaccharides as anticoagulants and antioxidants.

Dias *et al.* (2005) revealed the antiangiogenic and antitumoral properties. Although the latest action of sarg-a might be related to the inhibition of angiogenesis, the polysaccharides also exert cytotoxic effect on tumour cells. Because of its chemical characteristics and polyanionic constituents the polysaccharide from sarg-a might modulate the activity of heparin-binding angiogenic growth factors. Ye *et al.* (2005) investigated the effects of fucoidan extracts from *Cladosiphon novae* on invasion ability of human fibrosarcoma HT 1080 cells and found that fucoidan extracts significantly inhibit their invasion, possibility via suppressing matrix metalloproteinase. Further, they found that fucoidan extracts suppressed expression and secretion of angiogenesis factor vascular endothelial growth factor (VEGF), resulting in suppressed vascular tubules formation of tumour cells.

Wang *et al.* (2011) studied the anticoagulant potential of fucoidan from the brown seaweed *Laminaria japonica*, he observed that the fucoidan derivatives were able to inhibit both the intrinsic and extrinsic blood coagulants. Manggau *et al.* (2021) isolated sulphated polysaccharide from *Sargassum polycystum* and studied the *in vivo* anticoagulant activity where he observed the anticoagulant potential of the sulphated polysaccharide in 100mg/kg dosage group of mice and concluded that sulphate polysaccharide isolates have the potency to prevent and treat cardiovascular disease.

Conclusion

Oxidative stress has been linked to a wide range of diseases which leads to death, fucoidan from brown seaweeds has the antioxidant potential will inhibit the harm generated by excessive free radicals. This review chapter will take a lead towards the isolation of fucoidan from brown seaweeds for the deadly diseases.

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CHAPTER

03

SECONDARY METABOLITES IN ALGAE

Dr. Salve Niteen Ramdas*

A

bstract

The chapter covers the broad concepts of algae, metabolites, primary and secondary metabolites and the account of varied secondary metabolites such as the phenolics, alkaloids, and terpenoids found in algae. The phenolics such as phlorotannins, coumarins, polyphenols and flavonoids are more common in algae. Marine algae contain many types of alkaloids, including the indole, phenylethylamine, naphthyridine, and bis-indole types. Algae frequently include terpenoids such as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes, such as carotenoids. Algae also incorporate secondary metabolites such as the glycosides, amines, steroids, and polyketides. All these metabolites differ qualitatively and quantitatively in microalgae and macroalgae. The presence of a specific metabolite provides that particular alga its diagnostic characteristic.

Keywords: *Alkaloids, Macroalgae, Microalgae, Phytoplankton, Phenolics, Seaweeds, Terpenoids.*

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Introduction

Algae (singular: alga) is a heterogenous (polyphyletic) group of plants and hence it is quite difficult to define it precisely. But algae can broadly be defined as, “a group of small, simple, primitive, mostly eukaryotic, thalloid, non-flowering, seedless, non-embryonic, non-vascular, mostly aquatic (fresh water or marine), chlorophyllous (photosynthetic and autotrophic), plants/organisms with unicellular sex organs, if multicellular, these (sex organs) are without a jacket layer of sterile cells.”

Algae can be of two types such as macroalgae and microalgae. The macroalgae are macroscopic and multicellular commonly called as the seaweeds whereas the microalgae are microscopic and unicellular commonly known as the phytoplankton (Pereira, 2021; Samanthi, 2023). The macroalgae includes some forms of algae from Chlorophycophyta (green), Phaeophycophyta (brown), and Rhodophycophyta (red) (“Seaweed” Wikipedia, 2023). The microalgae constitute members from Cyanochloronta (blue-green algae), Chlorophycophyta (microscopic green algae), Chrysophycophyta (diatoms and golden-brown algae) Pyrrophyphyta (dinoflagellates) etc. (Beetul *et al.*, 2016).

The chemical substances called as the molecules or metabolites such as the proteins, lipids, nucleic acids, and carbohydrates present in living organisms are mostly made up of the elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur (Molnar & Gair, 2015). The term ‘Metabolite’ refers to a byproduct of metabolism that escapes into the environment or is produced in excess (it is often not a reserve food material) (Guiry and Guiry, 2023). A metabolic pathway is a set of chemical reactions that, step by step, convert a reactant into the product via a number of intermediate molecules. Metabolism consists of both the process of formation and breakdown of complex molecules, known as anabolism and catabolism, respectively.

For instance, in carbon metabolism, photosynthesis is the anabolic pathway and respiration is the catabolic pathway (Molnar & Gair, 2015). The metabolites which are produced during the primary (main/central) stream of plant metabolism are called as the primary metabolites, such as the carbohydrates, proteins, lipids, nucleic acids etc. Whereas the secondary metabolites are the ones which are not synthesized during the main/central stream of metabolism, but are produced as the by-products or intermediate products of the primary metabolism and primary metabolites. While the primary metabolism allows a plant to grow and develop in general, the secondary metabolism allows it to grow and develop in extremely specific environments.

Secondary metabolites are usually divided into three main categories: Phenolics, Alkaloids, and Terpenoids (Daniel, 2006). Any plant compound with one or more substituted hydroxyl group/s on its aromatic ring is referred to as a "phenolic compound" (Harborne, 1973). Alkaloids are a class of basic organic compounds found in nature that have at least one nitrogen atom arranged in a heterocyclic ring structure. In addition to alkaloids, notable nitrogen-containing secondary metabolites include amines, non-protein amino acids, cyanogenic glycosides, and glucosinolates (Wink, 1997). Terpenoids are isoprenoid compounds containing one or more isoprene (C₅H₈) units (Bramley, 1997). Terpenoids have

the highest percentage share of secondary metabolites in marine algae, followed by alkaloids and polyketides respectively (Blunt *et al.*, 2004).

Phenolics in Algae

The majority of phenolics (phenolic compounds) in plants such as the simple phenols, phenolic acids, flavonoids, tannins, coumarins, stilbenes, quinones, and lignin are produced by the shikimic acid (or shikimate) pathway by converting simple carbohydrate molecules [produced by the PPP (pentose phosphate pathway) and glycolysis] into aromatic amino acids such as phenylalanine, tryptophan and tyrosine (Kumara & Goel, 2019). Phloroglucinol (1,3,5-trihydroxybenzene) units are the building blocks of phlorotannins (Li *et al.*, 2017). In algae, Octaphlorethol A, a phlorotannin is isolated from *Ishige foliacea*, a brown alga (Lee *et al.*, 2012; Blunt *et al.*, 2014). Hartmann *et al.*, (2018) reported Dasycladin A and B coumarins, the natural benzopyrone derivatives from *Dasycladus vermicularis*, a green alga. Hartmann *et al.*, (2018) reported sulphated coumarins from *Dasycladus vermicularis*, a green alga and Ryu *et al.*, (2018) reported Ishophloroglucin A, a phlorotannin from *Ishige okamurae*, a brown alga (Carroll *et al.*, 2020). Lavoie *et al.*, (2019) reported Cladophorols, the polyphenols from *Cladophora socialis*, a green alga (Carroll *et al.*, 2021). As per Yoshie-Stark and Hsieh (2003), more flavonoids are found in red algae than in green or brown algae. According to Tsimogiannis and Oreopoulou (2019), lignins, the phenolic polymers are very rare or less explored in algae.

Alkaloids in Algae

Alkaloids are more prevalent in Rhodophycophyta than in Chlorophycophyta and less common in Phaeophycophyta. The indole type of alkaloids is the most abundant type in marine algae, followed by the phenylethylamine type and the naphthyridine type (Güven *et al.*, 2013). Li *et al.*, (2016) isolated four halogenated (brominated) indole-related alkaloids having antibacterial activities from the red alga *Laurencia similis*. Caulerchlorin, a halogenated (chlorinated) bis-indole type of alkaloid, was identified by Liu, *et al.* (2012) in green alga *Caulerpa racemosa*. The other bis-indole type of alkaloids such as Racemosins A and B have been detected by Liu, *et al.*, (2013) and Racemosins C by Yang *et al.*, (2014) from the same alga (Blunt *et al.*, 2016). The species of red algae such as *Polysiphonia*, *Gelidium*, *Gracilaria*, *Chondrus*, *Laurencia*, and *Lophocladia* and the species of green algal genera such as *Ulva*, *Caulerpa*, *Chaetomorpha* and *Ulvaria* yield various types of alkaloids (Güven *et al.*, 2013). Li *et al.*, (2018) reported alkaloid Rhodomelin A from *Rhodomela confervoides*, a red alga (Carroll *et al.*, 2020).

Terpenoids in Algae

Terpenes are simple hydrocarbons made up of basic isoprene units (C₅H₈). There is a slight difference between a terpene and a terpenoid. A terpene is a hydrocarbon, while a terpenoid is an oxygenated terpene (Perveen, 2018). Terpenoids, also called isoprenoids, account for more than half of the secondary metabolites identified in macroalgae (Stratmann *et al.*, 1992; Blunt *et al.*, 2004; Maschek and Baker, 2008; El-Beltagi *et al.*, 2019). Mevalonic acid is a link in the terpenoids biosynthesis, hence the pathway is known as the mevalonic acid (mevalonate)

pathway. Acetyl-CoA serves as the starting point in the synthesis of terpenoids. Through a series of events, it is transformed into six-carbon mevalonic acid, which is a precursor to five-carbon isoprene/isoprenoid units (Habtemariam, 2019).

Monoterpenes (C₁₀H₁₆)

The monoterpenes such as 1,8-Cineol, Linalool, p-Cymene, α -pinene, Geraniol, 2-Terpineol, d-Limonene, and others occur frequently in marine algae such as the species of *Ulva*, *Enteromorpha*, *Codium* (Chlorophycophyta), *Laminaria*, *Sargassum* (Phaeophycophyta), *Porphyra* (Rhodophycophyta) etc. (Naylor *et al.*, 1983). Prefuroplocamioi and plocamenol (from *Plocamium cartilagineum*, a red alga) (Díaz-Marrero *et al.*, 2002; Blunt *et al.*, 2004). According to Cikoš *et al.*, (2019) among all macroalgae, the genus *Plocamium* (Rhodophycophyta) is the most significant source of cyclic and acyclic monoterpenes.

Sesquiterpenes (C₁₅H₂₄)

Laurecomin and Okamurane, the chamigrane type of sesquiterpenes are reported from *Laurencia composita* and *L. okamurai*, the red algal species respectively (Li *et al.*, 2012; Liang *et al.*, 2012; Blunt *et al.*, 2014). Yicterpene, a chamigrane type of sesquiterpenes is identified from *Laurencia composita*, a red alga (Li *et al.*, 2013; Blunt *et al.*, 2015). Cheng *et al.*, (2016) identified Chlorellatin A and Chlorellatin B from (*Chlorella sorokiniana*- green alga), Ji *et al.*, (2015) isolated a cadinane and norcadinane sesquiterpenes (*Dictyopteris divaricate*- brown alga) while, Chen *et al.*, (2016) isolated brominated chamigrene and cuparane sesquiterpenoids (*Laurencia tristicha*- red alga) (Blunt *et al.*, 2018). Sabry *et al.*, (2017) showed the presence of Curcuphenol type of sesquiterpenes for the first time in green algae from *Udotea orientalis* and Oguri *et al.*, (2017) identified brominated/chlorinated sesquiterpenes along with non-halogenated sesquiterpenes called laurane from species of *Laurencia*, a red alga (Carroll *et al.*, 2019). The genus *Laurencia* (Rhodophycophyta) is the most potential source of sesquiterpenes among all macroalgae (Cikoš *et al.*, 2021). Kumagai *et al.*, (2018) confirmed a merosesquiterpene (terpenes of mixed origin) called iso-zonaroic acid in *Dictyopteris undulata*, a brown alga (Carroll *et al.*, 2020).

Diterpenes (C₂₀H₃₂)

According to Tringali (1991) the richest source of diterpenoids in algae is brown algae followed by green algae and red algae. The diterpenoids obtained from the brown algae include Pachydictyol A (from *Pachydictyon coriaceum*), epoxyxodolabelladiene (from *Dilophus ligulatus*), dictyol and dolabellane (from *Dictyota* sp.), Spatane from *Stoechospermum marginatum*, dolastane (from *Canistrocarpus cervicornis*) etc., Sargassinone (from *Sargassum crispum*) (Ayyad *et al.*, 2001; Blunt *et al.*, 2003). Stypolactone (from *Stypopodium zonale*, a brown alga) (Dorta *et al.*, 2002; Blunt *et al.*, 2004), Pachydictyol (*Dictyota dichotoma*, a brown alga) (Hirschfeld *et al.*, 1973; Blunt *et al.*, 2015). Meroditerpenoids such as cystodiones and cystones (*Cystoseira usneoides*- brown alga) (De los Reyes *et al.*, 2016; Blunt *et al.*, 2018), Maneesh and Chakraborty (2017) reported labdane type diterpenes from *Sargassum wightii*, a brown alga while Oguri *et al.*, (2017) isolated diterpene called omaezol from *Laurencia* species of red alga (Carroll *et al.*, 2019). Yan *et al.*, (2019) confirmed Dictyospiromide, a diterpenoid-maleimide in *Dictyota*

coriacea, a brown alga. Terpenoids called dictyols are obtained from various species of *Dictyota* (Carroll *et al.*, 2021).

Triterpenes (C₃₀H₄₈)

Ali *et al.*, (2017) reported Padinolic acid, a triterpene carboxylic acid from *Padina boergesenii*, a brown alga while Maneesh and Chakraborty (2017) found oleanene triterpenes in *Sargassum wightii* (Carroll *et al.*, 2019).

Tetraterpenes (C₄₀H₆₄)

The carotenoids are of two types: Carotenes (non-oxygenated hydrocarbons) such as α -carotene, β -carotene, γ -carotene, lycopene etc., and Xanthophylls (oxygenated hydrocarbons) such as lutein, astaxanthin, β -cryptoxanthin, violaxanthin, zeaxanthin, fucoxanthin etc. (Maoka, 2020) The β -carotene is present in almost all divisions of algae except Cryptophycophyta. This division consists of α -carotene. The α -carotene may be found in addition to β -carotene in the divisions such as Cyanochloronta, Chlorophycophyta and Rhodophycophyta. The carotenoid content in Rhodophycophyta (red algae) is type specific; the microalgal forms contain only β -carotene and zeaxanthin, whilst the macroalgal forms of red algae contain α -carotene, β -carotene, zeaxanthin and lutein (Takaichi, 2011). Some xanthophylls are also class- and division-specific in algae such as- siphonaxanthin (Chlorophyceae and Ulvophyceae of Chlorophycophyta); diadinoxanthin (Euglenophycophyta); fucoxanthin (Phaeophycophyta; Chrysophyceae and Bacillariophyceae of Chrysophycophyta); peridinin (Dinophyceae of Pyrrhophycophyta) etc. (Takaichi, 2011). Peng *et al.*, (2018) obtained Sargassumone, a norterpene (terpene that has lost methyl group) from *Sargassum naozhouense*, a brown alga (Carroll *et al.*, 2020).

Glycosides in Algae

The steroidal glycosides Iyengarosides A and B (*Codium iyengarii*, a green alga) (Ali *et al.*, 2002; Blunt *et al.*, 2004). The two diterpene glycosides called Peyssonoside A and Peyssonoside B are reported from *Peyssonnelia species* (a red alga).

Amines in Algae

The three different types of amines are recorded in algae: Aliphatic monoamines, Diamines and Polyamines, and the Alkaloids. Aliphatic monoamines such as Trimethylamine is found in Phaeophycophyta members that imparts the characteristic odor to these algae. Choline and ethanolamine are common in other classes but not found in Cyanochloronta. Diamines and Polyamines are chiefly present in microscopic green algae (Chlorophycophyta). The neurotoxic alkaloids such as saxitoxin and the gonyautoxins are observed in dinoflagellates (Pyrrhophycophyta) (Kneifel, 1979).

Steroids in Algae

Steroid named iyengadione (*Codium iyengarii*, a green alga) (Ali *et al.*, 2002; Blunt *et al.*, 2004). Mozukulin A and Mozukulin B (*Cladosiphon okamuranus*- brown alga) (cheng *et al.*, 2016; Blunt *et al.*, 2018). Jacob *et al.*, (2019) reported a sterol named Turbiconol from

Tubinaria conoides, a brown alga from India (Carroll *et al.*, 2021). Yang *et al.*, (2018) reported Dictyoptesterols, the cholestane-type of sterols from *Dictyopteris undulata*, a brown alga and Feng *et al.*, (2018) found dictyopterisins, the steroids from the same alga (Carroll *et al.*, 2020).

Polyketides in Algae

Polyketides account for approximately one fourth of all the known chemical substances from algae, and is the second most abundant class of compounds (terpenoids being the first) identified from macroalgae (Blunt *et al.*, 2004; Maschek and Baker, 2008). The polyketides such as the brevetoxin B, brevisamide and brevisin are isolated from the toxic dinoflagellate *Karenia brevis* whereas the amphidinoketide is reported from another dinoflagellate called *Amphidinium* (Sasso *et al.*, 2012).

Conclusion

Most of the secondary metabolites in marine algae are terpenoids, alkaloids, and polyketides, in a decreasing order. The genus *Plocamium*, a red alga, is the most important source of monoterpenes while the genus *Laurencia*, another red alga, has the greatest potential as a source of sesquiterpenes among all macroalgae. Alkaloids are found in greater concentrations in red algae than in green algae and very less in brown algae. In marine algae, the indole type of alkaloids is the most prevalent, followed by the phenylethylamine and naphthyridine types. The brown algae are the richest source of diterpenoids in algae followed by green algae and red algae. The flavonoids are more in number in red algae than in green or brown algae. The absence of β -carotene is a diagnostic character of division Cryptophycophyta. These algal forms consist of α -carotene. The red macroalgae and microalgae, contain specific type of carotenoids. Some xanthophylls belong to distinct classes and divisions of algae. Brown algae are known for their characteristic odour which is mainly due to presence of aliphatic monoamines like trimethylamine.

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CHAPTER

04

EUGLENOIDS IN FRESH WATER KHELNA RESERVOIR OF MAHARASHTRA

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A

bstract

Euglenoids are highly specialized unicellular flagellates which have features of both protozoa and algae. Phototrophic euglenoids are considered as algae because they show characteristics of algae. Euglenoids can be found in broad range of habitats. The present paper deals with the euglenoids of fresh water Khelna reservoir of Maharashtra. Taxa of *Euglena* and *Phacus* were observed and recorded. *Euglena acus*, *Euglena elongata*, *Euglena polymorpha* and *Phacus orbicularis* were found abundant in Khelna reservoir. Euglenoids often serve as indicators of water quality. Mainly euglenoids are studied for diversity, distribution, abundance and their ecological importance as indicators of water quality.

Keywords: *Euglenoids, Khelna Reservoir, Water Quality.*

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Introduction

Euglenoids are protozoan like protists which have features of both protozoa and algae. The derivation of the euglenoids is obscure, although there is some evidence that they have evolved from marine ancestors. Approximately 40 genera and 1500 species of euglenoids are known. Euglenoids were first observed in the 1600s by Antonie van Leeuwenhoek using his primitive single lens microscope. Euglenoids can be found in broad range habitats including fresh water, brackish water, snow, high and low pH conditions and both aerobic and anaerobic environments. Euglenoids are unicellular, have generally two flagella by means of which they can swim easily. One flagellum is short and one is long. Flagella are found on anterior part of body. Many euglenoids are highly metabolic and some may even adopt an amoeboid type of motility and apparently never develop flagella.

Euglenoids are distinguished by the absence of cell wall but they have protein rich layer called pellicle in place of cell wall. Pellicle is flexible made of protein, located under plasma membrane. All euglenoids are characterized by the presence of unique cell covering known as pellicles. Euglenoids have a definite nucleus and chlorophyll a and b is not scattered in granules but is localized in chloroplast as in higher plants. In addition to chlorophyll a and b xanthophylls pigment is also present. The chloroplasts of euglenoids are known to contain pyrenoids which are used in the synthesis of Paramylon, form of starch-B-1, 3 glucans, helps the organism to survive a long period of light deprivation. Euglenoids have red eye spot along with photoreceptors so they can respond and move toward light. Euglenoids are solitary cells that move about using a single whip-like tale. While some euglenoids are flexible and change shape frequently, other are rigid and do not change shape.

Euglenoids have three main modes of nutrition, some are phagotrophic and feed on bacteria and algae. Others contain chloroplasts and can produce their own food by photosynthesis known as Phototrophy. Some absorb nutrients from water by osmosis called Osmotrophy. Euglenoids have flexible body. Most of the euglenoids are between 5 to 50 μ , some can be several hundred microns or longer. Euglenoids reproduces by longitudinal cell division, a form of asexual mode of reproduction, although there are few reports of conjugation and of isogamete fusion.

Why phototrophic euglenoids are considered as algae?

Phototrophic euglenoids such as *Euglena*, *Phacus*, *Lpocinlis*, *Trachedomonas* and *Strobomonas* are considered as algae because they have features of algae. These are highly specialized unicellular flagellate algae. Phototrophic euglenoids contain chloroplast and produce their own food by photosynthesis. The main pigment is Chlorophyll a and b with Xanthophyll. These algae possess a complex vacuolar system. The chloroplasts of euglenoids are known to contain pyrenoids which are used in the synthesis of Paramylon, a polysaccharide which is a product of photosynthesis. Most of the euglenoids have one or two flagella which arises from anterior end. All these characters support that phototrophic euglenoids are considered as algae. G.M. Smith and F. E. Fritsch also considered phototrophic euglenoids as algae. G. W. Prescott also supported Smith and Fritsch. Now a days all over the world, Fritsch classification of algae is followed by the psychologists.

Phagotrophic Euglenoids

Ganpati and Chaco (1952) studied euglenoids from natural and polluted water. Haughey (1970) enumerated morphology and occurrence of *Euglena acus* in ponds. Munawar (1972) studied ecological distribution of euglenoids in polluted and unpolluted water. Ashtekar (1980) studied phototrophic euglenoids of fresh water habitats. Hosmani and Bharati (1983) reported *Euglena* and *Phacus* as most frequent euglenoids in fresh water bodies. Wringley and Toerin (1990) recorded dominance of *Euglena* and *Lepocinclis* in sewage ponds. Waghodekar and Jawale (2001) reported eight taxa of euglenoids from fresh water bodies. Kumawat and Jawale (2004) worked on ecology of fish pond in relation to euglenoids. Kumawat *et al.* (2007) reported 31 taxa of euglenoids from different water bodies. Talekar (2009) studied diversity and distribution of euglenoids in fresh water habitats. Hosmani (2012 and 2013) studied distribution of euglenoids in fresh water lakes and reported *Euglena* and *Lepocinclis* as indicator of water quality. Gour and Pal (2017) recorded 41 taxa of euglenoids from Indian sunderbans. Murulidhara and Murthy (2018) studied distribution and ecology of euglenoids in relation to physicochemical characteristics of water. Yadav (2020) recorded 60 taxa under 4 genera of euglenoids from different fresh water habitats. He observed abundance of euglenoids in summer season. The maximum number of euglenoids were found in polluted water.

Phototrophic Euglenoids of Khelna Reservoir

While studying algal flora of Khelna reservoir, authors came across some interesting taxa of phototrophic euglenoids. Khelna reservoir is fresh water reservoir. It is located in Sillod tehsil area of Aurangabad district of Maharashtra. Collection of algal samples were made at monthly intervals from October 2014 to September 2016. Acid washed collection bottles were used for collection of algal samples. Algal samples were brought immediately to the laboratory and observed under microscope and identified by referring to the standard literature on algae.

During present study, 6 taxa of euglenoids were identified and recorded (Table 1). *Euglena acus*, *Euglena elongata*, *Euglena polymorpha* and *Phacus orbicularis* were found abundant in Khelna reservoir. *Euglena Proxima* and *Phacus* sp. were also recorded. Ganpati and Chaco (1951), Munwar (1972), Hosmani and Bharathi (1980) and Wrigley and Toerien (1992) recorded dominance of *Euglena* and *Lepocinclis* in fresh water bodies. Talekar (2009) also recorded similar kind of observation. Yadav (2020) recorded 60 taxa of euglenoids from different fresh water bodies. He observed that summer season is the suitable season for abundance of euglenoids. Euglenoids often serve as indicators of organic pollution. The presence of large number of euglenoids is evidence of high dissolved organic content. Dominance of euglenoids indicates the presence of higher pollution load in water body. (Hosmani 2012 and Ajayan 2020). *Euglena* sp. isolated from sewage treatment plants had higher nutrient removal capability and growth rate than other algae (Mahapatra *et al.* 2013). The application of *Euglena* in environmental engineering has been studied for waste water treatment systems and bioindicators for environmental pollution. (Gissibl *et al.*, 2019). Euglenoids occupy shallow aquatic ecosystem in large number due to high concentrations of

organic materials in water body. The species richness and abundance are two most important factors that make this group unique to nutrient rich water body.

Table-1: Taxa of euglenoids from fresh water Khelna reservoir

Sr. No.	Euglenoid taxa	Frequency of occurrence
1	<i>Euglena acus</i> Ehrenberg	+++
2	<i>Euglena elongata</i> Schewiakolt	+++
3	<i>Euglena polymorpha</i> Dangeard	+++
4	<i>Euglena proxima</i> Dangeard	+
5	<i>Phacus orbicularis</i> Huebher	++
6	<i>Phacus</i> sp.	+

+ = Minimum, ++ = Moderate, +++ = Dominant

Conclusion

Phototrophic euglenoids such as *Euglena*, *Phacus*, *Lepocinclis*, *Trachelomons* and *Strobomonas* are considered as algae because they have characters of algae. They are highly specialized unicellular flagellate algae. From Khelna reservoir, 6 taxa of euglenoids were recorded. *Euglena acus*, *Euglena elongata*, *Euglena polymorpha* and *Phacus orbicularis* were found dominant. Euglenoids often serve as indicators of water quality. The presence of large numbers of euglenoids is evidence of high dissolved organic content. Mainly euglenoids are studied for diversity, abundance and their ecological importance as indicators of water quality.

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CHAPTER

05

IMPACT OF AIRBORNE ALGAE ON HUMAN HEALTH

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A**bstract**

Algal spores and fragments are one of the important components of air microflora. It is a well-known fact that algal cells contain protein in high concentration and it is of considerable importance with reference to allergic reactions in sensitive individuals. The airborne algal propagules have been considered to cause bronchial allergy in human beings. Hay fever, allergic asthma, allergic rhinitis, allergic cough and cold and dermatitis are caused by allergenic airborne algae. A few studies from abroad as well as India have already implicated algae as causative agents in allergic diseases in human being. While studying airborne algal flora of Khandesh and Marathwada region of Maharashtra, authors came across some interesting taxa of allergenic airborne algae. A total of 23 taxa of allergenic airborne algae have been recorded. This data is useful to physician and allergician for effective treatment of various allergic disorders in human beings.

Keywords: *Airborne Algae, Allergy, Human Health.*

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Introduction

Airborne algae are one of the important biocomponents of air. It remains viable in the atmosphere in the form of spores and filaments. Both aquatic and terrestrial algal forms may become airborne under certain environmental conditions. The algal mats formed in water bodies and on moist soil get dried up during dry weather in various seasons, with the exception of rainy season. They may be lifted into air in the form of dried filaments or resting spores, along with the wind currents. When suitable nutrient media are used for trapping and providing them with the growth promoting substances, they develop into the usual thalloid habit. Tropical climate of our country is most suitable for airborne algae. However very few investigations are being conducted on airborne algae and its impact on human health. Algal spores and filaments remain suspended and viable in air for a long time. When they come in contact with sensitive individuals it causes allergic diseases. It is a well-known fact that algal cells contain protein in high concentration and it is of considerable importance with reference to allergic reactions in sensitive individuals. Research on airborne algae all over the world is gradually gaining momentum. A few investigations from abroad as well as India have already implicated airborne algae as causative agents in human respiratory allergies. While studying airborne algal flora of Khandesh and Marathwada regions of Maharashtra, authors came across some interesting taxa of allergic airborne algae (Jadhav 1995, Jadhav and Quazi 2010, Jawale and Jadhav 2017).

Airborne Algae and its Impact on Human Health

Ehernberg (1844) was the first to study algae present in the dust. Salisbury (1866) is the first to implicate algae as the disease-causing organisms. Woodcock (1948) postulated the cause of respiratory difficulties. Heise (1949) demonstrated that the allergenicity of algae was responsible for hay fever and asthma. Bernstein and Safferman (1966) and Beniam Pinto (1972) observed airborne algae as a possible etiologic factor in respiratory allergy. Agarwal *et al.* (1977) studied the immunological role of airborne algae in chronic lung diseases. Beskow (1978) reported algal silicosis in diatomaceous earth factory workers. Mittal *et al.* (1979) performed intra dermal skin tests on 400 patients suffering from nasobronchial allergy. Santra (1987) reviewed the work related to algal allergy. Jadhav (1995), Jadhav and Quazi (2010) and Jawale and Jadhav (2017) reported allergenic airborne algae from Khandesh and Marathwada region of Maharashtra. Sharma and Rai (2008) worked on allergenicity of airborne Cyanophycean algae *Phormidium fragile* and *Nostoc muscorum*.

Allergenic airborne algae recorded from the atmosphere of Khandesh and Marathwada region of Maharashtra

Occurrence of airborne algae has been known since long time. Airborne algae have definite impact on human health. They are considered to cause bronchial allergy in human being. Hay fever, asthma, bronchial rhinitis, cough and cold are other diseases are caused by these organisms in different individuals. While studying airborne algae of Khandesh and Marathwada region of Maharashtra. Jadhav (1995), Jadhav and Quazi (2010) and Jawale and Jadhav (2017) recorded 23 allergenic airborne algae (Table 1) which have been already reported as aeroallergens. *Chlorella vulgaris*, *Nitzschia palea*, *Aphanothece nidulans*,

Phormidium jenkelianum, *Phormidium molle*, *Nostoc muscorum*, *Nostoc punctiformae*, *Plectonema gracillimum* and *Scytonema bohneri* were found dominant in air. Heise (1949) worked on symptoms of hay fever and asthma caused by algae. Goyal (1976) reported some allergenic airborne algae from atmosphere of Delhi. Agarwal *et al.* (1977) studied immunological role of airborne algae in chronic obstructive lung disease. Shwimmer and Schwimmer (1964) studied abundance of algae in air and allergenic diseases caused by them. Bernstein and Safferman (1966) examined allergenicity of six algal forms viz. *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorococcum botryoides* and *Ankistrodesmus falcatus* var. *acicularis* on 79 atopic patients.

Mittal *et al.* (1979) from Delhi, performed intradermal tests on 400 patients of respiratory allergy with extracts of 10 algal forms viz. *Lyngbya major*, *Phormidium angustissimum*, *Anabaena fertilissima*, *Scytonema bohneri*, *Chlorella pyrenoidosa*, *Westiellopsis prolifica*, *Anabaenopsis circularis*, *Oscillatoria simplicissima*, *Nostoc commune* and *Chlorococcum humicola*. Mc Elhenny *et al.* (1962) tested 4 green algae *Neochloris*, *Chlorosarcinopsis*, *Bracteococcus* and *Hormidium* on 120 children suffering from respiratory allergy and observed that 98 cases show positive reaction to intradermal test. Benaim Pinto (1972) from Caracas, Venezuela, tested an extract of *Chlorella pyrenoidosa* on adult patients and asthmatic children and 20% of this showed reaction. 23 airborne allergenic algae have been recorded from Khandesh and Marathwada regions of Maharashtra. This work supports the findings of other research workers.

Table-1: Allergenic airborne algae recorded in the air of Khandesh and Marathwada regions of Maharashtra.

Sr. No.	Allergenic airborne algae	Frequency of occurrence
1	<i>Chlorococcum humicola</i>	++
2	<i>Trebouxia humicola</i>	++
3	<i>Chlorella vulgaris</i>	+++
4	<i>Ankistrodesmus falcatus</i>	+
5	<i>Nitzschia palea</i>	+++
6	<i>Gloeocapsa rupestris</i>	+
7	<i>Gloeotheca palea</i>	+
8	<i>Aphanothece nidulans</i>	+++
9	<i>Myxosarcina burmensis</i>	++
10	<i>Phormidium abronema</i>	+

11	<i>Phormidium fragile</i>	+
12	<i>Phormidium jenkelianum</i>	+++
13	<i>Phormidium molle</i>	+++
14	<i>Phormidium tenue</i>	+
15	<i>Microcoleus acutissimus</i>	++
16	<i>Nostoc commune</i>	+
17	<i>Nostoc linckia</i>	++
18	<i>Nostoc muscorum</i>	+++
19	<i>Nostoc punctiformae</i>	+++
20	<i>Plectonema gracillimum</i>	+++
21	<i>Plectonema hansgirgi</i>	+
22	<i>Scytonema bohneri</i>	+++
23	<i>Scytonema schmidtii</i>	++

+ = Minimum, ++ = Moderate, +++ = Maximum

Conclusion

Airborne algae are important biocomponents of atmosphere which remains viable in the air in the form of spores and filaments. Algal cells contain protein in high concentration and it is of considerable importance with reference to allergic reactions. Allergenicity of algae is responsible for hay fever, asthma, nasobronchial allergy, allergic cough and cold, dermatitis, silicosis and chronic obstructive lung diseases. Tropical climate of our country is most suitable for airborne algae. 23 taxa of known allergenic airborne algae have been recorded from Khandesh and Marathwada region of Maharashtra. This work supports the findings of other researchers and will be helpful to the physician and allergician for effective and efficient mode of treatment of various allergic disorders in human being.

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CHAPTER

06

MARINE ALGAE AN OVERVIEW: UNLOCKING THE DEVELOPMENTAL PROSPECTIVE

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Rathod²

A bstract

The water world is the primary untapped source of naturally occurring products with structurally distinct potential that are primarily found in living things and are not competitive with land utilization or agriculture, providing a source of income, food, medicine, benefits to human health, ecological benefits, ecosystem services, and employment. Marine algae are important sources of chemical compounds that are beneficial for health and have been extensively utilized for decades. In addition to being novel nourishment with significant nutritional benefits, marine algae are speculated to serve as the primary producers of some of the highly bioactive substances found in marine resources. Algae, comprising seaweeds and microalgae, contribute to the production of seaweeds used in aquaculture around the world. Marine algae, one of the most significant potentially known renewable resources in the ocean and existing in a diversity of diverse environs and present in all prevailing bionetworks on Globe, comprise an extremely essential element of the marine ecosystem contributing a major feeding, breeding and nursery ground for marine animals as well sunrise sector resources for human being.

Keywords: *Macroalgae, Marine Algae, Seaweed, SDG, PMMSY.*

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Introduction

Marine algae are amongst the most primeval associates of the plant kingdom. They can be found in a staggering range of organisms, from single-celled organisms to gargantuan kelp that may reach up to 40 meters tall. The smallest species known to be consumed by human beings is the protein-rich *Spirulina*, composed of free-floating threads roughly one quarter of a millimeter long. The microscopic species are seldom exploited. Some of the edible *Codium* species generate soft pillows that adhere to the substrate resembling mushroom caps.

Marine algae are typically divided into three types according to their morphological pigmentations, and are rich sources of structurally diverse bioactive chemicals. Rhodophyta, a group of red seaweed, Chlorophyta, a group of green seaweed, and Phaeophyta, a group of brown seaweed, are these three types. Environmental factors including CO₂ levels, temperature, pH, salinity, and sunshine intensity can affect the chemical properties of the seaweeds. Complex carbohydrates are prevalent in marine algae from the genera *Gracilaria*, *Laminaria*, *Undaria*, and *Ulva*. Polysaccharides and oligosaccharides from marine algae show great potential in food, nutraceuticals, and pharmaceutical area due to the multiple bioactivities (such as anti-bacterial, anti-virus, anti-oxidant, anti-inflammatory, anti-cancer activities, and regulation of gut microbiota).

Multicellular marine algae that are large enough to be seen by the naked eye are referred to as seaweed. Some can reach lengths of up to 60 metres. They are not plants because they belong to the kingdom Protista. They lack the internal transport system (vascular system) found in plants as well as roots, stalks, leaves, flowers, or cones. They contain various pigments that can be tinted red, blue, brown, or gold and utilise the pigment chlorophyll for photosynthesis just like plants produce.

Seaweeds qualify as plants as they use solar energy to create carbohydrates from carbon dioxide and water through photosynthesis, they tend to be simpler than land plants primarily because they obtain every nutrient from the water surrounding them and lack roots or specialized conducting tissues (Kandale *et al.*, 2011).

Brief Overview

Total fisheries and aquaculture production reached a record 214 million tonnes in 2020, comprising 36 million tonnes of algae, largely due to the growth of aquaculture, particularly in Asia. Global algae production (including cultivation and wild collection) improved over 60 times from 0.56 million (wet) tonnes in 1950 to 35.82 million tonnes in 2019 with a Global Market Value: USD 12 Billion (FAO-2019); approximately all the development was contributed by seaweeds farming represents 97.1% by volume. Cultivation of algae globally grew by half a million tonnes in 2020 (FAO,2021)

Indian Seas have been reported to have 844 different kinds of seaweed, with a standing stock value of 58,715 tonnes (wet weight). India is home to over 434 species of Red Algae, 194 species of Brown Algae, and 216 species of Green Algae out of the total 844 seaweed species.

According to food and agriculture organization the term "algae" in this article refers to both the aquatic photosynthetic organisms known as microalgae and seaweeds (also known as marine macroalgae). Algae play a crucial role in the aquatic ecosystem by providing the energy base of the food web for all aquatic organisms. They also offer a variety of

environmental advantages and ecosystem services, including the reduction of eutrophication, the capture or sequestration of carbon dioxide, the amelioration of ocean acidification, the provision of habitat, and the protection of shorelines.

Seaweeds are regarded as a nutritious, low-calorie, and healthy food. Seaweeds are generally low in fat and include a variety of critical nutrients, including omega-3 and omega-6 polyunsaturated fatty acids, vitamins (A, C, E, and B12), iodine, dietary fiber, and antioxidants, even though the nutrient composition of seaweeds varies depending on the species. Beyond its nutritional value, seaweed consumption has been associated with several health advantages, including lowering blood pressure and reducing the risk of stroke (Fitzgerald *et al.*, 2011). Seaweed is one of the multiple sustainable alternatives that might support global food security, either as food or as feed, notably for aquaculture, in the context of an increasing global population and environmental issues (Cai *et al.*, 2021).

Seaweeds have a wide range of additional applications, in both the food and non-food industries, such as food additives, animal feed, pharmaceuticals, cosmetics, textiles, biofertilizers/biostimulants, bio-packaging, and biofuel (FAO, 2018). Biodiesels, biogases, bioalcohols biomaterials, biocomposites, bioadhesives agrichemicals, biopolymers, animal feed fine chemicals, ingredients, human food nutraceuticals, cosmeceuticals, bioactives in Markets and added value of seaweed products. However, the scientific community and enterprises associated to seaweed are typically the only ones who are aware of their contribution to these products.

The potential contributions of seaweeds to numerous Sustainable Development Goals (SDGs) (e.g. SDG 1, SDG 2, SDG 3, SDG 8, SDG 10, SDG 12, SDG 13 and SDG 14) have been documented, for example, in a "Seaweed Manifesto" due to their various social, environmental, and economic benefits and contributions (Bjerregaard *et al.*, 2016). Varied industries are becoming more interested in seaweeds, with a focus on their potential to provide nutrient-dense food to feed the world's increasing population and for the ecosystem services they offer, particularly in lowering greenhouse gas emissions (Parodi *et al.*, 2018; Duarte *et al.*, 2020). Researchers of seaweeds predict a future "Seaweed Revolution" due to the existence of large oceanic areas suited for seaweed farming.

Seaweed Reproduction

The life and reproduction of seaweeds can be very complex. While some seaweeds are annuals and only live for a year or two, others are perennials. Annual seaweeds often start growing in the spring and keep growing all summer. Some red seaweeds live for six to ten years.

Seaweeds have the ability to reproduce sexually by the union of specialised gametes, or male and female reproductive cells. The spores settle after being discharged from the sporophyte and develop into male and female plants known as gametophytes. Gametes (eggs or sperm) are produced by the gametophytes. Either the sperm and eggs are kept inside the gametophyte plant body or they are discharged into the water. A zygote is created when an egg and sperm combine to fertilise an egg. The life cycle continues as zygotes mature into sporophytes.

The before mentioned scenario is merely a generic illustration of one type of seaweed reproductive and life cycle, known as alternation of generations. Asexual and sexual reproduction alternate with each generation in a small number of organisms.

Seaweeds are able to reproduce asexually by division or fragmentation. This occurs when a plant's constituent parts separate and give rise to fresh individuals. Asexual reproduction produces only clones, which are genetically identical to both the parent seaweed and each other.

Microalgae

Microalgae are tiny algae that are typically not visible to the unaided eye. Phytoplankton called microalgae can be found in freshwater and marine ecosystems. Microalgae are a diverse group of unicellular algae species, including diatoms (Bacillariophyceae), green microalgae (Chlorophyceae; for example, *Chlorella* spp.) and *Nannochloropsis* spp. (Chua *et al.*, 2020), Schizochytrium spp. (EFSA NDA Panel, 2020), to name a few closely related to aquaculture. The production of commercial microalgae is relatively low compared to seaweeds. *Spirulina* and other cyanobacteria, commonly referred to as blue-green algae, are typically included in the category of microalgae (Hill, 2017).

Microalgae have other potential uses include wastewater treatment, the production of algal meal and oils, carbon sequestration, and the production of biofuels (Spolaore *et al.*, 2006; Khan *et al.*, 2018; Barkia *et al.*, 2019; Ragaza, 2020). Although some of these applications may not yet be fully commercialised, the markets for many of them are expanding quickly. Other aquaculture facilities cultivate significant amounts of microalgae to create animal feeds,

Three groups of seaweeds are recognized, according to their pigments that absorb light of particular wavelengths and provide them their distinguishing colours of green, brown or red. Because they required sunlight to survive, seaweeds are found only in the relatively shallow parts of the ocean shorelines. They occur in a variety of shapes and sizes, from the large kelps (certain brown seaweeds) that form forests on temperate coasts, to the hard “encrusting corallines” that appear alike pink glazing, but important in building and cementing coral reefs in the tropics. Some seaweed, especially many of the larger reds, are showy and attractive, while others may be small and inconspicuous, and grow in a low “turf” on the rocks.

Only macro-phytic marine algae that grow in saltwater, both naturally and artificially, are referred to be seaweeds. Seaweeds can be categorised botanically as green, brown, or red.

Red Algae (Rhodophyta)

These are between 6,500 and 10,000 different known species of marine algae, predominantly multicellular, including several well-known seaweeds and more than 160 freshwater species. The phycobiliprotein pigments give them their red colouring. Red algae come in a variety of sizes, from tiny to enormous and fleshy. Although they are often red, some species that reside at the top of the intertidal zone might be olive-brown.

All across the world, red algae can be seen growing adhering to the ground or other hard surfaces. Herbivores including fish, crabs, worms, and gastropods graze on them. Red algae generally grow more slowly than green algae and can reproduce both asexually and sexually. Of all species, they have some of the most intricate sexual cycles. Glycogen constitutes the manner in which most red algae store their carbohydrates. A lengthy chain of glucose molecules with numerous side branches builds up of glycogen. Compared to the starch that brown or green algae store, it is extremely different. Rhodophyta has three significant

compounds in their cell walls in addition to the typical cellulose cell walls that the majority of algae possess. Agar, carrageenan, and gelans (mucous sugars) are the compounds in doubt. Some red algae, like coralline algae, deposit calcium carbonate in their cell walls. This protects them from being devoured while giving them strength and stability. These algae play a significant role in the development of coral reefs.

Some red algae species have the ability to produce a thin mat that covers rocks and other hard surfaces; these species are known as crustose when they do so. Red algae that is erect and crustose attach and fill coral skeletons to create vast sedimentary structures strong enough to withstand wave action and erosion. Red algal gamete cannot swim as they rely on water currents to carry them to the female reproductive organs. Their reproductive cycles, which involve the alternation of generations, can be fairly complicated.

Several species, such as *Palmaria palmata* or dulse, are consumed as food. It is a popular snack food in Europe, and it has long been a significant fibre source in Iceland. Nori cultivation is a significant sector in Japan that employs over 300,000 people. Nori is often red or crimson in colour; it turns dark or almost black when dried and turns green when cooked. Sushi is made with nori, which is also used in breads, soups, and salads.

Green Algae (Chlorophyta)

Green algae are found in more than 6,000 different species. Green algae can be found in freshwater or marine environments and some species can even survive on land, growing on rocks, trees, and soil. Green algae can be colonial, multicellular, or unicellular. While some species develop colonies (lived as a loose aggregation of cells) or coenocytic (made of one giant cell without cross-walls; long filaments, or larger macroscopic seaweeds), others spend the most of their life as single cells.

In both temperate and tropical waters, green algae can be found. They cling to the bottom but do not grow to considerable depths because they need sufficient light to photosynthesize. They can frequently be found in tide pools and the intertidal zone of rocky coastlines. Many will settle in regions where rivers meet the sea because they can endure low salinity levels.

Green algae come in a wide range of forms and dimensions. On coral reefs, where they play an essential role, some of the most prevalent types of green algae are sea lettuce (*Ulva*), sea grapes (*Caulerpa*), and turtle grass (*Chlorodesmis*). On coral reefs, there are between 50 and 100 common green algae, which are the main source of food for most herbivorous fish and several other smaller species like crabs, worms, and gastropods. Like higher plants, they store food as starch, certain lipids, or oils. Green algae reproduce asexually through fission, budding, fragmentation or zoospores (motile spores), as well as through an alternation of generations cycle.

The hypersaline (high salinity water) green alga *Dunaliella salina* is grown in sizable ponds to produce organic beta-carotene, one of the green pigments in green algae. Lung cancer has been demonstrated to be one of the malignancies that carotene is highly good in preventing.

Brown Algae (Phaeophyta)

The major type of algae is brown algae. Brown or yellow-brown in colour, they are typically found in temperate or arctic oceans. Brown algae typically have a holdfast. There are

approximately 1500–2,000 diverse kind of brown algae. Fucoxanthin is an extra pigment that they possess. They come in various shades of olive green and brown. The precise coloration is determined by the quantity of fucoxanthin that occurs in the algae. The brown algae category features the largest and fastest-growing seaweeds. The stipes of giant kelp can grow up to 6 cm in a single day.

The cell wall of brown algae made up of two layers *i.e.* the inner layer, which provides strength, is made of cellulose, while the outside wall layer is primarily made of algin, a substance that resembles jelly when wet but hardens and cracks as it dries. Algin-derived substances are employed in industrial, pharmaceutical, and food products including ice cream as thickening, stabilizing, emulsifying, or suspending agents.

Brown algae can grow in a variety of shapes and sizes. The smallest individuals in the group develop as brief, feathery tufts of cells with a diameter of a few centimetres or less. The largest of all the algae is the enormous kelp *Macrocystis pyrifera*, while smaller kelps include the two-foot-tall sea palm *Postelsia*. The brown algae, which are found in the tropical waters of the Sargasso Sea in the center of the North Atlantic Ocean, take a variety of shapes, from tiny crusts or cushions to leafy free-floating mats.

Brown algae can be made up of foot-long flattened branches resembling a fan, as in *Padina*, or delicate felt-like strands of cells, as in *Ectocarpus*. Along stony seashores, several brown algae, including the *Fucales*, typically flourish.

Coralline Algae

Red algae are coralline algae. Due of calcium carbonate deposits found within the cell walls, they have a hard thallus. These algae typically have pink or other reddish hues as their colour; however, some species can also be purple, yellow, blue, white, or grey-green.

The majority are encrusting and rock-like, and they can be found in marine environments all over the world, from occasionally exposed intertidal zones to a depth of 270 m (about the maximum light penetration). In the ecosystem of coral reefs, they are crucial. Many young animals, such as sea urchins, chitons, and limpets, as well as parrot fish, sea urchins, and molluscs (such as limpets and chitons) that feed on coralline algae provide microhabitats that provide protection to these young creatures.

They are the most prevalent red algae in coral reef ecosystems, creating hard crusts on the reef surface, binding coral, and constructing the structure of the reef. On the underside of coral debris or in dark places, the coralline algae are very prevalent.

Coralline algae can be divided into two categories:

- **Geniculate** (articulated) corallines, are flexible, branched trees that are held to a surface by holdfasts that are crustose or calcified and resemble roots. Uncalcified parts (genicula) between longer, hard sections provide the plant's flexibility.
- **Nongeniculate** (nonarticulated) corallines have crusts that can be a few micrometers to several centimeters thick. They can grow on rock, coral skeletons, shells, other algae, or seagrasses and are frequently very slowly growing.

Advancement of sea-weed culture in India

Department of Fisheries, Government of India is been providing huge funding for the promotion of the seaweed farming practices, growth and development in different coastal states through Autonomous body like NFDB National Fisheries Development Board.

Various Central institutes coming forward for the development of seaweed sector in India and working for the seaweed cultivation *i.e.* Central Salt And Marine Chemical Research Institute (CSIR-CSMCRI); Central Marine Fisheries Research Intitutes (CMFRI); National Institute of Ocean Technology (NIOT); State Fisheries Departments.

PMMSY (Pradhan Mantra Matsya Sampada Yojana)

The goal set by the Department of Fisheries for FY 2020-25 is 11.2 Lakh MT of Fresh Seaweed Production in India which is envisaged to be achieved under Pradhan Mantri Matsya Sampada Yojana (PMMSY) during next 5 years.

PMMSY providing various opportunities to increase the income of the Seaweed Farmers and fund provided approximately Rs. 640 Cr. Investment. For Seaweed cultivation as well as additional Investment for interventions like establishment of seed banks, genetic improvement programs for high yielding cultivars, seaweed parks etc.

Main purpose of the scheme is to enhance the production and productivity in the seaweed aquaculture in the country for harnessing the potential of the coastal waters and reduce reliance on wild harvest. To improve the seaweed value chain and industrial product diversification to meet domestic demand and thus reduce dependence on imports to promote seaweed cultivation as a viable and sustainable livelihood amongst rural communities, especially for women.

Ranjan (2021) Intensive efforts will be made by the implementing agencies to prioritize formation and promotion of FFPOs, support to cooperatives and women SHG's in the potential coastal areas in the country. Women were the first and primary adopters of seaweed farming In India, which offered them an income within a safe environment (Krishnan and Narayanakumar, 2013).

Conclusion

Marine algae are boon for the economy through various industries as a great source of income generation, nutrition, livelihood, biodiversity, numerous Sustainable Development Goals and thousands of benefits which we cannot neglect. These papers inform the most significant spectrum of marine algae offers an extensive list of beneficial goods for us. Thus, marine algae are essential to mankind as food, vitamins, minerals, medications, commerce & industrial products and other requirements of daily existence. We concluded from this that marine algae have a key role in human welfare.

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CHAPTER

07

ALGAL POLYSACCHARIDES: THE POTENTIAL MEDICINES

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A**bstract**

Among polysaccharides in algae, starch and cellulose, are the most prevalent forms. These are called storage and structural polysaccharides, respectively. In addition to these, the agar, alginates, and carrageenans are also extracted from macroalgae. The bulk of commercial seaweed extracts comes from these three hydrocolloids. The ulvans, fucoidans and laminarin are the other important polysaccharides found in algae. The commercial applications of algal polysaccharides in industries such as the food, cosmetic, pharmaceutical, paper etc., are well known but the algal polysaccharides as potential medicines are less discussed.

Keywords: *Hydrocolloids, Macroalgae, Polysaccharide, Seaweed Extracts.*

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Introduction

Algae is a diverse (polyphyletic) category of plants, making it challenging to characterize these exactly. However, algae are generally referred to as an "assemblage of tiny, simple, primitive, mostly eukaryotic, thalloid, non-flowering, seedless, non-embryonic, non-vascular, mostly aquatic (fresh water or marine), green (photosynthetic and autotrophic), plants/organisms with single-celled sex organs, if multicellular in nature those (sex organs) are without any sterile layer of jacket cells."

Algae are divided into two types such as microalgae and macroalgae. The microalgae are microscopic and unicellular and are referred to as the phytoplankton while the macroalgae are macroscopic and multicellular commonly known as the seaweeds (Pereira, 2021; Samanthi, 2023). The macroalgae belonging to the division Chlorophycophyta, Phaeophycophyta, and Rhodophycophyta are commonly known as "green seaweeds," "brown seaweeds," and "red seaweeds," respectively. ("Seaweed" Wikipedia, 2023). The class of carbohydrates known as polysaccharides consists of units of simple sugars (monosaccharides) that are linked or joined together in a long chain through glycosidic bonds. The most common types of polysaccharides include starch, cellulose, glycogen, and chitin. Polysaccharides may be branched or unbranched. Cellulose and amylose, a constituent of starch are the examples of unbranched polysaccharides, whereas amylopectin, another constituent of starch, chitin and glycogen are highly branched polysaccharides (Rye *et al.*, 2016).

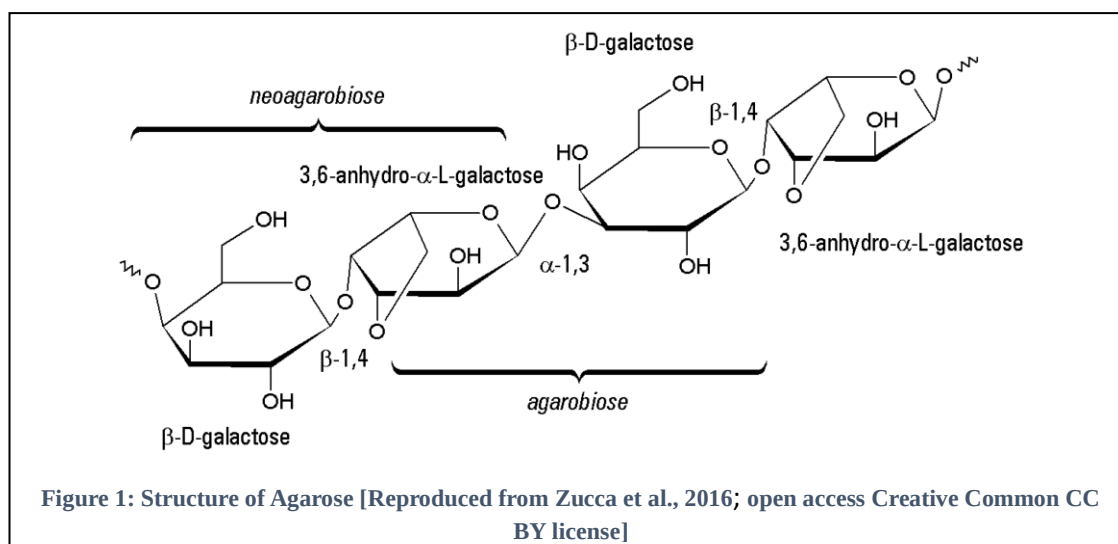
The polysaccharides such as agar, alginates, and carrageenans are the hydrocolloids that are derived from macroalgae and are utilized as thickening and gelling agents. Together, these three hydrocolloids make up the majority of commercial seaweed extracts. Hydrocolloid is a non-crystalline material with very big molecules that dissolves in water to produce a thicker solution (McHugh, 2003; Gualtieri and Barsanti, 2014). In brown algae, the cell wall is composed of two layers: an outer layer consists of alginic acid and fucoidan, and an inner layer is made of cellulose (Sharma, 2011).

The global market for algal polysaccharides has been growing gradually in the recent years and is predicted to increase by 7.5% CAGR (compound annual growth rate) from 2023 to 2030. By 2030, the market is expected to be worth USD 3.1 billion, up from the present valued USD 1.9 billion (in 2022). And brown algae are currently the market leaders with a value share of over 35%. The green algae market is anticipated to expand more quickly. Even though the food industry currently holds the top spot, there are encouraging prospects for algal polysaccharides' medicinal sector to expand in the coming years (Credence Research-report, 2023).

Agar-Agar

Agar (agar-agar) is a type of sulphated galactan (polymer of galactose) obtained from the cell walls of red algal (Rhodophycophyta) species, mainly the species of *Gelidium* and *Gracilaria* (Das, 2007). Such agar-containing seaweeds are known as agarophytes. *Gelidium* yields comparatively higher quality agar than *Gracilaria*. However, pre-treating *Gracilaria* with alkali before the extraction though reduces the yield, gives the high-quality agar (McHugh, 2003; Agargel, 2023).

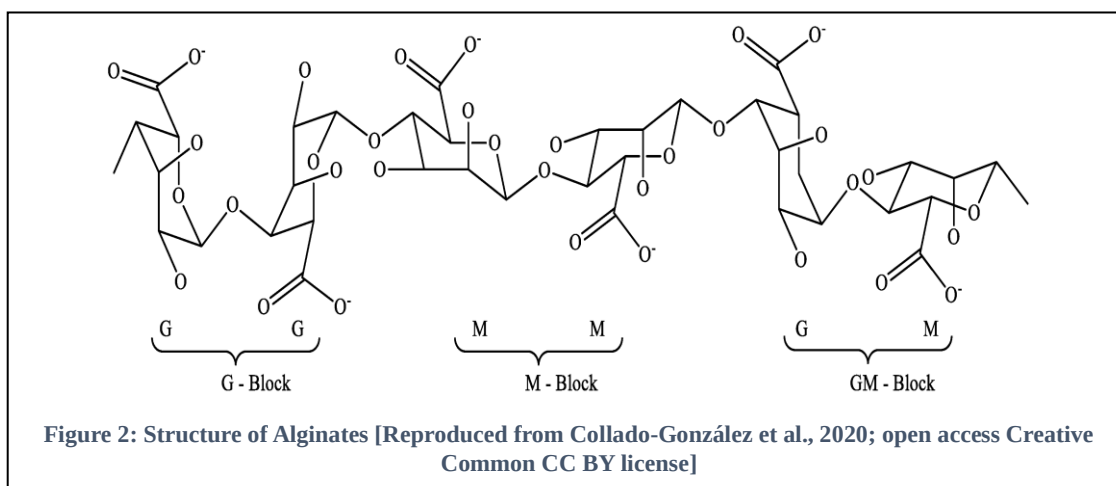
Agar is made up of agarose and agarpectin. Agarose is the major component made up of repeating units of two monosaccharides called β -D-galactose and 3,6-anhydro- α -L-galactose (Figure 1) that are linked by two different forms of glycosidic linkages, called β (1–4) and α (1–3) linkage forming a basic disaccharide unit of agarose polysaccharide. The agarpectin has the same repeating units with few substituents on 3,6-anhydro- α -L-galactose units (Zucca *et al.*, 2016).



The stomach is displaced in a patient with stomach prolapse, meaning that the top of the stomach is still in its normal position but the base is lower than usual. When such a patient is given a diet that contains dried agar-agar and is advised to drink a lot of water, the stomach returns to normal (Vashishta *et al.*, 1960; Vinmec, 2019). Agar is employed as a laxative in medicine (Daniel, 2006). In a study, conducted by Louala and Lamri-Senhadj, (2019) the protective effects of the high agar diet against HFD (High Fat Diet-) induced hepatic steatosis damage in rats was observed and they suggested that this agar diet may be a promising therapeutic approach to prevent the onset of Non-Alcoholic Fatty Liver Disease (NAFLD), which is a liver condition that is closely related to obesity. Agar-agar is helpful in treating the form of constipation linked to tiny, dry stools in both children and adults. The use of agar is safe and typically yields the best results (Morse, 1910).

Alginates

Alginate-containing seaweeds are called alginophytes (McHugh, 2003). These are obtained from the cell walls of the species of brown algae (Phaeophycophyta) such as those of *Ascophyllum*, *Lessonia*, *Macrocystis*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Fucus*, and *Sargassum* (McHugh, 2003; Das, 2007). The salts of alginic acid are known as alginates. The polysaccharide alginic acid is made up of two different forms of uronic acid units, mannuronic acid unit (M) and guluronic acid unit (G), (Figure 2) which combine in a specific fashion to form three different types of polymer portions of blocks: M blocks are made up of only M-M bonding, G blocks are made up of only G-G bonding, and M&G random blocks are formed of M-G random bonding (KIMICA, 2023).

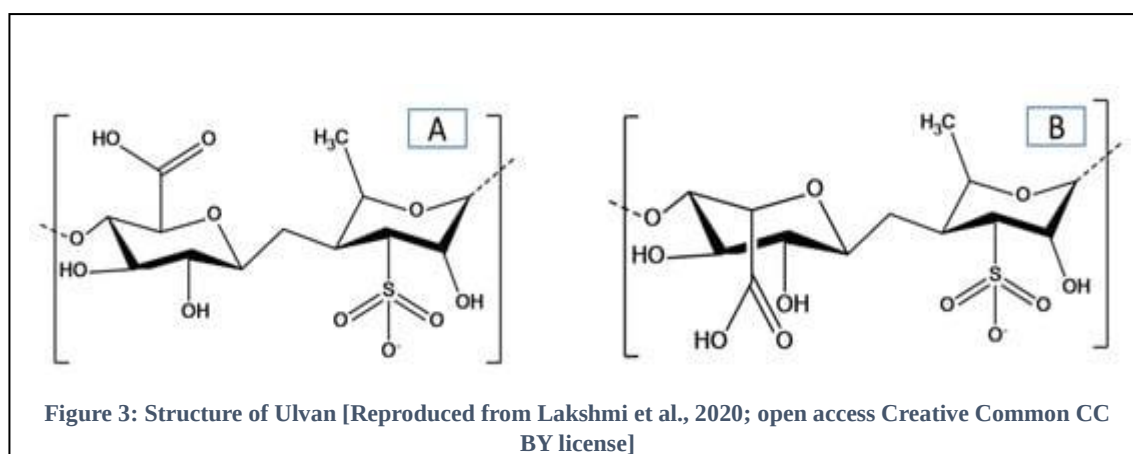


Alginic acid helps in blood coagulation process (Vashishta *et al.*, 1960). Alginate fibres, or calcium alginate, are employed as a swab for pathological testing or as a haemostatic dressing (Daniel, 2006). Since alginate hydrogels maintain a structural resemblance to the extracellular matrix in tissues, they have been used in tissue engineering, drug delivery, and wound healing. Pancreatic islet allografts and xenografts are micro-encapsulated in alginate gel during tissue engineering, protecting the grafts from the host immune system without the need of immunosuppressive medications. This approach is used to help cure animal model type I diabetes by grafting (Lee and Mooney, 2012).

Ulvans

These are structural polysaccharides obtained from the cell walls of various species of *Ulva*, the green seaweed. *Enteromorpha* also yields the ulvans. (Kim *et al.*, 2011) The ulvans are sulphated and water soluble heteropolysaccharides (Boubakri, 2020). The ulvan is made up of the repeating disaccharide units of glucuronic acid and iduronic acid (Figure 3) each linked to rhamnose-3-sulphate sugar (Lakshmi *et al.*, 2020).

Ulvan has the potential to be used as a biomaterial for wound dressings, and Sulastri *et al.* (2021) successfully prepared this on the basis of its biopolymer qualities, such as its antibacterial and antioxidant properties. According to the findings of Zhao *et al.*, (2020), mice models treated with ULP (*Ulva lactuca* polysaccharide) demonstrated strong anticancer activity and immunoregulatory effects.

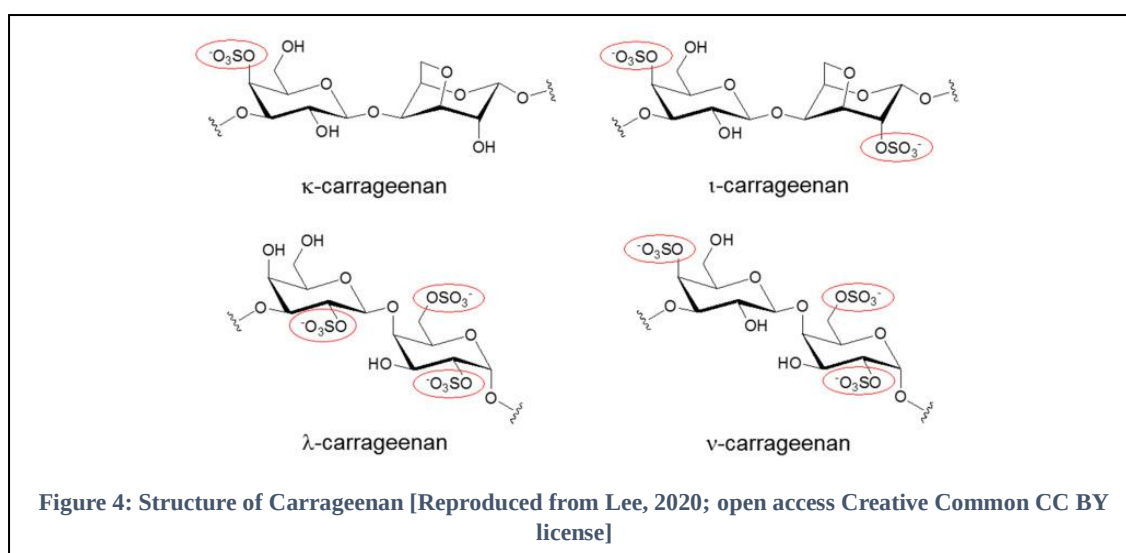


Carrageenans

Carrageenan is obtained from the cell walls of a red algal (Rhodophycophyta) species known as *Chondrus crispus*. The substance/ product extracted is known as carrageenan because this species is commonly called as ‘Carraigin’ in Ireland and ‘Irish Moss’ or ‘Carrageen Moss’ in England (Necas & Bartosikova, 2013). It is a type of sulphated galactan (Das, 2007). Carrageenans are galactans like agar, but have a larger concentration of sulphate esters and D-galactose rather than L-sugar (Figure 4). There are different polysaccharide complexes of carrageenan. Carrageenans produce an insoluble and a soluble component when treated with 0.25 KCl solutions. The κ -carrageenin is the insoluble portion. On alkali treatment, the KCl soluble fraction generates both soluble and insoluble components. λ -carrageenin is the name of the polysaccharide that is soluble in alkalies. The μ -carrageenin fraction is the alkali-insoluble fraction (Daniel, 2006).

Carrageenan-containing seaweeds are known as carrageenophytes. A mixture of kappa and lambda carrageenans, which are difficult to separate during commercial extraction, are present in the wild *Chondrus crispus* species. However, the two different *Eucheuma* species produce specific types like the kappa- or iota-carrageenan. (McHugh, 2003).

Carrageenin/ carrageenan assists in blood coagulation process (Vashishta *et al.*, 1960). In medicine, carrageenins are employed as an emulsifier (Daniel, 2006). Carrageenin is frequently employed as a cough suppressant and an emulsifying agent in pharmaceuticals (Kumar and Singh, 1979).



Despite the fact that Carrageenans' anticipated antiviral benefits could not be confirmed in human research, Lee (2020) comes to the conclusion that these compounds have superior antiviral potency and favourable safety profiles in subclinical tests. It is one of the suitable candidates for developing the broad-spectrum microbicides in these lines because it has antiviral potential against a variety of viruses, including the human immunodeficiency virus, human papilloma virus, herpes viruses, respiratory viruses, dengue virus, and some other viruses. These activities simply need to be reevaluated.

Fucoidans

Fucoidans are complex, heterogeneous sulphated water-soluble polysaccharides with a significant proportion of L-fucose and sulphate ester groups (Figure 5). These are obtained from brown algae (Phaeophycophyta) (Li *et al.*, 2008).

Fucoidan is used to manage hypertension (blood pressure higher than normal), reduce inflammation (swelling), prevent blood clots, and stimulate your immune system (MSKCC , 2023). Wang *et al.*, (2019) discussed the preclinical anticancer, antioxidant, antithrombotic-anticoagulant, immunoregulatory, antiviral, and anti-inflammatory activities of fucoidans and referred to them as the classic activities with potential in further clinical trials.

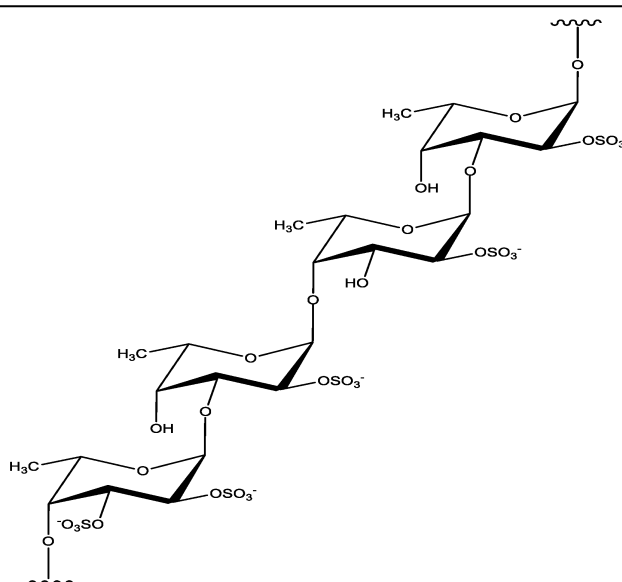
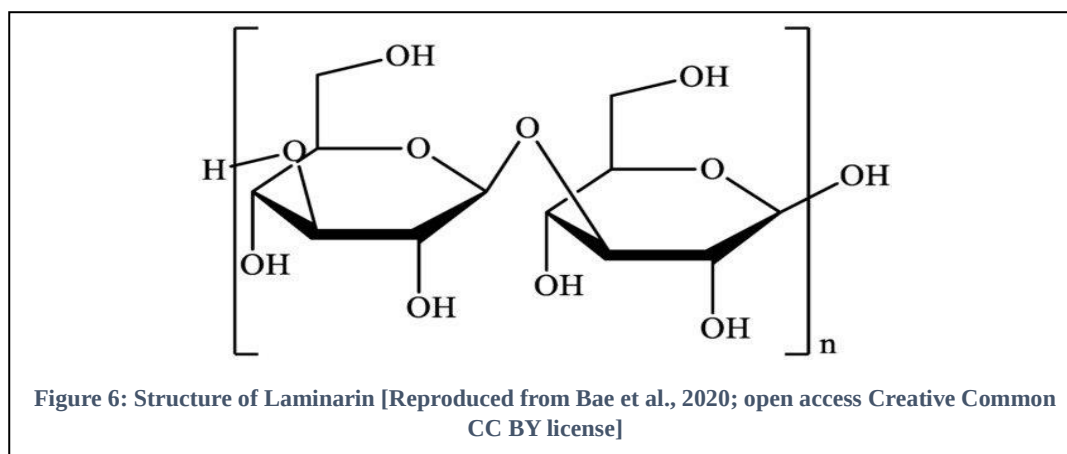


Figure 5: Structure of Fucoidan [Reproduced from Van Weelden *et al.*, 2019; open access Creative Common CC BY 4.0 license]

Laminarin

Laminarin, also called laminaran, is the reserve food material of almost all brown algae (Phaeophycophyta). It is a storage polysaccharide of glucose called as glucan. It is a linear polysaccharide, consisting of a β (1→3) glucan with β (1→6) side chains (Figure 6) present in a ratio of 3:1 (Nisizawa, 1963).

Ji *et al.* (2012) investigated the molecular basis of antitumor activity of laminarins and established that laminarin causes death of human colon cancer LOVO cell via a mitochondrial pathway. Malyarenko *et al.* (2017) investigated the molecular basis of anticancer activity of laminarins and their sulphated derivatives *in vitro*. And they observed such activity in human breast, melanoma, and colorectal cancer cells. Bae *et al.*, (2020) noticed suppressive activity of laminarin against ovarian cancer cell growth in a zebrafish xenograft model.



Starch and Cellulose

The true starch is the primary reserve food material in green algae (Chlorophycophyta) and stoneworts (Charophyta). It is in the form of amylose or amylopectin. The starch is therefore the storage polysaccharide. It is a polymer of glucose. The sugar/glucose molecules are joined together by alpha 1,4 linkages. While glucose is soluble, the starch is insoluble in water. The cellulose present in the cell walls of algae is the structural polysaccharide. It is also the polymer of glucose. But, the sugar/glucose molecules here are joined together by beta 1,4 linkages. The starch is the reserve food material in Chromophycophyta and Euglenophycophyta too. But the glucose molecules here are joined together by beta 1,3 linkages and is stored outside the chloroplast (Britannica, 2023).

Table-1: The various types of starches present in algae.

Sr. No.	Algal Division* (Bold and Wynne, 1978; 1985)	Common Name	Reserve Food Material
1.	Cyanochloronta	Blue Green algae	Cyanophycean/Myxophycean starch
2.	Chlorophycophyta	Green algae	Starch (alpha 1-4 linkage)
3.	Charophyta	Stoneworts	Starch (alpha 1-4 linkage)
4.	Euglenophyta	Euglenoids	Paramylon starch (beta 1-3 linkage)
5.	Phaeophycophyta	Brown algae	Laminarin (beta 1-3 linkage)
6.	Chrysophycophyta	Golden brown algae	Leucosin and Chrysolaminarin (beta 1-3 linkage)
7.	Pyrrhophycophyta	Desmids and Dinoflagellates	Starch (alpha 1-4 linkage) or oils

8.	Cryptophycophyta	Cryptomonads	Starch (alpha 1-4 linkage) or oils
9.	Rhodophycophyta	Red algae	Floridean starch (alpha 1-4 linkage)

*Bold and Wynne System of classification (1978, 1985) is followed here.

Note that, as per the System of Classification of Algae proposed by G. M. Smith (1955):

- Division Chlorophyta is divided into two classes Chlorophyceae and Charophyceae.
- Division Phaeophyta is divided into three classes Isogeneratae, Heterogeneratae and Cyclospora.
- Division Chrysophyta is divided into three classes Xanthophyceae, Chrysophyceae and Bacillariophyceae.
- Division Pyrrophyta is divided into three classes Cryptophyceae, Desmokontae and Dinophyceae. (Smith, 1955)

The dusting talcum powder is made using the starch for application over the skin. Starch serves as an antidote for iodine poisoning, a pill and tablet disintegrating agent, and a diluent in dry extracts of crude drugs (Pharmacy180.com, 2021). It is frequently utilized as a disintegrant, diluent, binder, and lubricant in conventional tablets and capsules (Builders and Arhewoh, 2016). Due to its biodegradability, biocompatibility, nontoxicity, hydrophilicity and antimicrobial properties, cellulose is utilized in wound dressing, controllable drug delivery system, tissue engineering, blood purification, and other applications (Sindhu *et al.*, 2014).

Miscellaneous

'Naviculan' is a sulphated polysaccharide that was isolated from the diatom *Navicula directa*. *In vitro*, it demonstrated antiviral activities against HSV-1 (Herpes Simplex Viruses Type 1), HSV-2, IFV-A (Influenza A Virus), and HIV (Human Immunodeficiency Virus) (Lee *et al.*, 2006).

Conclusion

The variety of polysaccharides in algae are having the potential to be used as medicine. The polymers such as agar, alginates, and carrageenans together make up the majority of commercial seaweed extracts. The agar-containing, alginate-containing, and carrageenan-containing seaweeds are known as agarophytes, alginophytes and carrageenophytes, respectively. The other significant polysaccharides present in algae include ulvans, fucoidans, and laminarin. The polysaccharides in general from algae are reported to have anticancer and antitumour, antioxidant, antithrombotic-anticoagulant, immunoregulatory, antiviral, antihypertensive, and anti-inflammatory activities. The preclinical *in vitro* or animal models provide the majority of knowledge on algal polysaccharides as potential medicines. But to support the widespread use of algal medicines, the clinical research on these topics has to advance and grow so that it would be supplementary to the modern medicine practices and

would also rejuvenate the traditional knowledge of seaweeds being used as medicines. Despite the fact that the algal food industry currently dominates the international market, there are promising signs for the growth of the medical industry of algal polysaccharides in the upcoming years.

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CHAPTER

08

EXPLORING ALGAE AS A NOVEL SOURCE FOR SUSTAINABLE TEXTILES

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A bstract

This chapter delves into the innovative potential of algae as a novel source for sustainable textiles, investigating their diverse applications and unique properties. Algae, encompassing macroalgae and microalgae, offer a promising avenue for transforming the textile industry, given their rapid growth, low resource demands, and ecological benefits. The chapter explores extraction techniques, and biofabrication methods, that merge biology and design and it also examines the environmental impact of algae-based textiles compared to traditional counterparts. By showcasing case studies and collaborations, the chapter demonstrates the feasibility and multifaceted advantages of these textiles, including biodegradability, UV resistance, and moisture-wicking properties. Amidst challenges related to scaling production, cost, and public perception, the chapter emphasizes the importance of ongoing research and partnership to overcome obstacles. Ultimately, algae-based textiles emerge as a pivotal force in shaping a more sustainable and eco-friendly textile landscape, aligned with principles of circular economy and innovation. Thus, it shows a harmonious coexistence between fashion and the environment.

Keywords: *Algae-Based Textiles, Biofabrication, Biodegradability, Circular Economy, Sustainable Fashion.*

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Introduction

The conventional methods of textile production have raised significant environmental concerns, ranging from excessive water usage and chemical pollution to energy consumption and waste generation. As the global demand for textiles continues to rise, the need for more sustainable alternatives becomes increasingly urgent. This chapter delves into the unexplored realm of algae as a potential revolutionary source for sustainable textiles, addressing its promise to mitigate the environmental impacts of traditional textile production processes.

Algae as a unique and sustainable alternative

In the face of mounting ecological challenges posed by conventional textile manufacturing, the exploration of innovative and sustainable alternatives is imperative. Algae, often overlooked in the realm of textile production, presents a unique opportunity to revolutionize the industry. Unlike resource-intensive traditional methods, algae offer a promising source of sustainable textiles due to their rapid growth rate, minimal resource requirements, and potential to thrive in diverse environments. By harnessing the inherent qualities of algae, we have the potential to reshape the textile landscape while addressing pressing environmental concerns. Lim *et al.*, (2020)

Unveiling Algae's Potential-

While algae have traditionally been associated with food, biofuels, and bioremediation, their potential as a textile source is gaining attention for its ecological benefits. The varied and versatile nature of algae species, including macroalgae and microalgae, opens up a spectrum of possibilities. Algae can be cultivated using significantly less land, water, and fertilizers compared to traditional textile crops like cotton. Moreover, algae's ability to absorb carbon dioxide during growth offers an avenue to mitigate greenhouse gas emissions. This chapter seeks to unveil this lesser-known aspect of algae and highlight its potential to revolutionize the textile industry's sustainability quotient.

A Symbiotic Path to Sustainability-

As the world grapples with the imperative of sustainable practices across industries, the textile sector stands at a crossroads. The utilization of algae as a textile source represents a symbiotic approach to addressing multifaceted challenges. Algae-based textiles possess intriguing qualities, from biodegradability and moisture-wicking properties to UV resistance. By embracing algae as a source of textiles, we not only mitigate the environmental footprint of the industry but also open avenues for innovation and cross-disciplinary collaboration. The uncharted territories of algae-driven sustainable textiles, urge us to envision a future where ecological responsibility and creative design converge. Park *et al.*, (2019)

Algae: A Versatile Resource

Algae are the versatile natural resource that have several roles. Some are as-

Exploring Algae Diversity in Textiles-

Algae, a remarkably diverse group of photosynthetic organisms, hold immense potential as a resource for textiles. The two primary categories, macroalgae (seaweeds) and microalgae (single-celled algae) offer unique properties that can be harnessed for textile production. Lim *et al.*, (2020). Macroalgae, with their size and structural complexity, provide a source of fibrous materials suitable for weaving. On the other hand, microalgae, owing to their rapid growth and efficient nutrient absorption, offer consistent and abundant biomass that can be processed into textiles using innovative biofabrication techniques. This distinction in types opens a spectrum of creative opportunities for textile designers and researchers alike.

Diverse Algae Species and Their Applications-

Algae encompass an astonishing variety of species, each possessing distinct characteristics that can be tapped into for textile applications. For instance, certain macroalgae species, like kelp, contain natural polymers that lend themselves to fiber production. These fibers can exhibit properties such as elasticity and strength, making them potentially suitable for diverse textiles, including clothing and industrial fabrics. Park *et al.*, (2019). On the microalgae front, species like spirulina and chlorella not only grow rapidly but also contain valuable compounds like proteins and pigments. These properties suggest their potential incorporation into textiles with added functional benefits, such as color variation or even UV protection. Teufel *et al.*, (2020)

The Appeal of Rapid Growth and Minimal Resources-

One of the most compelling aspects of algae lies in their ability to thrive under conditions that would otherwise be unsuitable for conventional crop cultivation. Algae's remarkable adaptability enables them to flourish in a range of aquatic environments, from oceans and lakes to controlled cultivation ponds. Their rapid growth rate, often outpacing terrestrial crops, accelerates the biomass production cycle, reducing the time required to yield usable material. Moreover, algae's efficient use of resources, including water and nutrients, positions them as a sustainable alternative to resource-intensive crops like cotton. This efficiency aligns seamlessly with the goal of establishing a more sustainable textile production process, prompting further investigation into algae's potential as a versatile textile resource. Van Beilen *et al.* (2019)

Algae-based textiles production and bio fabrication-

The journey from algae to textiles involves a fascinating array of extraction and processing methods that underscore the innovation in this field. When extracting fibers from macroalgae, techniques such as mechanical crushing, enzymatic treatment, and chemical dissolutions are employed to break down the complex structures and extract the usable fibers Zhu *et al.*, (2019). Microalgae. Microalgae, being unicellular, undergo processes like centrifugation and filtration to concentrate the biomass before converting it into a usable material (Park *et al.*, (2019). What makes algae-based textile production even more intriguing is the concept of

biofabrication. Biofabrication involves using living organisms, like microalgae, to create materials. In this context, microalgae are cultivated and manipulated to secrete fibers, which are then harvested and processed into textiles. This fusion of biology and design presents an entirely novel approach to sustainable textile production, challenging traditional methodologies. Kim *et al.*, (2020), Lim *et al.*, (2020), Mancini *et al.*, (2020).

Comparative Environmental Impact and Properties-

Algae-based textiles hold the promise of a significantly reduced environmental footprint compared to their conventional counterparts. Traditional textiles, especially those derived from resource-intensive crops like cotton, often require extensive land use, water consumption and chemical inputs. Algae-based textiles, on the other hand, require considerably less land and water, as algae can be cultivated in controlled environments. Furthermore, the carbon dioxide absorbed during algae growth can contribute to the mitigation of greenhouse gas emissions. The inherent properties of algae-based textiles, such as their biodegradability and moisture-wicking capabilities, align with sustainability objectives while offering unique functional advantages. These textiles possess the potential to redefine the industry's landscape, paving the way for a more ecologically responsible and forward-looking approach to clothing and fabric production. Rufino *et al.*, (2020).

Properties of Algae-Based Textiles and Specialized Applications

Algae-based textiles offer a distinctive array of properties that hold the promise of revolutionizing various industries. Their inherent biodegradability addresses the growing concern of textile waste, presenting a solution that aligns with circular economy principles. Furthermore, algae-based textiles exhibit remarkable UV resistance, which could find valuable application in outdoor gear, sun-protective clothing, and even architectural textiles. Additionally, the moisture-wicking nature of these textiles enhances comfort by efficiently absorbing and evaporating moisture, making them well-suited for activewear and sports gear. With these unique qualities, algae-based textiles transcend conventional applications, opening doors to a range of specialized uses that amalgamate functionality, sustainability, and innovation. Lim *et al.*, (2020).

Case Studies and Research

Numerous case studies and research papers underscore the feasibility and burgeoning potential of algae-based textiles. Noteworthy research by Kim *et al.* (2020) explored the conversion of microalgae biomass into functional fibers using biotechnology techniques. Their study demonstrated the viability of producing textiles with enhanced properties, such as UV protection and moisture absorption, from microalgae-derived materials. Additionally, a pioneering work by Smith *et al.* (2019) investigated the integration of algae cultivation systems with textile production processes, illustrating the symbiotic relationship between algae growth and fiber production. These examples highlight the ongoing efforts to bridge the realms of biology, materials science, and design, unlocking the transformative capabilities of algae-based textiles.

Innovative Projects and Collaborations

The marriage of scientific inquiry and creative expression has given rise to exciting collaborations among researchers, designers, and companies in the realm of algae-based textiles. The "Biocoutre" the project led by designer Suzanne Lee exemplifies this synergy. Lee worked with biologists to cultivate cellulose-producing bacteria, eventually creating biodegradable textiles through a process reminiscent of kombucha fermentation. Similarly, companies like Algiknit have taken up the mantle of sustainability, using microalgae to produce biodegradable yarns that can be woven into textiles. Collaborative initiatives such as these not only advance the scientific understanding of algae-based textiles but also shape the direction of sustainable fashion, challenging conventional paradigms and inspiring novel approaches that harmonize creativity and ecological consciousness. Ferrari *et al.*, (2018)

Challenges and Future Directions in Algae-Based Textile Production

Scaling up algae-based textile production presents a series of intricate challenges that demand careful consideration. While the concept holds immense promise, hurdles such as the optimization of cultivation techniques, are addressed for large-scale viability. Moreover, the cost-effectiveness of algae-based textiles in comparison to established materials remains a key concern. As the industry navigates these hurdles, public perception becomes pivotal; raising awareness about the environmental benefits of algae-based textiles will be crucial to garnering acceptance and interest. Looking ahead, the horizon is rife with innovation. Exploring different types of algae for their unique properties or fine-tuning processing techniques to enhance material performance, holds the potential to reshape the textile landscape and propel the industry toward sustainable, cutting-edge solutions. Patel *et al.*, (2020)

Environmental Impact and Sustainability of Algae-Based Textiles

When considering the environmental impact, algae-based textiles emerge as a promising solution that challenges the status quo of traditional textile production. A comprehensive analysis reveals that algae-based textiles could substantially reduce the ecological footprint compared to their conventional counterparts. Rufino *et al.*, (2020). Through meticulous life cycle assessments, it becomes evident that algae's rapid growth rate and minimal resource requirements translate into reduced land use, water consumption, and chemical inputs.

Moreover, the potential to absorb carbon dioxide during their growth presents a unique opportunity to contribute to greenhouse gas mitigation. Van Beilen *et al.*, (2019). Algae-based textiles seamlessly align with sustainable fashion principles and the circular economy by embodying concepts of renewability, biodegradability, and low environmental impact. As the world seeks ecologically conscious alternatives, algae-based textiles stand poised to drive a paradigm shift more harmoniously. Smith *et al.*, (2019)

Conclusion

In conclusion, the exploration of algae-based textiles underscores a realm of unique advantages and transformative potential. The inherent properties of algae, from their rapid growth to their sustainable cultivation, biodegradability, and functional attributes, position them as a game-changing resource for the textile industry. As we stand at the precipice of environmental challenges posed by conventional textile practices, algae-based textiles offer a beacon of hope. However, to fully realize their promise, ongoing research, and collaborative efforts are imperative.

Overcoming challenges related to scalability, cost, and public perception necessitates a collective endeavour. The undeniable role of algae-based textiles in shaping a sustainable and eco-friendly sustainable textile industry cannot be understated. Their integration into the fabric of sustainable practices aligns harmoniously with the trajectory toward a greener and more responsible future for fashion and textiles.

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CHAPTER

09

A REVIEW OF THE FABRICATION OF METAL NANOPARTICLES USING ALGAE

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Abstract

Algae is a rich source of bioactive components with antibacterial and antioxidant properties. There are two main groups of algae: microalgae and macroalgae, Both the group have numerous bioactive compounds. The algae are found in the natural aquatic habitat abundantly thus, scientists gained much interest in the utilization of algae for the fabrication of nanoparticles. There is a great advantage of using biological sources (Plants, Bacteria, Fungi, and Algae) for the fabrication of nanoparticles over physical and chemical methods. The term phyco-nanotechnology denotes to the algae-mediated fabrication of nanoparticles and their applications. The algal nanoparticles have unique physical and chemical properties, high surface area, small size and less toxicity. The algal fabrication is an easy, simple and non-toxic method owing to the easy cultivation, handling process, and less harmful effect. The different groups of algae are involved in the fabrication of various nanoparticles, including organic, inorganic, carbon-based nanoparticles. Recently, Metal nanoparticles gained great attention due to their low –cost and unique characteristics. The algae and their bioactive compounds are used as bio-reducing agent for the synthesizing of metal nanoparticles such as silver, gold, iron, platinum, zinc and oxide nanoparticles. The algal nanoparticles have many applications in different sectors including food, pharmaceutical, medical, environmental and other fields. This review focused on the fabrication of metal nanoparticles using algae as bio-reducing agent and their applications.

Keywords: *Algae, Biogenic Synthesis, Nanoparticles, Metal Nanoparticles.*

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Introduction

Algae is a photosynthetic eukaryotic organism, it is a large and diverse group that exists from ocean, river, lake, pond, brackish water to extreme conditions. Algae are found in either unicellular or multicellular organisms. They are free-living and can form a symbiotic relationship. There are two important forms of algae Microalgae and Macroalgae, Macroalgae is multicellular and generally known as seaweeds, it can be seen with the naked eye. Macroalgae are found in marine, it seems big plants in the sea. Macroalgae is classified into three types red algae, green algae and brown algae based on their colour. Microalgae is found in rivers, ponds, lakes and sea. Microalgae can be seen via a microscope, they are unicellular, small plant-like organisms known as phytoplankton. They have photosynthetic pigments and accessory pigments. There are different colours in microalgae including blue-green, yellow, brown or orange microalgae. The diatoms and dinoflagellates are the two important groups of microalgae. The pigments of algae are divided into three such as chlorophylls, carotenoids and phycobilins. The use of microalgae and macroalgae as a basis of natural valuable bioactive compounds due to the huge demand for the natural ingredients for food, feed, nutraceutical and other industries (Soha 2012).

Algae metabolites have attained great attention globally for many applications, there is a wider range of secondary metabolites including alkaloids, flavonoids, glycosides, terpenoids and phenazines, Other than secondary metabolites algae have proteins, carbohydrates, lipids, polysaccharides, polyols and phycobiliproteins. There is a commercial impact of algal metabolites in different fields including food, feed, aquaculture, biomedicine veterinary medicine, cosmetic industries and health (Rao *et al.*, 2017). The secondary metabolites of the algae possess antibacterial, antifungal, antiviral, antioxidative, anticancer, neuroprotective and chemo-preventive activities (Eze *et al.*, 2023). Algae involves various applications because of their rapid growth, high nutrient content and low requirement in different sectors such as energy production (biogas, bioethanol, biobutanol, bio-oils, biohydrogen), food, agriculture, pharmaceuticals, bio-ink, bio-plastic, paper-making industries (Arora *et al.*, 2021).

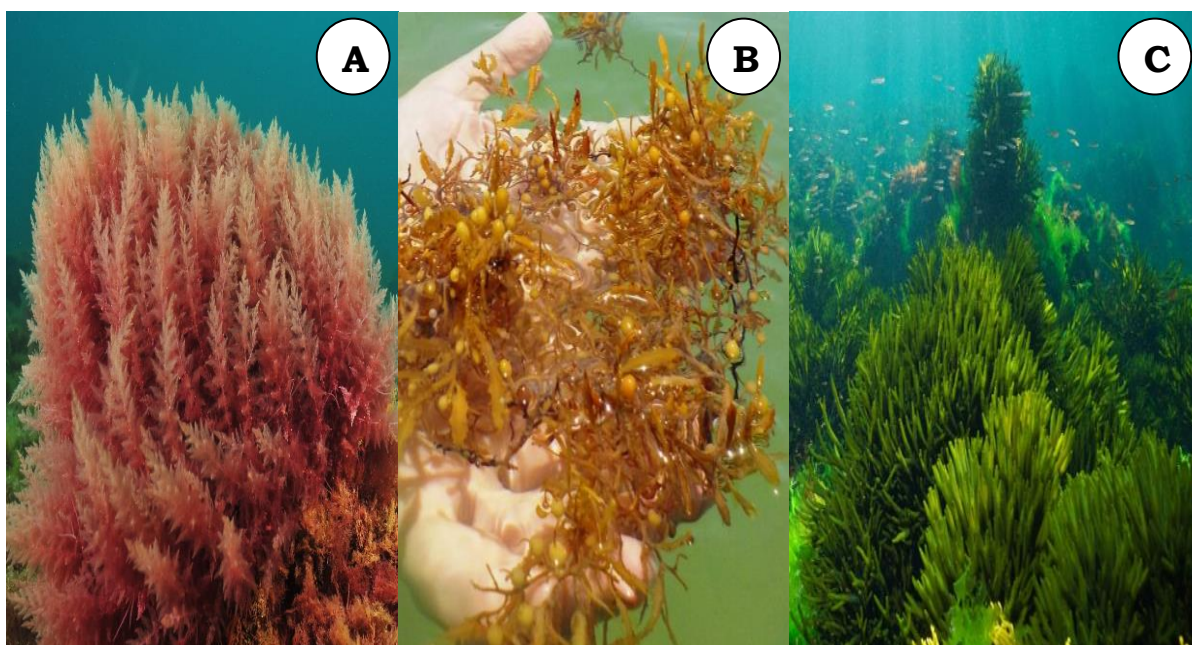


Figure-1: Red (A), Brown (B) and Green Algae (C) (Image credit: 100% pure, University of Florida, American Ocean Website)

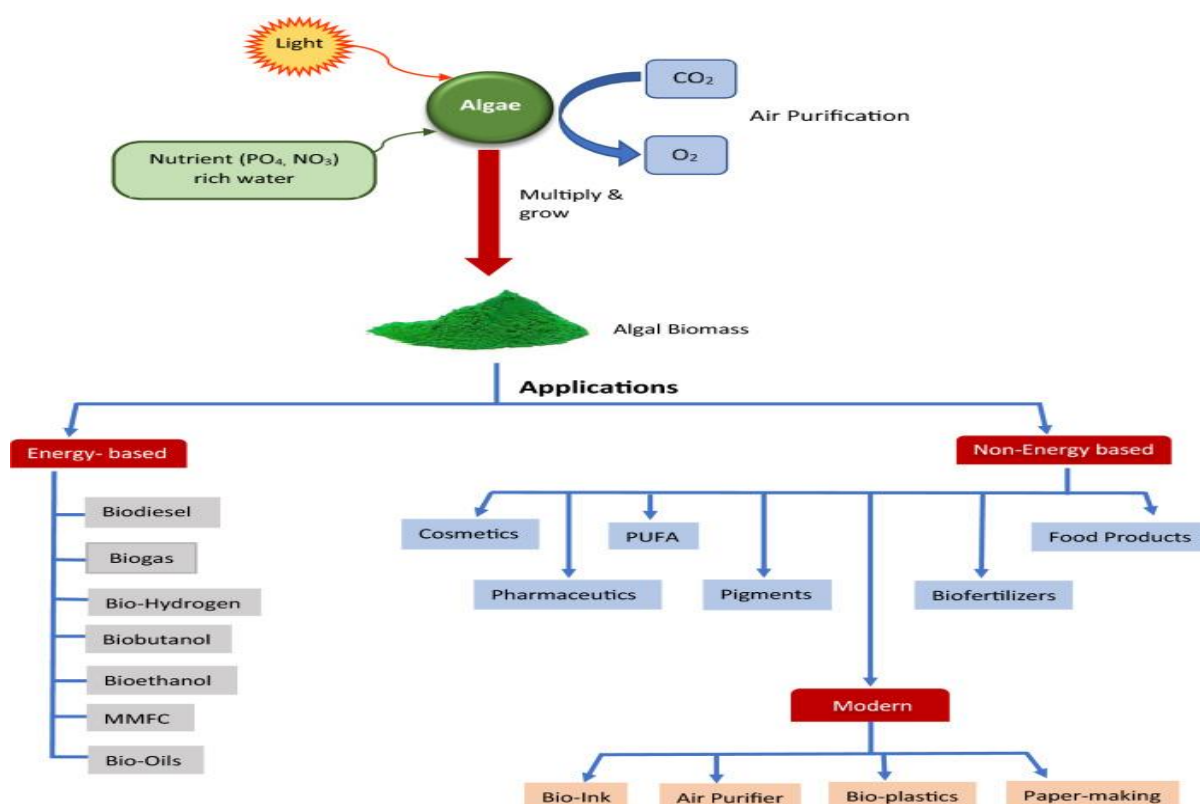


Figure-2: Versatile applications of Algae (Arora *et al.*, 2021)

Algal Nanotechnology

Green nanotechnology has gained attention to fabricate different nanoparticles using biological sources including the cells of plants, bacteria, fungi and algae and their biomolecules like enzymes, pigments, and vitamins owing to overcoming the disadvantages

of chemical and physical methods. The biological sources possess the reducing and stabilizing agents such as enzymes, polysaccharides, and proteins for the synthesizing of nanoparticles. Presently, biological fabrication of nanoparticles has great interest owing to their eco-friendly method with low or no harmful yield, production of nanoparticles with effective physicochemical and biological features like small size, large surface area, high stability, thermal and electrical conductivity, low toxicity and have the capacity to coat with other materials such as antibiotics and drugs (Hamida *et al.*, 2022). In recent times the utilization of algae has gained great interest in the field of nanotechnology. Algae involved in the green synthesis of nanoparticles have great advantages such as easy handling of the algal culture at low temperatures, maximum reduction of metals and a higher rate of synthesis. The term phyco-nanotechnology is the new branch of algal research involved in the algal-based biosynthesis of nanomaterials (Priyam Bose, 2022).

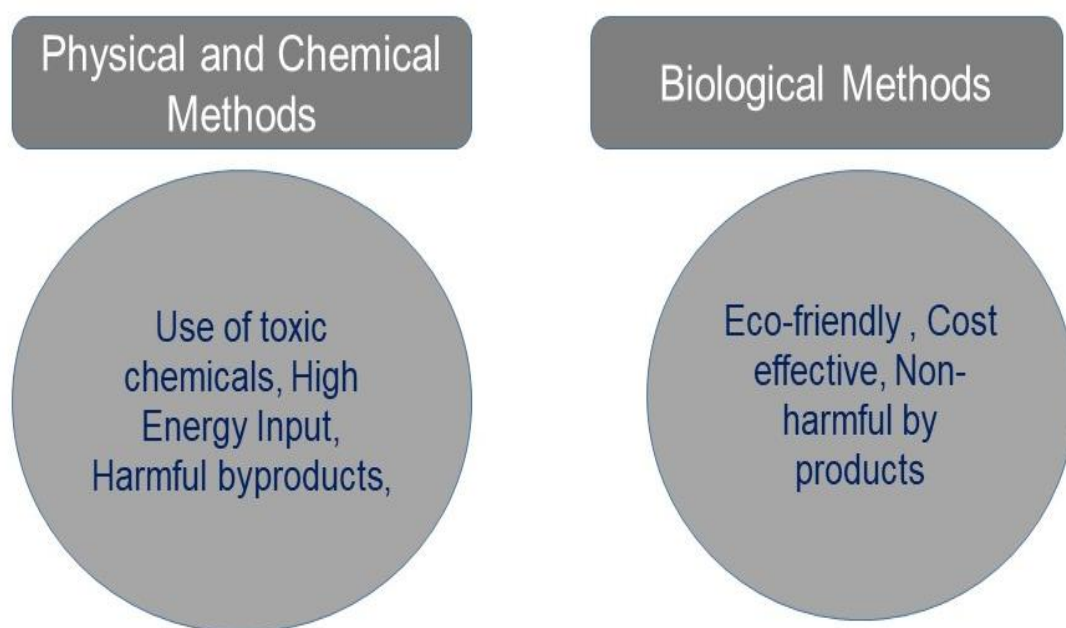


Figure-3: Comparison of Physical, chemical and biological methods for the fabrication of Nanoparticles.

Algae are a preeminent alternative to the physical and chemical methods for the fabrication of nanoparticles, Nanoparticles can be produced by both living and dead material of algae thus algae are known as bionanofactors. The algae phyla include Cholorophyceae, Phaeophyceae, Cyanophyceae, Rhodophyceae and other species of diatoms that are commonly involved in the fabrication of metal nanoparticles. The use of algae can minimize metal ions that build inside, Algae are easy to handle, and they form less harmful substances during the fabrication process and less danger to surroundings. The algae-mediated fabrication is energy efficient. Overall mediated fabrication of nanoparticles has several advantages over physical and chemical processes of fabrication of nanoparticles. The algal-mediated fabrication is more rapid than the synthesis of nanoparticles using any other biological sources. The collection of microalgae and macroalgae is possible throughout the year. The culturing of algae is easy without the addition of chemicals or fertilizers, they are doubling their bulk ten times faster

than higher plants. The extraction of algae is taken by boiling or heating the algae in solvent or water followed by the extract is used for the fabrication of nanoparticles. The molar concentration of the ionic metal compound is mixed with algal extract and incubated for 24 hours to 48 hours with proper mixing and culture conditions (Michael *et al.*, 2022; Mehta and Gaur, 2005; Mukherjee *et al.*, 2021).

The following steps are involved in the fabrication of nanoparticles using algae-

- The culturing of algae or collection of algae from natural sources.
- Dry the algae culture in shadow.
- Extraction of algae in solvent/water by boiling it.
- Addition of molar concentration of metal compound to the algal extract.
- Incubate it for some time with continuous stirring.
- Colour change is observed after the addition of a metal compound.

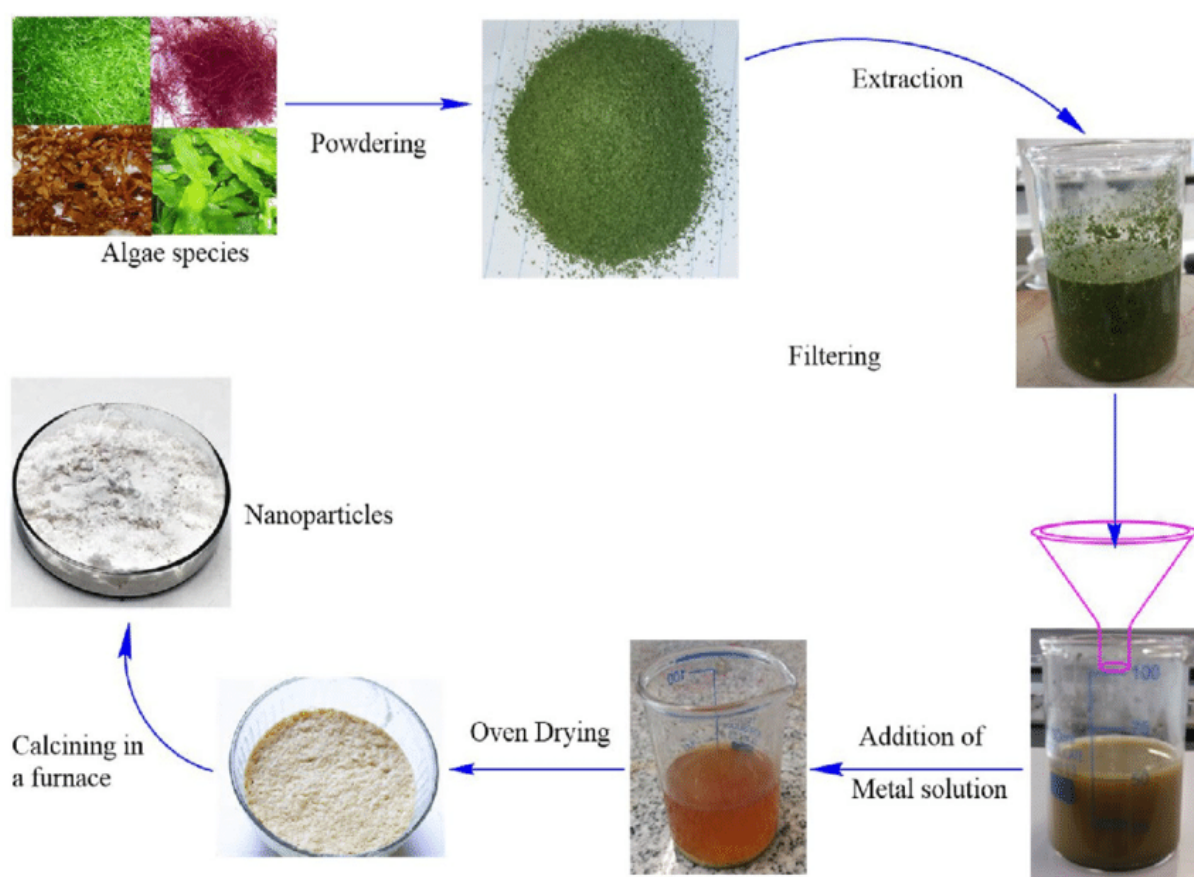


Figure-4: Fabrication process of algal Nanoparticles (Hagos and Getaye, 2019).

Metal Nanoparticles

There are various types of nanoparticles such as organic nanoparticles, polymer nanoparticles, liposomes, carbon-based nanoparticles and inorganic nanoparticles. Among various nanoparticles, inorganic or metal nanoparticles have gained great interest due to their cost-effective, nonbiodegradable, biocompatible and low toxicity. The metal nanoparticles can be moulded into various shapes because of their distinct optical and electrical qualities. There are many inorganic materials including silver, gold, iron, magnesium, silica, graphene

and oxides nanoparticles are metal nanoparticles. Table 1: Shows the different algae-mediated synthesis of metal nanoparticles (Yadav *et al.*, 2022). The nanoparticles are synthesized in different sizes using algae as the biological source.

Table-1: Various algae mediated fabrication of Nanoparticles.

S.N.	Organism Name	Nanoparticle	Size (nm)	Reference
1	<i>Sargassum wightii</i>	Gold	8-12	Singaravelu et al., 2007
2	<i>Eolimna minima</i>	Gold	5-100	Feurtet-Mazel et al., 2015
3	<i>Galaxaura elongate</i>	Gold	3.85-77.13	Abdel-Raouf et al., 2013
4	<i>Amphiroa fragilissima</i>	Silver	104	Ramalingam et al., 2018
5	<i>Chlorococcum humicola</i>	Silver	4-16	Jena et al., 2013
6	<i>Lobophora variegata</i>	Silver	20 -60	Thennarasan and Murugesan, 2014
7	<i>Caulerpa racemosa</i>	Silver	5-25	Kathiraven et al., 2015
8	<i>Chlorella vulgaris</i>	Palladium	5-10	Arsiya et al., 2017
9	<i>Sargassum wightii</i>	Zirconia	4.8	Kumaresan et al., 2008
10	<i>Sargassum longifolium</i>	Copper oxide	40-60	Rajeshkumar et al., 2021
11	<i>Chlorella vulgaris</i>	Gold	40-60	Luangpipat et al., 2011
12	<i>Nostoc ellipsosporum</i>	Gold	33-69	Parial et al., 2012
13	<i>Ecklonia cava</i>	Silver	43	Venkatesan et al., 2016
14	<i>Sargassum muticum</i>	silver	21	Belattmania et al., 2018
15	<i>Padina pavonica</i>	Gold	30-100	Isaac and Emilin Renitta, 2015
16	<i>Padina gymnospora</i>	Platinum	5-50	Ramkumar et al., 2017
17	<i>Chlorella sorokiniana</i>	Gold	-	Gursoy et al., 2021
18	<i>Plectonema boryanum</i>	Gold	10-25	Lengke et al., 2006
19	<i>Spirulina platensis</i>	Bimetallic	6-10	Govindaraju et al., 2008
20	<i>Laminaria ochroleuca</i>	Silver	10-20	Kaidi et al., 2021

Applications of Algal Nanoparticles

The algal nanoparticles have good antibacterial activity, the brown algae-mediated synthesized copper oxide nanoparticles showed bactericidal action against *Enterobacter aerogenes* and *Staphylococcus aureus*, and the antifungal activity also showed effective against *Fusarium dimerum*, *Mucor indicus* (Marimuthu *et al.*, 2011). The algae *Sargassum vulgare* mediated synthesized silver nanoparticles showed anticancer activity against malignant human myeloblastic leukemia cells HL60 and cervical cancer cells HeLa (Govindaraju *et al.*, 2015). The silver nanoparticles fabricated using *S. tenerrimum* and *T. conoides* showed catalytic activity against the organic dyes (Ramakrishna *et al.*, 2016). The titanium oxide nanoparticles synthesized by *sargassum myriocystum* have strong larvicidal activity against mosquitoes (Elgamouz *et al.*, 2020). The silver nanoparticles from algae showed tremendous optical thus they are useful for identifying the hormone types and numbers in our body which is an important assistance to the diagnosis of cancer (Zhang *et al.*, 2012).

Conclusion

This review stated the developments of algae-mediated fabrication of metal nanoparticles. The diverse group of algae is involved in the synthesis of metallic nanoparticles. The biogenic method using algae as the biological source has several advantages including low cost, non-toxic, and less energy thus the synthesized nanoparticles have versatile applications in many fields such as agriculture, food, environment, medical and pharmaceutical industries.

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CHAPTER

10

AGRICULTURAL IMPORTANCE OF ALGAE

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D. Arunkumar and T. Purushothaman*

A**bstract**

Algae are the microorganisms which involves in the photosynthesis to capture the energy from sunlight. Algae are the soil stabilizers and can also act as the biofertilizer. Algae are also known as seaweeds, which can be used as the fertilizer. These organisms are also used as the human food supplements. They can produce the carbon neutral food. It can be grown in the minimal amount of freshwater. Seaweeds are the importance source of iodine. Feeding milk cattle with seaweeds can increase the quantity of iodine in milk. Large brown algae and red algae are used as the organic fertilizer. Cyanobacteria are able to reduce atmospheric nitrogen to ammonia for the nitrogen fixation. Seaweed extract are bioactive at low concentrations. It helps in the growth stimulating factors in the plant. Seaweeds can also be used in the preparation of poultry and fish feeds. It is mainly used for seed germination, growth of the plant and resistance to the plants from the pest control.

Keywords: *Algae, Ammonia, Bio-Fertilizer, Cyanobacteria, Seaweed.*

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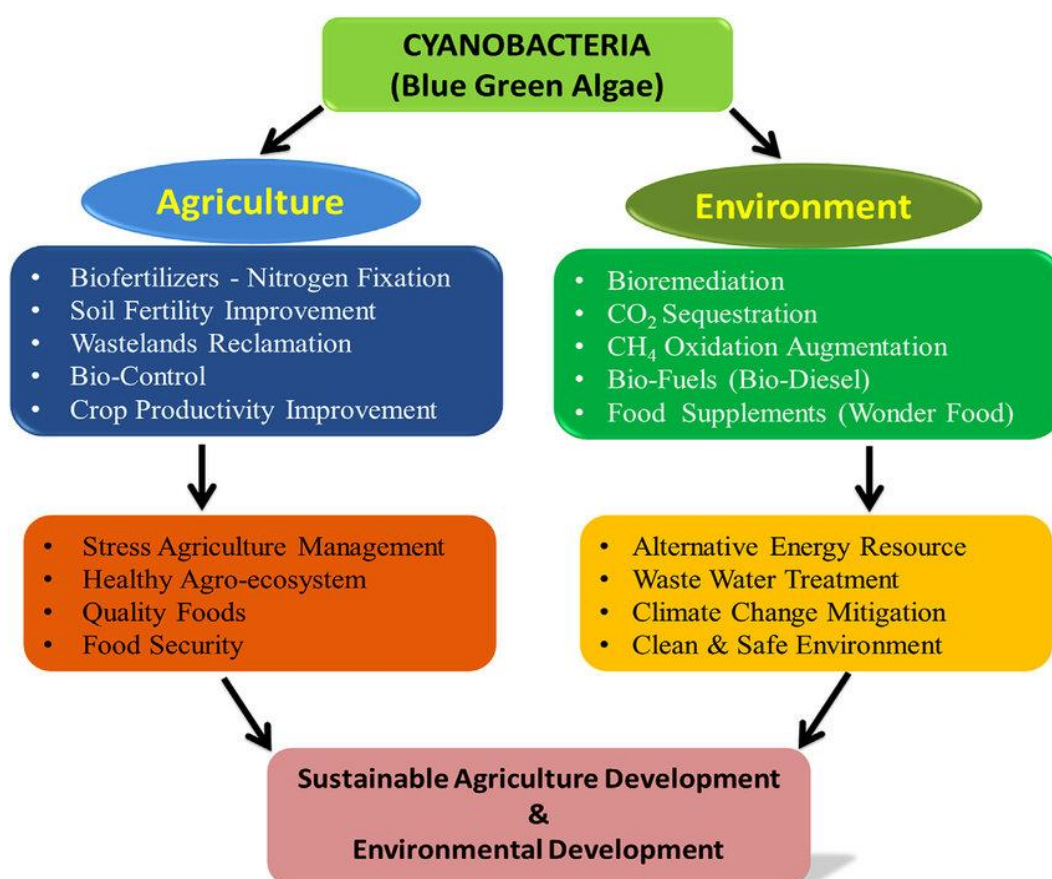
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Introduction

Microalgae and cyanobacteria are the biologically active compounds which contains phenolic compounds, polysaccharides, proteins act as the antioxidant agents. It also helps in the growth factor in plants. It can be both the prokaryotes and eukaryotes. These organisms can be grown in the low quality of water such as waste water. It can intake the CO₂ and from the atmosphere and reducing the carbon footprint in agriculture.

Algae

Algae are the leafy structure of seaweeds. It is commonly divided into two groups' microalgae and macroalgae. Microalgae are the large algae commonly known as seaweeds it is mostly present in freshwater and marine species. It is the microscopic algae found as the part of phytoplankton in all the aquatic and surface area of the soil. Microalgae are the most common carbon input to the soil. Some of the microalgae compounds contains antimicrobial, antiviral and antiprotozoal activities. The fern *Azolla sp. salviniaaceae* has been used as green manure to increase productivity in rice fields. This technology is based on the natural symbiotic association between *Azolla sp.* and the cyanobacterium *Anabaena azollae*. Cyanobacteria can grow on other macrophytes in rice fields forming epiphytic associations.



Cyanobacteria

It is a prokaryote and similar to the algae and higher plants which can be known as oxygenic photosynthesis process. Sunlight is their energy source in water they can generate oxygen. It

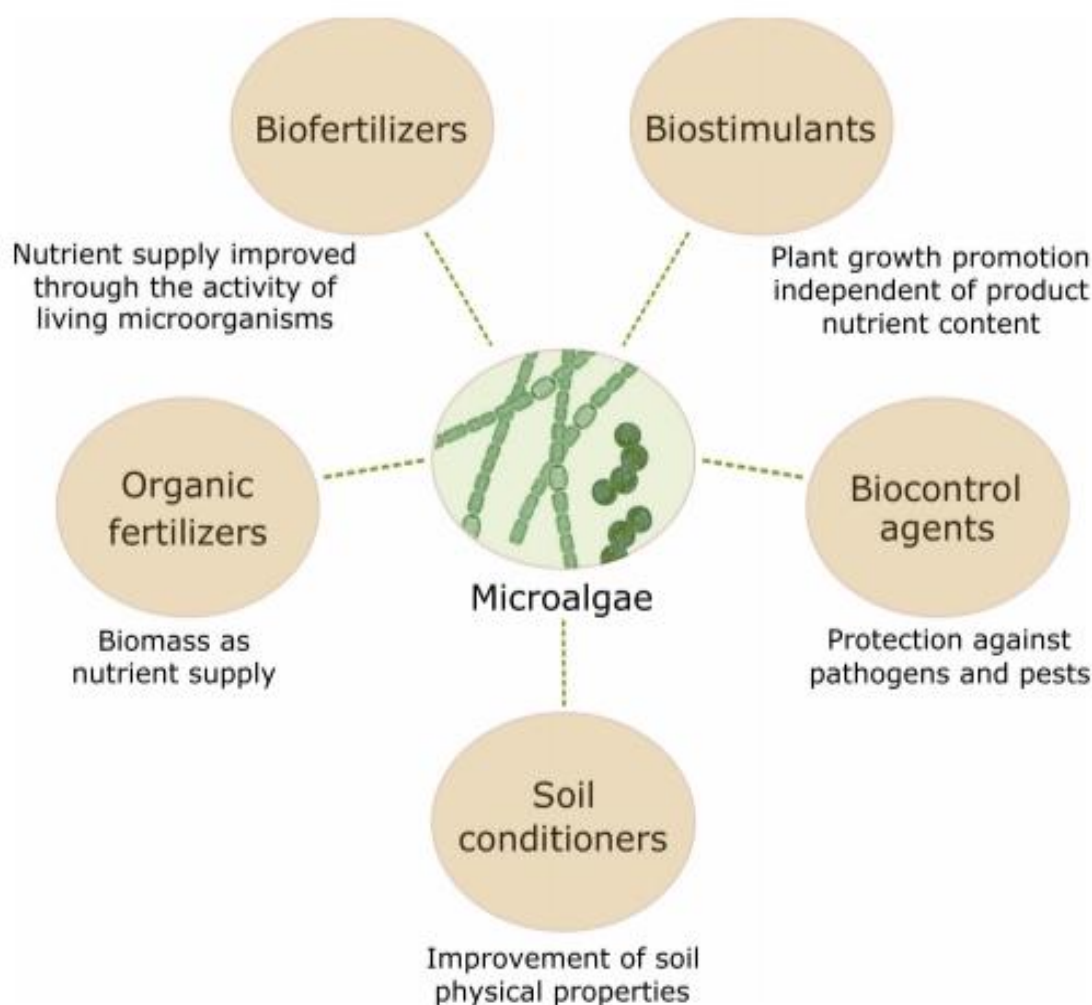
helps to maintain the fertility of soil, produces the substance which can be biologically active. It can able to absorb the heavy metals which the process is known as bioremediation.

Uptake of P and N

Cyanobacteria have soil phosphate solubilizing species. Phosphorous is the second most nutrient which can be required after the nitrogen source for the plant. In the inner part of algae, it can do several functions involving the biochemical and physiological adaptations. It can excrete the substance to enhance the availability of nutrient in the soil. Algae excrete the extracellular phosphates to maintain the normal level of nutrient in soil. Algae can store the excess amount of P for their immediate purpose.

Source of Organic Matter

Algae are the important source of organic matter in soil. It can be formed from the death and decayed matter of algae which can be mixed with the soil and act as a good bio-stimulant and nourishes the texture of the soil and increases the nitrogen and other organic substance present in the soil. These algae can help in the seed germination and increases the fertility of soil. It can act the bio fertilizer or the soil conditioner. Biofertilization is the technique which can enhance the plant to increase the availability of mineral in soil.



Biofertilizer

Bio fertilizer is the live microorganism that improves the chemical, biological aspects of the soil. It can restore the soil fertility and can encourage the plant growth. Plants require nitrogen to flourish the natural crop growth otherwise the fertilizer are been used. Soil micro flora has been used for the biomass productivity of the plant growth. It is a bio-based fertilizer. The bioactive compounds are highly rich in natural resources.

Plant	Cyanobacterial strains	Detected phytohormones	Improved growth/performance parameters
A - Seed incubation with live cell suspensions			
Wheat	<i>Phormidium</i> spp.	Auxins	- Seedling root and shoot length - Seedling number of roots and leaves - Seedling weight
Wheat	<i>Anabaena</i> sp. <i>Oscillatoria</i> sp. <i>Phormidium</i> sp. <i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA Cytokinins	- Seedling shoot length - Lateral roots - Fresh and dry weights - Shoot length, spike length and weight of 100 seeds in mature plants
B - Seedling root incubations with live cell suspensions			
Wheat	<i>Leptolyngbya</i> sp. <i>Phormidium</i> sp. <i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA	- Seedling shoot length - Shoot and root weight - Root and leaves number - Shoot and root auxin content
C - Culture supernatants applied to seeds in vitro			
<i>Lupinus termis</i>	<i>Anabaena flos-aquae</i> <i>Nostoc muscorum</i>	IAA Cytokinin Gibberellin	- Germination percentage - Carbohydrates in seedling shoots - Phytohormones in shoots
Pea	<i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA	- Lateral roots - Root length^a
D - Biomass extracts applied to seeds in vitro			
Pea	<i>Scytonema bohneri</i>	IAA IBA	Seedling root and shoot length
E - Extracts or hydrolysates applied on leaves			
<i>Petunia x hybrida</i>	<i>Arthrospira platensis</i>	IAA Cytokinins Gibberellins Ethylene Absciscic acid Other hormones	- Root and flower weight - Flower number - Enhanced N and other nutrients foliar concentrations^b
<i>Petunia x hybrida</i> (salinity conditions)	<i>Arthrospira platensis</i>	IAA Cytokinins Gibberellins Salicylic acid Jasmonic acid	- Salt stress tolerance - Leaf number and plant dry weight at high salinity - Flowering in spring season
F - Biomass water extracts sprayed on pot surface			
Cucumber, squash, tomato	<i>Anabaena vaginicola</i> <i>Nostoc calcicola</i>	IBA (predominant) IAA	- Plant height - Plant root length
Peppermint	<i>Anabaena vaginicola</i> <i>Cylindrospermum michailovskoense</i> <i>Nostoc calcicola</i>	IAA IPA	- Essential oil percentage - Plant root/shoot length - Stem and leaf dry weight - Leaf number and ramification
G - Soil inoculations with fresh biomass or cell suspensions on pot surface			
Chamomile	<i>Nostoc carneum</i> <i>Nostoc punctiforme</i> <i>Wollea vaginicola</i>	IAA IPA IBA	- Root length and weight - Weight of essential oil
Pea	<i>Nostoc entophyllum</i> <i>Oscillatoria angustissima</i>	IAA Cytokinin Gibberellin acid Absciscic acid	- Germination percentage - Seedling root and shoot length, dry weight and leaf area - Seedling pigments, carbohydrate, total N and P, and protease and amylase activities
<i>Plantago major</i> L.	<i>Anabaena vaginicola</i> <i>Cylindrospermum michailovskoense</i>	IAA IPA	- Carbohydrate and protein in seeds at fruiting stage - Phenolic and flavonoid content - Leaf number, root length, leaf and root weight, length of inflorescence
Wheat	<i>Nostoc khlmani</i> <i>Anabaena cylindrica</i>	IAA Cytokinins Gibberellins	- Germination percentage - Seedling root length and dry weight - Seedling shoot length and fresh weight
Rice	<i>Nostoc carneum</i> <i>Nostoc commune</i>	IAA	- Seedling growth promotion (root, shoot) - Biofertilizer plus 50% of chemical fertilizer showed similar yield parameters as full dose fertilizer (spikes and grains)

^a Root length increase was dependent on filtrate concentrations; a decrease was observed at lower filtrate dilutions.

^b Decreased leaves, stems and petioles dry weight.

Nitrogen Fixation

Blue green algae are able to fix atmospheric nitrogen into other nitrogen forms which can be easily assimilated by higher plants. Cyanobacteria can increase the availability of nitrogen in soil which helps in the growth factor. Microalgae and cyanobacteria store the biomass by the intake of nitrogen and phosphorus in the atmosphere. They can easily repair the pH of the damaged soil; control the salinity of the plant. These algae can play the defense mechanism in the plants.

Spirulina

It is an organism which can grow in both the fresh water and salt water. It is a single celled microbe which can be called as blue green algae. It can produce energy from sunlight through photosynthesis. It is a natural and healthy fertilizer.

Azolla

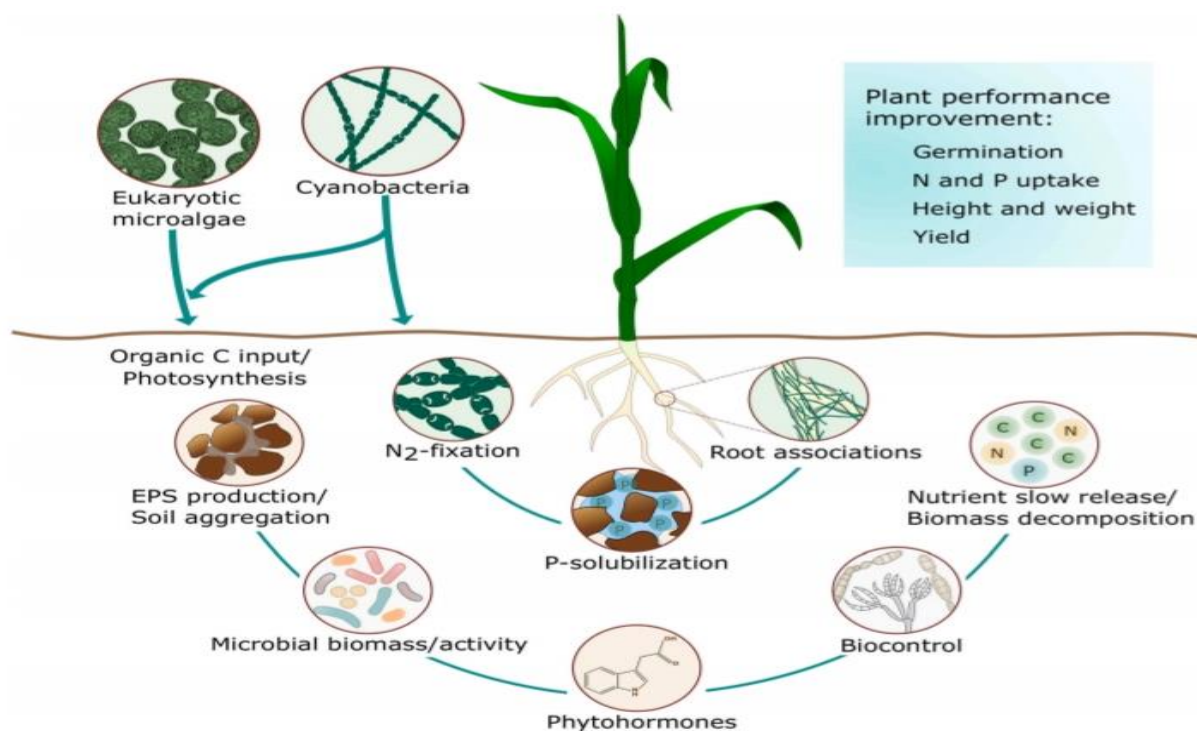
It consists of a floating stem, short, branched, bearing roots which can float on the surface of the water. It contains green chlorophyll by which the leaves are arranged in the alternative order. It creates the symbiotic relationship with the blue green algae.

Geitlerinema

It is a simple filamentous cyanobacteria. It acts as the supplied as the electron donor for photosynthesis. Its phallus is thin and delicate mostly in blue green colour, rarely in violet or brown in colour. It is a renewable energy source.

Nitrogen From Blue Green Algae

Nitrogen consists of one to two percent of total dry weight of plant and in unfertilized soil it contains of about often limit for plant growth.



The blue green algae contain of chlorophyll a carotene. Some algae contain the O₂ sensitive enzyme nitrogenizes for the nitrogen fixation. The reduction of atmospheric nitrogen to ammonia is known as biological nitrogen fixation and the organisms takes place in the process is diazotrophs. Some of the cyanobacteria can fix the N₂ in the highly specialized filaments which is present inside the cell and is known as heterocyst. Heterocyst are photosynthetically inactive N₂ fixing cyanobacteria is used as the green manure to increase the crop production in rice fields. Seaweeds are most widely acted as the good bio-stimulant.

Conclusion

Seaweeds are mostly been used for the agricultural purposes and in animal production. Seaweeds are used for the soil stimulation purpose. Bio-stimulants are different from the fertilizer as they are used in very small amount for the plant growth. Soil algae are more specifically rich in the photosynthetic organisms. By the water flow the soil erosion can be avoided. The algae can control the agricultural pest and agricultural waste water treatment.

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CHAPTER**11****ROLE OF ALGAE IN HUMAN WELFARE**

Jige Sandipan Babasaheb*

A

bstract

The algae are unicellular or multi-cellular occurs in water, the phytoplankton is occurring free floating microorganism composed primarily of unicellular algae. The algae also occur in soil on the surface of wood and rock moist surface. The algae live with fungi like lichens without any affect each other. In the Chinese literature the algae cited as food early 2500 years ago, it consume in different parts of Asia, South America and Mexico. The algae provide mostly vitamins, nutrients, in food industry agar used as gelatine substitute used in the manufacture of jellies, sauces, creams, custards, processed cheese, puddings and frozen dairy products. In the jam it also used for gelling and thickening, it used as anti-drying agent in pastries and breads, in ice cream and other deserts it used as thickener. In the countries like Sweden, Norway, South America, Ireland, Scotland, France, Japan, Germany, China and North America algae used as food ingredient.

Keywords: *Biomass, Diatomite, Ecosystem, Eukaryotic, Lichens.*

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Introduction

The study and use of algae is done from ancient peoples including Romans, Greeks and Chinese. The algal some species are also microscopic so its study waits after the invention of the microscope. The Dutch scientist Anton van Leeuwenhoek in the 1674 has invented the first microscope and observed bacteria in first time. In the science in early 1800s the study of phycology was born; the work of Nathanael Pringsheim a German scientist has published many methods of reproduction in algae. The Carl Adolph also works on the algae and attempting name and classification of various species of the algae. On the earth about 70% oxygen produced by algae, they play vital role in food chain in many aquatic ecosystem like saltwater, brackish water and fresh water ecosystem. The algae have eukaryotic organism but not have roots, stem, leaves and flower it contains chlorophyll and other pigments and carrying out photosynthesis process.

The European nation like Sweden, Norway and Denmark used algae as fodder to feed livestock like cattle and chickens. The algae have rich minerals and vitamins so it used as liquid fertilizer for repairing level of nitrogen in the soil. In the fish farming it used as food for fishes it also maintains health of ecosystem. The blue-green algae help in the reduction of the soil high concentration alkalinity of soil. The water pollution also checked by algae like chlorella and euglena. The death of diatomite cover accumulated in bottom of water and deposits particles called diatomite.

The algae are red, green, blue-green, brown and green in color depending upon pigment contained. The various algal species and their metabolites shows variety of best pharmaceutical activities the micro alga biomass applied in the form of tablets, powder and water agent. The various algal species and their metabolites shows variety of best pharmaceutical activities the micro alga biomass applied in the form of tablets, powder and water agent. The algae also used as food, fodder, and fertilizer and binding agent; it has considered as healthy food for human. It also used main ingredients of some local dishes in Japan and China, also used as salad, accompanying meat in fried dish, topping on oatmeal and liquid from as extract in nutritive smoothie. The algae consist Vitamins A, B, C and E with minerals like iron, calcium, potassium, magnesium, manganese and zinc. In the aquatic ecosystem the algae play as producer the primary consumer eat algae and secondary consumer eats primary consumer and keeps balance of the ecosystem. These balance patterns occur all over the earth without producer entire chain will collapse. In the algae asexual and sexual both reproduction system occurs with vegetative reproduction. The asexual reproduction occurs by fragmentation, spore formation and binary fission. In the sexual reproduction both sex cells fused together and from zygote which has diploid, it develops into sexual spore which is gamete at favorable condition it reproduce and produce haploid organism this pattern of reproduction known as alternation of generations.

In algal world about 300,000 species has been present but we documented only 30,000 species, these algae live in natural habit and adapt rapidly in extreme condition. The algae have many industrial applications it used in food industry, in wastewater purification process and pharmaceutical formulation. The algae used as food, fodder additives, cosmetics, antioxidant, anti-inflammatory, dyes and feed for aquaculture. The algae also used in bio-

films, it also used as biological indicator with ecological indicator. The algae produce wide variety of bioactive metabolites and contain different compounds like phenolic compounds, polysaccharides, phycobin protein, chlorophyll, fatty acids and carotenoids. The algal member of blue-green algae like *Nostoc*, *Anabaena* has fixed the atmospheric nitrogen and biomass accumulates organic material in soil and act as the bio-fertilizer. The substance like butyric acid, acetic acid and indol has release from the algae into the soil it stimulates the plants growth. The demand of food for growing population has constant search new food source. The land given about 90% food and remaining 10% get from the water sources. The aquaculture farming of fresh water, brackish and seawater has almost known from ancient agriculture. The marine organism alga has one of promising food resource, it is edible from richer with protein, vitamins, minerals and iodine. In the India most of lands having not fit for cultivation due to its high alkaline condition, the blue-green algae grow there profusely. In the rainy season the bunds constructed for retain the rain water and inoculated with nitrogen fixing algae. In this field these algae grow as like thick green mats adding many organic matters and which helps to lower the alkalinity of the soil. After two to three times repealing this process, we can improve the soil quality and growing crops like rice, wheat and sugarcane. These ways algae have plays important role in the human life.

Objectives

- To study the role of algae in human life
- To study the economic importance of the algae
- To focus the importance of the algae in lifestyle changing period
- To aware the young generation about the algal world
- To study algal role in the development of human and national economy

Discussion and Results

The essential polyunsaturated fatty acid which synthesized by plants and animals also algae synthesized. The algae and algal products also potential food value, the food products are daily consumed with other food items. The Japanese person has eaten seaweed food from 600 B.C. and the Chinese from 6th century A. D. In the vegetarian meal the algae have only one source of single cell protein. So, the Mexico, India, Africa and Taiwan like countries cultured it. The export of this food also gives foreign currency. The algae like *Chlorella* and *Spirulina* have cultured large scale by Central Food Technological Research Institute Mysore. The algae rich with protein, lipid and contains different types of vitamins, it also nutritive value equivalent to spinach and soybean. The algae after harvesting washed and pigment extracted, the dried mass of algae ground and stored as powder. The Taiwan has produced about 1500 tons dried algal mass annually. The marine algal food both conventional and delicacy in Japan, Thailand, China, Philippines and Korea the algae like *Ulva lactuca*, *Gelidium*, *Caulerpa*, *Enteromorpha* and *Laurencia* has eaten as raw salad. The spinach and other vegetables also cooked with *Ulva* and *Gelidium* in India.

In the Bible when Israelites were starving in wilderness the God provided manna as flake like thing lying on ground. It gathered and baked into bread it believes that that manna is like

lichen. In Vietnam the monk Khong Minh Khong discover the rice was more productive when azolla planted in paddies. The monk died the farmer build his temples because of food productivity increase. A thousand year ago the invention of efficient plough in Europe allows easier tilling of soil. Then European cut the forest for the cultivation of crops. In 19th century the industrial revolution introduced mechanized agriculture climaxing in the green revolution exported from United Nation in 1970. The modern agriculture boosted term productivity by using hybrid seeds and massive fertilizer, pesticides, water and energy outputs. It achieved many hidden costs like consumption of non-renewable fossil fuels for fertilizer and machinery.

Importance of Algae

The seaweed like *Porphyra* has contained up to 35% proteins, 45% carbohydrates and rich different vitamins. The mature *Porphyra* harvested and dried after pressed into sheets, these sheets toasted and cut into small pieces, it has eaten with vegetables, raw fishes and rice. It also used for soups and sushi flouring, these algal farming in Japan occur in 60,000 hectare area in the sea by placing concrete blocks on the sea floor to enhance the growth bamboo cum rope network also used. In Asia and Pacific countries seaweed harvested while foraging the shore and are consumed as food. The most common algal species present in the sewage oxidation ponds are *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Euglena*, *Eudorina* and *Pandorina*. Tests have shown that the algae recovered from sewage ponds can be used as animal food and in certain regions it may be a valuable source of fodder. There may be more than 25,000 species of algae, living every- where. They range in size from a single cell to giant kelp over 150 feet long. Most algae live off sunlight through photosynthesis, but some live off organic matter like bacteria. The macro algal seaweed also important about 70 species used for food, food additives, animal feed, fertilizer and bio-chemical. The microalgae in ocean known as phytoplankton which are base of food chain and support all higher life.

Algae as Bio-fertilizer

In the world the increase of population also necessary increase in food, it yields by crop plant increasing for that large scale chemical fertilizer used. The increase in fertilizer and pesticide the people today realized its effect on health, soil and environment. This fertilizer produced in factories by using nonrenewable energy sources like crude oil and natural gas it is not available after some time when exhausted. The crop added fertilizer also washed by irrigation water or rain and reaches water sources like lakes, ponds and rivers. The algae and bacteria of that water produce its pollution. The chemical fertilizer also shows physical properties it made soil unfit for crop growing. The traditional agriculture waste and bio-fertilizer has nutrient rich and developed crop yield without any harmful effect to environment, health and soil. The seaweed also used as fertilizer at costal area it washed ashore it collected and compost I consist minerals like phosphate, potassium and sulphate. The seaweed with boiled water made extract is known as Algifert in Norway SM3 in Englin also used for crops, like this cyanobacteria *Calothrix*, *Aulosira* and *Cylindrospermum* extract used for vegetables and crop yield. The thick mat like growing mass of algae dried and kept in bags it used in summer as fertilizer in empty field. This material contains nitrogen, phosphorous and other minerals.

The nitrogen fixing algae has directly added in paddy field. The Azolla like water fern commonly found in India, China, South East Asian countries and Vietnam used as fertilizer and fodder for animal and poultry. The azolla also known as nitrogen fixer with cyanobacteria anabaena in leaf pocket and grows rapidly when inoculated in rice field. The blue-green algae fix atmospheric nitrogen which is important for building proteins and amino acids. The atmosphere contains 78% nitrogen gas which is usable for plant and animal. The soil added nitrogen is very important for the production of the crops. The nitrogen fixing blue-green algae developed as nitrogen fertilizer.

Algae as Energy source

The fossil fuel reserves like coal, peat, crude oil products (hydrocarbons) and natural gas on the earth are limited. At present, they are consumed at much faster rate than before due to the rapid increase in industrialization. Unfortunately, they are non-renewable and it is estimated that they will soon be depleted. Therefore, serious efforts are being made to find alternate renewable sources of energy. Algae are identified as one such potential source. Algal biomass is found quite suitable for use in biogas plants for producing methane gas. It can be fermented in anaerobic digesters as sole substrate or along with sewage sludge. It has been shown that *Spirulina* when added to sewage sludge doubles the production of methane. In Bhavnagar *Sargassum tenerrimum* has been successfully used in biogas plants. Algae synthesize the energy-rich molecules like long chain hydrocarbons, glycerol and lipids. When some algae are grown without nitrogen and silicon, there is an increase in the synthesis of lipids. These energy rich chemicals can be converted into petrol and diesel.

Algae for Industry

The Glycerol required in pharmaceutical industry has produced from algae like *Chlamydomonas*, *Astromonas* and *Dunaliella*. The alginic acid, *Agar* and *Carrageenans* has high molecular weighted polysaccharides has colloidal properties. The cell wall constituent is brown and red seaweed it used as viscofers, emulsifiers and lubricants in food, paper, textile, cosmetic and drug industries. The alginates have used for making flame proof fabrics and plastic articles. It also contains large quantity of water it used as highly absorbent gauze in international operation for stop bleeding effectively. The non-toxic and colloidal property used for making antibiotic capsules. The agar is gelatinous substance used in solidification of culture media in microbiology and tissue culture experiments. The mixture of agarose and agar has extracted from the eighty algal species of seaweed. The algalic acid has used in manufacture of puddings, ice cream, jellies and soups. Its laxative property used for treatment of constipation, the stabilizer used in leather, pharmaceutical and cosmetics industry. The Irish moss *Chondrus crispus* used as carrageenan which used in dairy industry.

Algae as human food

The algae used as human food in various part of the world like Scotland, Norway, Sweden, Ireland, China, North and South America and Japan. The algae have used as salad, cooked with meat and eaten as vegetables. It also used as added flavor to various dishes the extract also used as beverage. The algae have high nutritional value they contain proteins,

carbohydrates, fats and vitamin A, B, C and E. The sea lettuce, Irish mass, *Nostoc* and *Porphyra* used in China, Japan and many Asian countries. The *Chlorella*, *Spirulina* cell is rich with protein and vitamins with amino acids it is essential for nutrition to the animal and humans.

Algae as fodder

The seaweed also used as fodder in many countries like Denmark, America, China, New Zealand, Sweden, Norway and Scotland. In the Norway, *Rhodomenia palmate* has used as sheep's weed. The *Laminaria focus*, *Sargassum* and *Ascophyllum* used as cattle feed. The egg from hens fed on seaweed meal increase iodine content while increase butter fat content in milk the cattle used diet is supplement with seaweed meals.

Algae in pisciculture

The fresh and marine algae have floating and attached forms provide primary food for the fishes and other aquatic animals. In the pond culture for fishes the fed with various algae, the species of green algae, diatoms and blue-green algae widely used as food to fishes. The different vitamins found in fish ultimately has traced in phytoplankton's which they feed. The algae are source of fish they absorb carbon dioxide and enriching water with oxygen by photosynthetic activity.

Algae for alkaline land

In the India many lands not cultivated for crops because of high alkalinity of the soil, these commonly called as usar soil. These lands made cultivable if its pH could lower and organic content and water holding capacity of soil increased. These all work done by the blue-green algae when used species like *Anabaena*, *Nostoc*, *Scytonema* and *Aulosira*. This algal growth helps in the reclamation of usar lands. In this process the algal crop growth in water logged condition its several series down the soil pH and organic content increase in soil with water holding capacities. In this land transplanted paddy crop grow with yield of 715-907 kg/acre.

Algae for medicine

The algae or its content used in medicine the *Alaria* is used in strengthening of stomach and restoring the appetite after sickness. The alginates used for his haemostatic nature the fucoidin and sodium laminarinsuophate used as blood anticoagulant. The *Digenia simplex* member of rodophyceae has given anthelmintic drug. The algae *Chlorella* has given chlorellin as antibacterial product which act as antibiotics. The algal extract of *Cladophora* and *Lygbya* has kill strain of *Pseudomonas* and *Mycobacterium* and exhibits antiviral activity. The algal species euglena, chlorella used in sewage treatment plants providesby photosynthesis the oxygen important for rapid decomposition of sewage by the bacteria. It breaks it into simple inorganic compounds like ammonia.

Algae Spirulina as super food

The *Spirulina* algae have safe for consumption documented by traditional peoples. It yields more proteins than the soybean, so it is best vegetable for protein source. Its content 65%

protein which is higher than any natural food. It also contains vitamins, minerals and other nutrients. In the 10-12 grams amount it give beta carotene, vitamin B and B12, iron and essential trace minerals. It also rich phytonutrients and functional nutrients it is important for health. In the America it introduced in 1981 as food supplement. In Japan it takes four grams daily and used for long term health maintains. It helps in low energy, poor digestion, it used to aggravate arthritis and rise cholesterol. The mature women also need calcium and magnesium it is important for prevention of osteoporosis. The algae are higher photosynthetic efficiency and release more oxygen and produce large food than other plants. The *Spirulina* is filamentous multicellular blue-green microalgae found in all over world. It is important for all terrestrial and aquatic animals as natural source. It contains bio-active compounds, mineral, polyunsaturated fatty acids, carotenes and other pigment of antioxidant activity.

Conclusion

In the Chinese literature the algae cited as food early 2500 years ago, it consumes in different parts of Asia, South America and Mexico. The algae provide mostly vitamins, nutrients, in food industry agar used as gelatin substitute used in the manufacture jellies, sauces, creams, custards, processed cheese, puddings and frozen dairy products. In the jam it also used for gelling and thickening, it used as anti-drying agent in pastries and breads, in ice cream and other deserts it used as thickener. In the countries like Sweden, Norway, South America, Ireland, Scotland, France, Japan, Germany, China and North America algae used as food ingredient. The European nation like Sweden, Norway and Denmark used algae as fodder to feed livestock like cattle and chickens.

The demand of food for growing population has constant search new food source. The land given about 90% food and reaming 10% get from the water sources. The aquaculture farming of fresh water, brackish and seawater has almost known from ancient agriculture. The algae used as food, fodder additives, cosmetics, antioxidant, anti-inflammatory, dyes and feed for aquaculture. The algae also used in bio-films, it also used as biological indicator with ecological indicator. The algae produce wide variety of bioactive metabolites and contain different compounds like phenolic compounds, polysaccharides, phycobin protein, chlorophyll, fatty acids and cartenoids. In the India most of lands having not fit for cultivation due to its high alkaline condition, the blue-green algae grow there profusely. In the rainy season the bunds constructed for retain the rain water and inoculated with nitrogen fixing algae. In this field these algae grow as like thick green mats adding many organic matters and which helps to lower the alkalinity of the soil. After two to three times repealing this process, we can improve the soil quality and growing crops like rice, wheat and sugarcane. These ways algae have plays important role in the human life.

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CHAPTER

12

THE TYPICAL SUSPECT: HOW TO COLLABORATE ON THE DEVELOPMENT OF HEALTHIER MEAT PRODUCTS

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A**bstract**

Meat substitutes have recently been offered as a healthier and more sustainable alternative to reduce if not completely eliminate, the rising demand for meat consumption. Microalgae, a novel protein source, are being touted as a possible element for meat substitutes. The increasing challenges faced by global food production and its environmental consequences necessitate novel approaches to sustainability in order to meet the needs of a growing population. The negative effects of traditional cattle production on land use, water consumption, and greenhouse gas emissions necessitate creative alternatives. Algal meat, a new concept, demonstrates the use of algae in meat analog to overcome these difficulties. The research also looks at recent advancements in algae cultivation techniques. Algal meat has the potential to reshape the future of food production. Because of the high protein content and presence of many nutraceutical substances, the food industry may be able to manufacture meat analogues with great nutritional value.

Keywords: *Algal meat, Healthier meat product, Nutritional, lectin, Phycobiliprotein.*

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Introduction

Food production is one of the most pressing global challenges, as food demand is expected to rise in the next decades. Animal and plant protein sources must be linked with consumer needs in this regard. The most often consumed protein source has been animal-based proteins, specifically dairy and meat. They are, however, doing various investigations into challenges of sustainability as well as bad human health. Plant-based proteins have piqued the interest of those interested in low-fat and vegan diets, as well as those concerned about renewable and sustainable alternatives to animal-based proteins (Nikbakht *et al.*, 2021) with increased consumer worries about the health and environmental ramifications of animal-based proteins, a race to develop sustainable and appetizing substitutes is on, and the greatest alternative may literally be green.

Producers in the food, drink, and nutritional supplement industries are quickly adopting plant-based alternatives that can match, if not outperform, the benefits of meat and seafood. Ingredients are important, and in order to keep up with customer demand, producers are looking for adaptable, healthful, and allergen-free inputs that can expand to suit the needs of the industries. With increased consumer concern about the health and environmental ramifications of animal-based proteins, a race to develop sustainable and appealing substitutes is on, and the greatest alternative may literally be green. In this regard, rising demand should take into account not only the protein's sustainable production technique, but also nutritional and safety issues. Plant protein sources such as beans, soybeans, and oil seeds have been sought after. (Ahmad, *et al.*, 2022).

Algae are a group of autotrophic photosynthetic, aquatic, and non-embryonic creatures. They might be unicellular, colonial, filamentous, or made up of simple tissues (Guiry, *et al.*, 2012). Microalgae are tiny or eukaryotic photosynthetic microorganisms that grow and exist in suspension water columns compounds (Mata, *et al.*, 2010). They are normally unicellular or small multicellular creatures that are only visible under magnification. Microalgae proteins are dried cells that are typically offered as protein supplements, albeit they may include some beneficial substances (Mata, *et al.*, 2010). Which is the protein-rich genus (Echave, *et al.*, 2021)? However, the food industry's use of proteins derived from seaweed protein as an ingredient has several significant downsides. Microalgae proteins are dried cells that are typically offered as protein supplements, albeit they may include some beneficial substances (Mata, *et al.*, 2010). Which is the protein-rich genus (Echave, *et al.*, 2021). However, the food industry's use of proteins derived from seaweed protein as an ingredient has several significant downsides.

Protein extraction from algae

Protein contents in micro and macroalgae

The necessity for a protein shift from animal diets to alternative sources is obvious today. The world population is constantly growing, and the production of food animals as the primary protein source has severe downsides due to its enormous carbon footprint. (Santamarina *et al.*, 2020). Because of the health risks associated with meat consumption, there is a growing

trend towards reducing meat consumption by shifting to more plant-based or plant-forward diets and foods (Lang *et al.*, 2020). In the case of microalgae, proteins account for between 3 and 36% of the biomass, with most species having values close to 40% (Wang, *et al.*, 2021), depending on the species and growth conditions, although some species, such as *Arthrospira platensis*, can contain up to 70% of protein (Torres-T, *et al.*, 2020). Algal proteins are typically cross-linked to polysaccharides via disulfide bonds inside the cell wall assembly, which limits their extraction (Santamarina *et al.*, 2020).

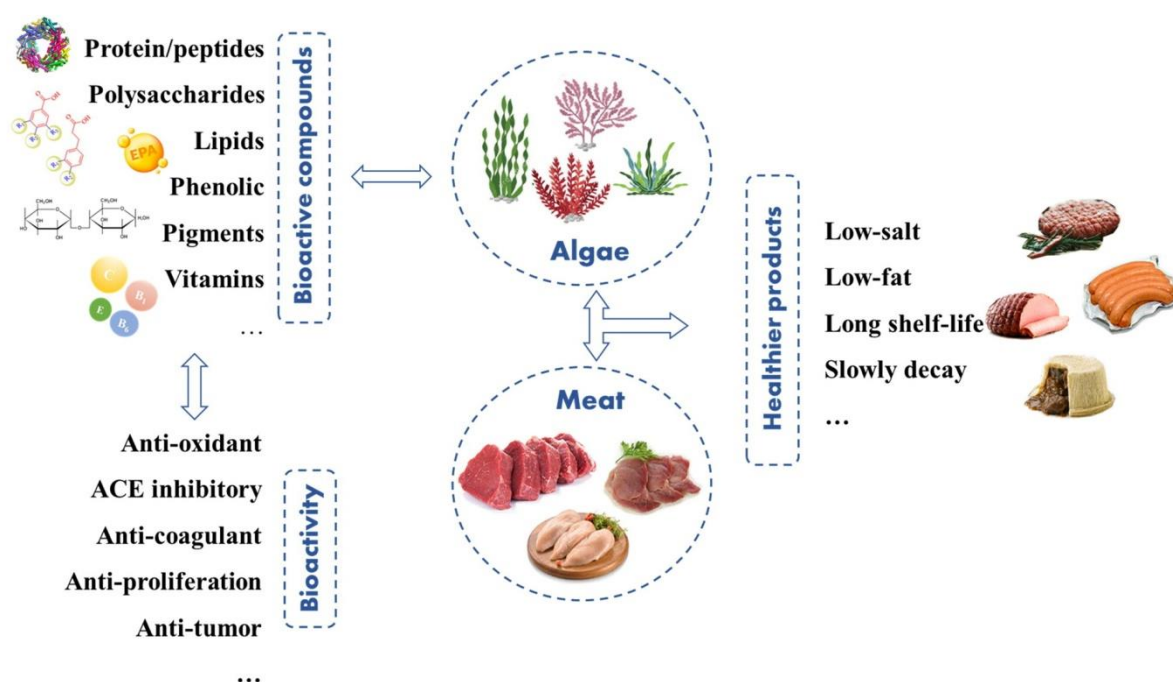


Figure-1: Showing Algal meat from algae (Wang, *et al.*, 2022).

Protein types in algae

Lectins and phycobiliproteins are two families of bioactive algal proteins that have been used in a variety of commercial applications. Lectins are often recovered from macroalgal sources, whereas phycobiliproteins are typically isolated from microalgae (Hayes, *et al.*, 2013). Phycobiliproteins, particularly phycoerythrin, can account for a sizable amount of the overall protein content in red algae, with levels as high as 1.2% (total DW) observed for *P. palmata*. Lectins are present at comparable levels, and a yield of 1% lectins was previously achieved from *Eucheuma Serra* (Rhodophyta). (Kawakubo, *et al.*, 1997).

Lectins

Lectins are glycoproteins that bind carbohydrates with great selectivity without beginning alteration via related enzymatic activity. (Weis, *et al.*, 1996). Lectins are involved in a number of biological processes, such as host-pathogen interactions, cell-cell communication, apoptosis induction, cancer metastasis, and antiviral activity (Hayes, *et al.*, 2013). Because of their extraordinary selectivity in carbohydrate binding, lectins are used in blood grouping,

anti-viral (including human immunodeficiency virus type 1 (HIV-1)) biomarkers, cancer biomarkers, and drug delivery targets (Naeem, *et al.*, 2007). Algae-derived lectins have not received the same amount of characterization as other plant-derived lectins. Nonetheless, algal lectins have been shown to exhibit mitogenic, cytotoxic, antibacterial, anti-nociceptive, anti-inflammatory, anti-viral (HIV-1), platelet aggregation inhibition, and anti-adhesion properties (Harnedy, *et al.*, 2011).

Phycobiliproteins

Phycobiliproteins are water-soluble proteins that play a significant role in photosynthesis in cyanobacteria, Rhodophyta, and cryptomonads (Glazer, *et al.*, 1994). Phycobiliproteins are components of phycobilisomes, which are enormous light energy-capturing complexes linked to thylakoid membranes (De Marsac, *et al.*, 2003). Phycobiliproteins are classified into four groups depending on their color and absorption properties: phycoerythrin, phycocyanin, allophycocyanin, and phycoerythrocyanin. (Sekar, *et al.*, 2008). *Spirulina sp.* (cyanobacterium) and *Porphydrium sp.* (Rhodophyta macroalgae) are the two most important commercial producers.

Phycobiliproteins are used in fluorescent labelling, flow cytometry, fluorescent microscopy, and fluorescent immunohistochemistry (Kronick, *et al.*, 1983; Aneiros, *et al.*, 2004). Phycobiliproteins are utilized in fluorescent labeling, flow cytometry, fluorescence microscopy, and fluorescent immunohistochemistry (Kronick, *et al.*, 1983; Aneiros, *et al.*, 2004) Many patents have previously been submitted for health benefit bioactivities of phycobiliproteins for nutraceutical purposes such as anti-oxidative, anti-inflammatory, anti-viral, anti-tumor, neuroprotective, and hepatoprotective properties (Aneiros, *et al.*, 2004)

Protein extraction from algae

The decreased efficiency of obtaining proteins from algae is mostly owing to the more complex and heterogeneous structure of marine algal cell walls than those of terrestrial plants (Samarathunga, *et al.*, 2022). Algal cell walls are made up of sulfated and branching polysaccharides that are linked together by proteins. As a result, treatments are required to destroy these structures in order to facilitate protein extraction (De Souza, *et al.*, 2023). Whatever technique is employed, protein recovery from seaweed involves two distinct steps: cell rupture and protein extraction, followed by a protein concentration process. (De Souza, *et al.*, 2023). Cell damage attempts to tear cell walls in order to liberate the intracellular fraction of biological materials, including free and associated proteins (Echave, *et al.*, 2021). Mechanical crushing, osmotic shock, alkaline treatment, freeze-thawing, and ultrasonic sonication are among the most commonly utilized cell disruption procedures (Samarathunga, *et al.*, 2022).

The most frequent protein extraction method is alkali extraction and acid precipitation. (Sun, *et al.*, 2022). After the cell walls have been ruptured, the protein extraction procedure begins. Solid-liquid extraction, enzyme-assisted extraction, electric pulse, high hydrostatic pressure extraction, ultrasound-assisted extraction, and microwave-assisted extraction are the most often utilized extraction methods for this purpose today. (Echave, *et al.*, 2021).

Algae as a protein meat substitute ingredient

Algae include a variety of technologically functional and bioactive chemicals, including hydrocolloids, pigments, vitamins, minerals, fatty acids, antioxidants, proteins, and bioactive peptides (Gullón, *et al.*, 2020; Kumar, *et al.*, 2022). Because of their high protein concentration (over 50% of dry weight) and nutritional value during the protein transition from animal to vegetable proteins, both microalgae and macroalgae have been widely recognized as nutritious and sustainable options (Fu, *et al.*, 2021; Singh, *et al.*, 2021).

Yet, in order to be incorporated in food systems, proteins must have acceptable technofunctional capabilities. Previous revisions concluded that microalgae proteins have emulsifying, foaming, gelation, and solubility properties comparable to or even superior to other vegetable proteins such as soy, which is frequently used as an ingredient in meat formulations (Fu, *et al.*, 2021; Grossmann, *et al.*, 2019).

Actually, the common approach has been to substitute soy protein with algal protein or the entire algae as an ingredient replacer in meat replacements. The primary challenge in producing meat alternatives based on microalgae is the early stage of research and development of successful techniques to texturize algal biomass, the need to remove the undesirable odors and colors typically provided by algae, and the lack of improvements in cultivation processes to achieve efficient scaling-up (Fu, *et al.*, 2021). The use of HME to make meat analogs by replacing lupin proteins with *Spirulina platensis*. The effects of *Spirulina* concentration (15, 30, and 50%), barrel temperature (145 to 170 degrees Celsius), feed moisture (50 to 60%), and screw speed (500 to 1,200 revolutions per minute) were studied. Higher *Spirulina* concentrations (30 and 50%) changed the color from yellow to dark green, decreased the cutting force, indicating a decrease in fibrous structure production, and resulted in poorer cooking yields.

Protein from algae as a source of bio functional peptides

Although the earliest attempts at algae utilization were mostly focused on polysaccharide obtention, such as carrageenan, the extraordinary protein concentrations present in seaweeds and microalgae have opened up a new avenue for algae utilization. Aside from protein extraction and purification for supplementation or replacement, the production of bioactive peptides has lately gained attention due to the functional qualities attributed to these molecules. Bioactive peptides are short-chain amino compounds that typically contain 2-20 amino acids, but can have up to 40 amino acids, and are inactive until they are released, resulting in good health consequences (Lafarga, *et al.*, 2020). Milk, meat, fish, eggs, legumes, and cereals are examples of protein-rich sources and byproducts that have been treated to the generation of bioactive peptides (Sarker, *et al.*, 2022).

Cultivation systems

The open raceway pond (ORP) is a traditional form of photobioreactor that is used for industrial-scale algae culture. The fundamental benefit of ORP is that it is easy to create, customize, and operate; it is also energy-efficient and scalable. The main disadvantage of using ORP is that it is susceptible to biological and chemical contamination, as are all open-

loop systems. Infection is a key concern in the manufacture of food-grade materials; thus, the use of ORP for microalgae culture is primarily limited to the production of non-dietary algal biomass. ORP has been successfully used in the industrial production of *Arthrospira sp.* and *Chlorella sp.* The former is an alkaliphilic cyanobacterium, and the high pH in the cultivation media makes it impossible for common biological contaminants to grow; *Chlorella sp.*, on the other hand, relies on a rapid growth rate to out-compete potential contaminants. (Lam, *et al.*, 2018).

Closed-loop photobioreactors (PBR) provide a more controlled environment, which is revolutionary in terms of contaminant management. PBRs of various shapes and sizes have been developed, including tube, plastic bag, flat-panel, and column PBRs. Light distribution inside the reactor, mixing efficiency and mass transfer efficiency, temperature control, and capital and operating expenses are all important issues in the design of PBRs. (Jiang, *et al.*, 2017)

Whole seaweed/microalgae and extract use in meat products

Application	Seaweed or Microalgae	Meat product	Effect of technological and organoleptic properties	References
Antioxidant activity	<i>Himanthalia elongata</i> , <i>Undaria pinnatifida</i> , and <i>Porphyra umbilicali</i>	Creating a meat emulsion	↑ antioxidant capacity due to soluble polyphenolics ↑ antioxidant activity in <i>H.elongata</i> sample	López-López, Bastida <i>et al.</i> , (40)
Source of nutrient	<i>Fucus</i> and <i>Laminaria</i>	Cutlets, meatballs, quenelles, and griddle sausages are all options.	<i>Fucus</i> were good Se and I sources <i>Laminaria</i> was not recommended due to very high I and Se content	Kryzhova <i>et al.</i> (74)
Replacement of soy protein	<i>Spirulina</i> and <i>Chlorella</i> proteins	Turkey breast preparation	↓ soy allergen	Marti-Quijal <i>et al.</i> (4)

Conclusion

In this review, we look at the potentials and protein extraction from algae of employing microalgae as ingredients to make meat substitutes. Algae appear to be the perfect match in the search for newly promising and sustainable protein sources for the development of meat

alternatives, offering high protein contents, low growing requirements, high photosynthetic efficiency, and meeting the sustainable requirements with a low carbon footprint. Algal protein, particularly partially substituting soy proteins, can be employed in the manufacture of meat analogues employing high moisture extrusion as a texturizing method. Furthermore, the knowledge gained from the use of whole algae and extracts in meat products can be used to develop meat analogues with lower salt and fat contents or to replace refined texturizing agents, synthetic antioxidants, or phosphates with algal ingredients, which may help to improve their perception of naturalness. Due to increased biomass density, more consistent protein emulsification, and lighter colour than other modes, heterotrophic culture is a viable strategy for producing meat analogues from microalgae. Furthermore, heterotrophic production has the lowest environmental impact, making it a more sustainable option.

In conclusion, microalgae are appealing ingredients for developing meat substitutes. More research is required to build a mature technological pipeline for producing microalgae-based meat analogs.

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CHAPTER

13

A QUALITATIVE AND QUANTITATIVE EVALUATION OF THE MICROALGAE AND SEAWEED AS FEEDSTUFF FOR RUMINANTS

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A bstract

Algae have gained significant attention as a potential feedstuff for ruminants due their high nutritional value and sustainable production characteristics. This research paper aims to explore the utilization of single-cell algae species as feed for ruminants, the opportunistic use of al by-products, and the feasibility of on-site algae production systems. The paper provides an overview of the nutritional composition of algae, their potential benefits for ruminant health and performance, and the challenges associated with their incorporation into ruminant diets. Additionally, it discusses the utilization of algal by-products generated from various industries and their potential as alternative feed ingredients. Furthermore, the paper explores the concept on-site algae production systems, highlighting their advantages, limitations, and potential integration with ruminant farming operations. The information presented in this research paper will contribute to a better understanding of the role of algae in ruminant nutrition and provide insights into the sustainable utilization of algae as a feedstuff.

Keywords: *Bioavailability, Feedstuff, Ruminant, Seaweeds, Single-Cell Algae.*

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Introduction

There are several usage and customisation potential for sustainable ruminant production due to the enormous diversity of microalgae between and within species. High-protein species have the potential to replace traditional protein feeds in ruminant diets. Although ruminal degradability of protein is one of the important properties of a feed regulating the effectiveness of the ruminant's usage of protein, information on ruminal degradability of microalgal protein and the effects of various microalgae production conditions on it is still lacking. In comparison to standard protein meals, microalgae often have higher methionine but lower histidine concentrations.

However, it appears conceivable, at least in part, to adjust the AA composition of microalgae using N supply on the growing medium (arginine, asparagine, cysteine, glutamic acid, and lysine). However, the ratio of lipid to protein or protein to carbohydrates, NPN concentration, and lipid profile of microalgae can also be significantly impacted by N supply. Future potential for increasing the economic competitiveness of microalgae could include the ability to manufacture products with additional value or reduce climate change by feeding ruminants microalgae by Lamminen, 2021.

The increased demand for animal feed protein, the need for substitutes for the conventional soybean and animal protein feed, as well as food market restrictions pertaining to livestock feeding have all had an impact on the usage of seaweed in ruminant diets. Studies done to date on the use of seaweed in bovine, caprine, and other ruminant nutrition have mainly concentrated on adding small amounts of various macroalgal species to the feed and then evaluating the animal to see if there was any potential for prebiotic activity and improved animal performance. There is little available information on the use of green seaweeds in ruminant feed. Male lambs may receive up to 20% of their food as *Ulva lactuca* without experiencing any unfavourable effects on taste. It has low protein degradability (40%) and moderate energy digestibility (60%) and is suited for use with feeds that have a high energy/low protein ratio, such as cereal grains (Arieli *et.al.*, 1993). It is comparable to a medium to low grade forage. Also used to feed developing lambs, *Chaetomorpha linum* (Chlorophyta) with a 20% seaweed meal had a mildly negative effect on growth and feed conversion ratio, presumably because of the high ash content, (Misurcova *et.al.*, 2011; Rjiba Ktita, 2010). As previously shown, red seaweed has received greater consideration for use in cattle feed than for other ruminants (Misurcova *et.al.*, 2011). Red seaweed has been used in certain cases to successfully buffer the rumen's pH (a 70% concentration of *Phymatolithon calcareum*, also known as *Lithothamnion calcareum* extract, was fed at a ratio of 0.5 g/kg) but it hasn't been shown to promote fiber digestion or alter rumen fermentation (Misurcova, *et.al.*, 2011; Montañez-Valdez, *et.al.*, 2012). This is consistent with the literature because the species *Ulva* has low ash levels, making it an excellent choice for bioavailability research in the future. For instance, feedlot cattle supplemented with the brown seaweed *Ascophyllum nodosum* had their fecal shedding of *Escherichia coli* reduced (Makkar, *et.al.*, 2016; Montañez-Valdez, 2012).

More study is being conducted with the addition of seaweed in caprine feed. Orkney sheep from North Ronaldsay Island are known to eat predominantly brown seaweed for the majority of the year. Species such as *Laminaria digitata*, *Laminaria hyperborea*, and *Saccharina latissima* (Phaeophyceae) account for 90% of this sheep's summer feed, meeting a significant proportion of nutrient requirements due to them up to 13% crude protein content. Other seaweed species consumed by Orkney sheep include *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus* sp. (brown seaweed), *Palmaria palmata* (red seaweed), and certain green algae. Sheep consume J. Mar. Sci. Eng. 2020, 8, 559 14 of 24 seaweed in sufficient quantities to meet maintenance needs, but they suffer from mineral overload due to its high mineral concentration (Makkar, *et.al.*, 2016; Hansen, *et.al.*, 2003).

Some studies also suggest that up to 30% *Macrocystis pyrifera* can be used as a supplement in goat feed without influencing digestibility, degradability, or ruminal fermentation parameters (such as pH and ammoniac nitrogen). The rumen pH, water intake, and urine excretion all increased. *Sargassum* species are also researched for this purpose. We now know that it can be introduced at up to 30% in the diets of developing sheep and goats without affecting intake, growth performance, or diet digestibility (FAO: Rome, Italy, 2014;). Eating *Sargassum* sp. increased water consumption, most likely due to the high content of minerals, primarily Na and K, which may make *Sargassum* less suited for feeding during dry period. (Marín *et al.*, 2009) Further research could include determining bioavailability in a blend of *Ulva lactuca* and one or more of the red seaweed choices suggested. This will allow us to determine whether such a blend may be employed as a prebiotic while keeping the benefits of both species present. There have been several reports of ruminant meals containing mostly brown and red seaweed. However, with the exception of a few isolated examples, the data is insufficient to begin R&D work in order to produce new products for the ruminant feed industry.

Several studies on seaweeds have been conducted to evaluate their effects as livestock feeds and their potential to regulate rumen fermentation and methane generation, and it has been discovered that the formulation of the basal feed is critical. Many seaweed species have been shown to lower rumen methanogen methane generation, although with varying effects on fermentative health and substrate digestibility.

This shows that *Aspargopsis* sp. can be a crucial player in lowering methane emissions in ruminants without causing side effects; however, the seaweed must be introduced to the standard feed as a feed supplement to be effective. The effect of *A. armata* is not simply methane reduction; it can also give vital nutrients for ruminant growth and dietary digestibility, but evidence on the latter is limited, therefore additional research is needed in this area.

The most abundant groups of microalgae are eukaryotic diatoms (Bacillariophyceae), green algae (Chlorophyceae), and golden algae (Chrysophyceae). There are also cyanobacteria (Cyanophyceae or blue-green algae), which are prokaryotic creatures yet are classified as microalgae. More than 100,000 species have been identified, with diatoms accounting for a

sizable proportion. However, only a small portion of this total, about 30,000 species, has been investigated (Richmond, *et al.*, 2004).

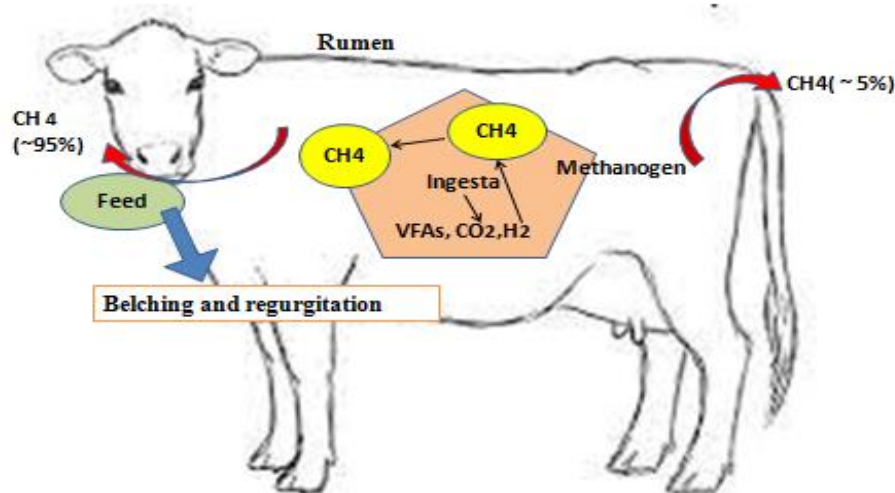


Figure-1. Depicts a comprehensive overview of methane generation and emissions from ruminant animals. The rumen undergoes enteric fermentation of ingested foods, where the bulk of CH_4 is expelled from the mouth via eructation. VFAs (volatile fatty acids); CO_2 (carbon dioxide); H_2 (hydrogen).

Microalgae characterization

However, there are heterotrophic microalgae that get their energy from organic carbon rather than sunlight. Some of these microalgae can utilise economically advantageous carbon sources to produce biomass. Furthermore, heterotrophic microalgae can be easily cultivated and controlled in standard bioreactors. Some microalgae merit a closer look because of their potential application in cattle feeds. *Arthrospira*, first and foremost, is a significant prokaryotic cyanobacterium that may be used in animal feed (Bishop and Zubeck, 2012). Another notable example is *Chlorella*, which is frequently sold as a supplement and is regarded an essential source of nutrients in many locations (Bishop and Zubeck, 2012). Because of its high lipid content (over 20%), *Isochrysis galbana* is a valuable source of n-3 long-chain (>C18) PUFA (n-3 LCPUFA), primarily eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Batista *et al.*, 2013). *Dunaliella salina*, an autotrophic microalga, is a potentially beneficial β -carotene producer (Pirwitz *et al.*, 2016) and an organism for the industrial production of *Haematococcus pluvialis* for astaxanthin production. It has been observed that even after extracting up to 90% of the pigment, the biomass remains untapped. Because the lipid component is often taken along with the pigment, the remaining biomass could be a rich source of carbohydrates for feeds (as a particular polysaccharide supplementation or as the primary energy ingredient). *Porphyridium* microalgae are red microalgae that contain dietary fibre and release exopolysaccharides. Because of the presence of uronic acids and sulphate groups, *Porphyridium* polysaccharides have large molecular weights and are negatively charged.

These latter have beneficial effects on gastrointestinal physiology and lipid metabolism, and hence can help livestock output (Arad and Levy-Ontman, 2010 & Pignolet et al., 2013 & Marcati et al., 2014). *Thraustochytrids* such as the genus *Schizochytrium*, are producers of microbial oils, notably DHA-rich ones. As a result, as this microalgae characterisation and synthetic overview show, microalgae have the ability to create feed ingredients as well as high added value supplements for livestock production. Because the majority of the aforementioned biomolecules are not biosynthesized in the animal body, they are classified as essential.

Nutritional features of microalgae as a ruminant feedstock

Ruminants Microalgae have been utilized to increase growth performance in ruminants as an ingredient or feed additive. A cost-effective supplementing strategy with *Arthrospira platensis* was obtained at the 10% level for optimal liveweight increase in weaner lambs, but had no effect on ADG (Holman et al., 2012). When employing the same microalga as a feed supplement, EL-Sabagh et al. (2014) determined that *Arthrospira platensis* may be successfully included in lamb diets as a growth promoter feed additive, validating previous findings of (Kulpys et al., 2009) using dairy cows.

Although feed intake was lowered, providing marine supplement *Schizochytrium sp.* had no significant effect on dairy cow performance (Franklin et al., 1999). It is unknown if the decrease in intake was due to lower palatability of the 14 algae meals, increased fat intake, or intake of specific fatty acids; nonetheless, the total fat content of the diets was not significantly different. *Schizochytrium sp.*, administered as DHA-Gold extract, showed no influence on lamb growth rate (Meale et al., 2014; Hopkins et al., 2014), which is to be expected given that the diets were calorie and protein balanced (Hopkins et al., 2014).

Seaweeds

Seaweeds inhabit aquatic areas and are mostly consumed by coastal communities (Makkar et al., 2016). Many seaweed species are commonly utilized in raw form in medicine, human diets, animal feeds, and agricultural soil development as fertilizers (Jamal et al., 2017). They are high in potassium, sodium, calcium, magnesium, and phosphorus, as well as iron, manganese, copper, zinc, cobalt, selenium, and iodine (Corino et al., 2019). Little is known regarding their nutritional bioavailability. Seaweeds are basic organisms that use sunshine to transform carbon dioxide into sugars and oxygen through the photosynthesis process. The most common edible algae varieties are *Neopyropia/Porphyra/Pyropia spp.*, *Undaria pinnatifida*, *Saccharina latissima*, *Palmariapalmata*, and *Chondrus crispus*, which have many health benefits such as lowering blood pressure, preventing spills, and serving as a valuable protein source (Probst et al., 2015).

Seaweed biomass is an excellent alternative feed for cattle. Proteins, minerals, lipids, and fibres varied greatly across macroalgae. Seaweeds have a high mineral content because of their ability to absorb inorganic compounds from the environment; they have a limited quantity of lipids, primarily polyunsaturated fatty acids (PUFAs), but are high in polysaccharides. Seaweed has only a little amount of lipids (1-5%), yet the majority of those

lipids are PUFAs. Brown and red seaweeds have higher levels of the PUFAs 20:5 n-3 (EPA) and 20:4 n-5 (arachidonic acid) than green algae (Misurcova *et al.*, 2011).

Nonprotein nitrogen, such as free nitrates, is abundant in algae, resulting in nitrogen-to-protein conversion coefficients of 5.38, 4.92, and 5.13 for brown, red, and green seaweed, respectively (Miurcová *et al.*, 2011). There are several edible seaweeds for human consumption that have a high protein content and varying essential amino acids (Moreda *et al.*, 2012). They also take minerals from seawater and have 10 to 20 times the amount as land plants (Gaillard *et al.*, 2018). Green and red algae are often thought to have more nutritional value than brown algae due to their low protein and high mineral content.

Brown algae, on the other hand, have a greater and more diverse amount of bioactive compounds with high economic potential (Misurcova *et al.*, 2017). As a result, algae can offer energy, minerals, and proteins to animal feed and have the potential to serve as an alternative protein source for ruminants (Angell *et al.*, 2016). Analysis of protein quality and concentration is required to identify the nutritional value of algal biomass so that it can be used fresh, dried, liquefied, or cooked. This analysis is significant because it determines the concentration of essential amino acids (EAAs) (Maehre *et al.*, 2014).

In comparison to cereal and soy flour, algae have a comparatively high protein level. More than 75% of seaweed has more total essential amino acids than wheat flour, and 50% more than soy flour, rice, and corn (Maehre, 2014; Wang *et al.*, 2018). Methionine and lysine levels are comparable to those seen in typical protein sources. In terms of methionine content, algae are often richer than soy flour but poorer than wheat flour. Algae, on the other hand, contains less lysine than soy and wheat flour (Maehre & Malde *et al.*, 2014).

Nutritional qualities of seaweeds as ruminant feed

As previously proven, seaweeds (or macroalgae) are classified into three broad groupings based only on their colour. There are 10,100 identified seaweed species worldwide, which can be found in all seawater habitats, with certain seaweeds also present in freshwater, Koizumi, J.;et.al., (2018). Green seaweeds (Chlorophyta), of which 2200 species are known, can grow to a height of 1 m. The presence of chlorophyll influences their colour (Corino, *et al.*, 2019; Kidgell, 2019). About 6100 species, red seaweeds (Rhodophyta) are adept at photosynthesizing in deeper seas. Their length fluctuates, and they look like green seaweed. Their colour is caused by the presence of pigments called phycoerythrin and phycocyanin, which mask -carotene, lutein, zeaxanthin, and chlorophyll (Corino, *et al.*, 2019; Peng, 2015). Only 1% of the almost 1800 species of brown seaweeds (Ochrophyta, Phaeophyceae) are known to survive in fresh water, and their length can reach 50 m. The dark colour is due to the carotenoid fucoxanthin, which masks -carotene, violaxanthin, diatoxanthin, and chlorophyll. *Laminarin*, *Fucoidans*, *Agar*, *Carrageenans*, *Porphyran*, *Ulvars*, and *Alginates* are the most common seaweed polysaccharides. The growing demand for animal feed protein, the need for alternatives to standard soybean and animal protein feed, and food market rules governing livestock feeding have all influenced the usage of seaweed in ruminant diets. There is little information on the use of green seaweeds in ruminant feed. *Ulva lactuca* could be fed to male lambs at up to 20% of their diet without altering

palatability. It has a low protein degradability (40%) and a moderate energy digestibility (60%), making it comparable to medium to poor grade forage and ideal for use with high energy/low protein diets like as cereal grains (Arieli, *et al.* 1993). *Chaetomorpha* (Chlorophyta) was also fed to developing lambs as a 20% seaweed meal, which had a somewhat negative effect on growth and feed conversion ratio, possibly due to the high ash content. As previously indicated, red seaweed has attracted greater attention in bovine feed than in other ruminant feed (Misurcova *et al.*, 2011). Red seaweed (a 70% concentrate of *Phymatolithon calcareum*, *Lithothamnion calcareum* extract administered at a ratio of 0.5 g/kg) has been used to buffer rumen pH, but it did not increase fibre digestion or change rumen fermentation (Montañez *et al.*, 2012). This is consistent with the literature, as the species *Ulva* has low ash levels, making this seaweed an excellent candidate for further bioavailability investigations. Supplementing feedlot cattle with the brown seaweed *Ascophyllum nodosum*, for example, was observed to minimize fecal shedding of *Escherichia coli* (Makkar 2016; Montañez *et al.*, 2012). More study is being conducted with the addition of seaweed in caprine feed. Orkney sheep from North Ronaldsay Island are known to eat predominantly brown seaweed for the majority of the year. Species such as *Laminaria digitata*, *Laminaria hyperborea*, and *Saccharina latissima* (Phaeophyceae) account for 90% of this sheep's summer feed, meeting a significant proportion of nutrient requirements due to them up to 13% crude protein content. Other seaweed species consumed by Orkney sheep include *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus sp.* (brown seaweed), *Palmaria palmata* (red seaweed), and certain green algae. There have been several reports of ruminant meals containing mostly brown and red seaweeds. However, with the exception of a few isolated examples, the data is insufficient to begin R&D work in order to produce new products for the ruminant feed industry. There is numerous research that must be conducted in order to support seaweed as a feed supplement in ruminants.

Table-1. Nutrient (% dry weight) and mineral (mg 100 g⁻¹ dry weight) composition of some edible seaweeds.

Nutrient Composition (%)						Mineral Composition (mg.100 g ⁻¹)				
Green seaweed, red seaweeds & brown seaweed										
Species	Protein	Ash	Dietary	Fiber	Carbohyd rate	Na	K	P	Ca	Mg
<i>Caulerpa lentillifera</i>	10–13	24–37	33	38-59	0.86–1.11	8917	700–1142	1030	780–1874	630–1650
<i>C. racemosa</i>	17.8–18.4	7-19	64.5	33-41	9.8	2574	318	29.71	1852	384–1610
<i>Codium fragile</i>	8–11	21-39	5.2	39-67	0.5–1.5	-	-	-	-	-
<i>Ulva compressa</i>	21–32	17-19	29-45	48.2	0.3–4.2	-	-	-	-	-
<i>U. lactuca</i>	10–25	12.9	29-55	36-42	0.6–1.6	-	-	140	840	-

<i>U. pertusa</i>	20–26	28.6		47	-	-	-	-	-	-
<i>U. rigida</i>	18–19	-	38–41	43–56	0.9–2.0	1595	1561	210	524	2094
<i>U. reticulata</i>	17–20	-	65.5	50–58	1.7–2.3	-	-	-	-	-
Red seaweeds										
<i>Chondrus crispus</i>	11–21	24–37	33	38–59	0.86–1.11	8917	700–1142	1020	780–1674	300–632
<i>Crassiphycus chani</i>	6.9	7–19	64.5	33–41	9.8	2574	318	29.71	1652	465
<i>Agarophyton chilense</i>	13.7	21–39	5.2	39–67	0.5–1.5	-	-	-	-	160–510
<i>Palmariapalmata</i>	8–35	17–19	29–45	48.2	0.3–4.2	-	-	-	-	665
<i>Neopyropiatenera</i> <i>Neopyropiatenera</i>	28–47	12.9	29–55	36–42	0.6–1.6	-	-	120	440	270
<i>Porphyra umbilicalis</i>	29–39	28.6		47	-	-	-	-	-	250
<i>Neopyropia yezoensis</i>	31–44	-	38–41	43–56	0.9–2.0	1165	1251	120	324	1054
Brown seaweeds										
<i>Alaria esculenta</i>	9–20	9.72	42.86	46–51	1–2	-	-	-	-	-
<i>Eisenia bicyclis</i>	7.5	-	10–75	60.6	0.1	-	-	-	-	-
<i>Fucus spiralis</i>	10.77	14–30	63.88	-	-	-	-	-	-	-
<i>F. vesiculosus</i>	3–14	27–36	45–59	46.8	1.9	2450–5469	2500–4322	315	725–938	670–994
<i>Himanthalia elongata</i>	5–15	38	33–37	44–61	0.5–1.1	4100	8250	240	720	435
<i>Laminaria digitata</i>	8–15	29.47	37–37	48	1.0	3818	11.5–79	-	1005	659
<i>L. ochroleuca</i>	7.49	27–33	-	-	0.92	-	4350–5951	-	-	-
<i>Saccharina japonica</i>	7–8	34.78	10–41	51.9	1.0–1.9	2532–3260	-	300	-	757

<i>Sargassum fusiforme</i>	11.6	26–40	17–69	30.6	1.4	-	5500–6810	-	1860	687
<i>Undaria pinnatifida</i>	12–23	-	16–51	45–51	1.05–45	1600–7000	-	235–450	680–1380	405–680

Challenges with Algae

Different physiologically active chemicals are produced by macro- and microalgae. A number of these substances can have antinutritive and sometimes even more severe negative effects. Algae toxins, such as domoic acid, can cause major health problems. Other components found in some macroalgae, such as high iodine concentrations (Mabeau *et al.*,1993), could be harmful if fed in excess by animals. Iodine levels should not exceed 8 mg I/kg DM of total diet. These chemicals may be useful in some situations, but in excess, they may be harmful to rumen microbes and/or the animal. Recent studies on the usage of the macroalgae *Asparagopsis* show significant potential in terms of reducing enteric methane emissions, but they also emphasize the necessity to examine and comprehend the ramifications of bromoform or other halogens transferring into meat or milk. found no evidence of bromoform buildup in animal tissue, however the substance was expelled in dairy cow urine and milk. Studies on the use of seaweed in bovine, caprine, and other ruminant nutrition have focused on adding small amounts of different macroalgal species to feed and then assessing the animal for possible prebiotic activity and improved animal performance.

The latter is a concern that must be addressed quickly before large-scale commercialization begins.

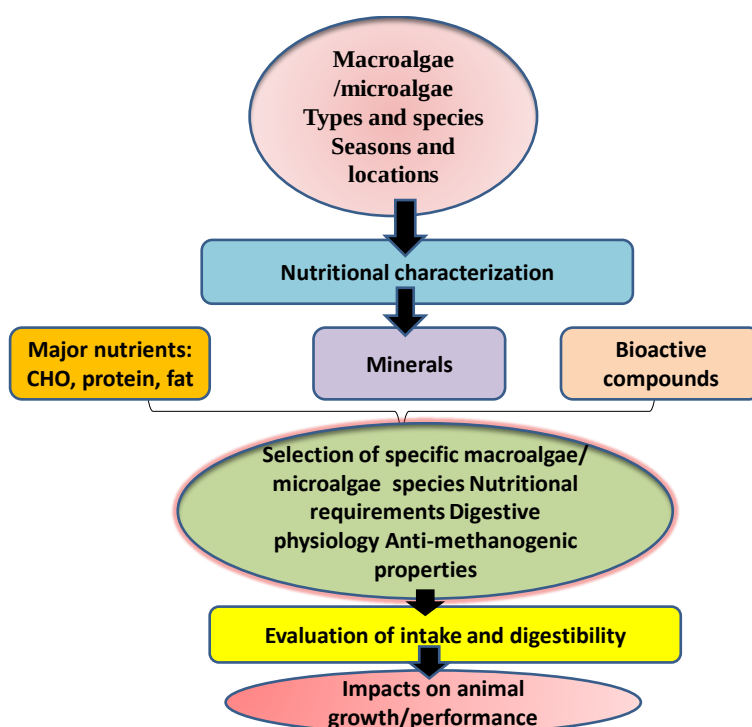


Figure-2: A flowchart for the future evaluation of macroalgae and microalgae as ruminant feed ingredients. Carbohydrates are abbreviated as CHO.

Future Prospective

In this scenario, economic and environmental concerns must be addressed while limiting rivalry with plant-based human food chains Countries in Europe. For animal feed, microalgae are a viable source of protein and other nutrients. However, the incredible richness of physiologically active molecules makes these creatures particularly appealing as feed ingredients, as their importance extends far beyond nutrient provision. Due to the restricted use of microalgae as animal feed or food ingredients, there will be little competition between microalgae-based feed and food chains. (European Commission. 2013). This study attempts to synthesize current knowledge on minor pulses and other protein-rich seaweeds and microalgae as alternatives to traditional animal protein sources, with an emphasis on production, availability, and nutritional qualities. Strengths, weaknesses, opportunities, and threats related to the use of these protein sources in animal feeding are separately examined using a SWOT analysis to underpin future research and/or technological development needs that could help valorize these nutrient sources as feed ingredients. The utilization of microalgae proteins is not a new concept, however concerns such as economically sustainable manufacturing remain unresolved.

Conclusion

Several ruminant feed management strategies emphasize the addition of nutritional supplements, increasing the quantity of starch, or supplementing with high-energy lipids. These initiatives aim to increase animal output while lowering methane emissions. Algae supplements are being researched as innovative ingredients for reducing methanogenesis, with the potential creation of algal biomass also contributing to lower greenhouse gas emissions. As a result, using algal biomass as a feed concentrate in nutritional supplementation is a sustainable and environmentally friendly technique. The anticipated future demand for very nutrient-dense animal food items will compel the animal production system to seek out new sources of high-quality protein feedstuffs. As a result, it can be stated that the plant kingdom can provide intriguing chances that will require correct insights and evaluations to help meet the protein hunger of the livestock and aquaculture sectors, as well as humans, in the near future. (Giuliana & Francesca, 2020).

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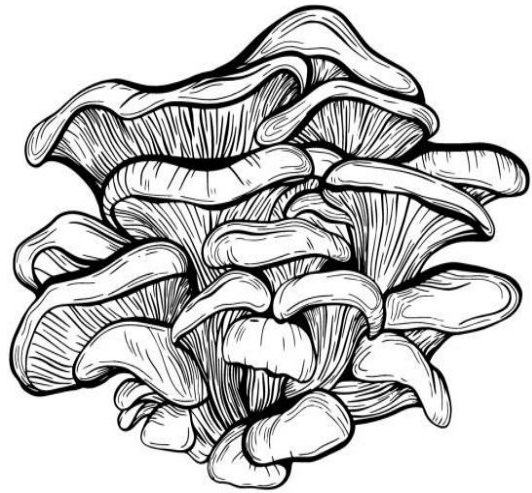
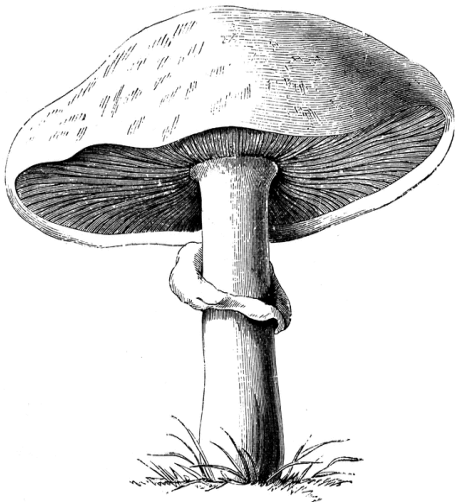
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Section-II

MYCOLOGY



FUNGAL RESEARCH

CHAPTER**14****CHECKLIST OF FUNGI FROM
KALSUBAI AREA, MAHARASHTRA
STATE- PART 1**

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Ranadive K. R.*

A

bstract

This is the highest peak of the state of Maharashtra. During this visit 14 fungi were collected from which Aphyllophorales are found to be dominant in the same area. This is the first effort to document the diversity of the Kalsubai area.

Keywords: *Aphyllophorales, Basidiomycetes, Diversity, Fungi.*

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Introduction

Kalsubai is the highest peak of the Maharashtra state and western ghats also which is 5,400 feet or 1646 meters above the sea level. It's easily accessible from Pune. Kalsubai Harishchandragad wildlife sanctuary includes forests of Kalsubai (361.71 sq. km.) and Bhimashankar (130.78 sq. km.) Kalsubai among the Sahyadri hill ranges of Maharashtra. Variety of plants, fungi and animals are found on Kalsubai Hill. The actual mountain range lies within the Kalsubai-Harishchandragad Wildlife Sanctuary. As far as the history is concerned, the name is given after one of the three sisters Kalsubai, Ratnabai and Katrabai. Ratangad is the other peak named after Ratnaba. (Wikipedia, 2022).

As we all know that the Western Ghats of Maharashtra, at a prodigious elevation of 1646 metres lies the highest peak of the state: it's known as the 'Everest of Maharashtra'. Surrounded by huge mountain ranges and lush greenery. A local deity temple named Kalasubai temple is a sacred temple. (Wikipedia, 2022). At every Tuesday and Thursday there is prayer by priest. In Navratri festival special Pooja is arranged. Complete Kalsubai area is having vast stretches of forests nestled along their slopes and valleys. Excellent diversity is observed here around (Wikipedia, 2022) (Figure-1).

Materials & Methods

The fungal materials were collected in a paper bag and numbered by standard methods. The photographic documentation has been done with the help of camera. (Nikon Digital camera Z5). The collected materials are preserved by standard methods. Identification was done by using standard literature. The samples are submitted to Department of Botany, Annasaheb Magar Mahavidyalaya, Hadapsar.

Observations and Results

As this is the first try to study the diversity of fungi from Kalsubai, the present data gives the glimpses of diversity. Total 14 different fungal species were collected from Kalsubai area. All these during this survey we are found some non-lichenized group of fungi. In this survey total 14 fungi (non-lichenized) were found. From which total 14 forms were belongs to non-lichenised group. As per species type the members which are found shows healthy climate and good rainfall also. Diversity of the funga from Kalsubai area includes the members as follows *Ganoderma multiplicatum*, *Hypoxylon hoemotostrama*, *Anthracophyllum archeri*, *Coprinellus radians*, *Auricularia aurinellus*, *Schizophyllum commune*, *Flavodon flavus*, *Fomes* sp. *Tryblidaria maharastrensis*, *Phellinus gilvus*, *Pseudofavolus tenuis* (Figure-2, Table 1).

Table-1: List of fungi

Name of the species	Family
<i>Anthracophyllum archeri</i> (Berk.) Pegler 1965,	Omphalotaceae
<i>Auricularia cornea</i> Ehrenb.	Auriculariaceae

<i>Coprinellus radians</i> (Desm.) Vilgalys, Hopple & Jacq. Johnson	Psathyrellaceae
<i>Flavodon flavus</i> (Klotzsch) Ryvarden	Meruliaceae
<i>Fomes</i> sp.	Polyporaceae
<i>Ganoderma multiplicatum</i> (Mont.) Pat. 1889,	Ganodermataceae
<i>Hypoxylon haematostroma</i> Mont. 1845	Hypoxylaceae
<i>Leucocoprinus cepistipes</i> (Sowerby) Pat.	Agaricaceae
<i>Oxyporus</i> sp.	Schizoporaceae
<i>Peniophora</i> sp.	Peniophoraceae
<i>Phellinus gilvus</i> (Schwein.) Pat.	Hymenochaetaceae
<i>Pseudofavolus tenuis</i> (Fr.) G. Cunn.	Polyporaceae
<i>Schizophyllum commune</i> Fr.	Schizophyllaceae
<i>Tryblidaria maharastrensis</i> Anahosur	Patellariaceae

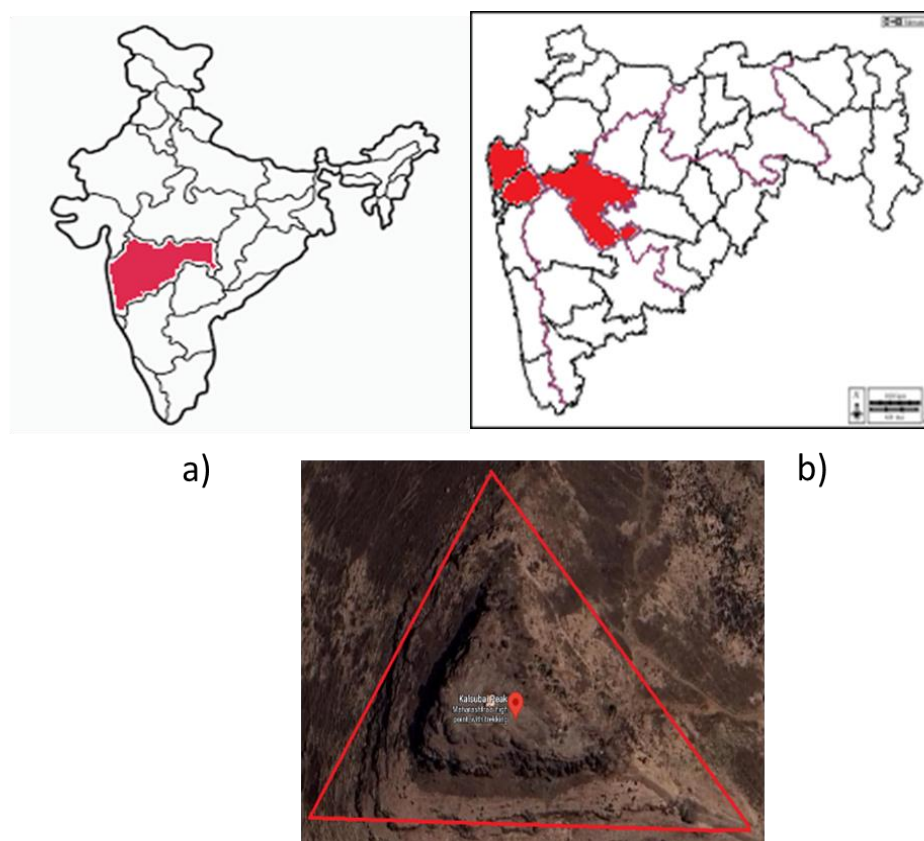


Figure-1: a) Map of India showing Maharashtra State b) Map of Maharashtra State showing Nashik District and c) map of showing Kalsubai area in Nashik district.



Figure-2: A] *Ganoderma multiplicatum* (Mont.) Pat., B] *Hypoxylon hoemotostrama* C] *Anthracophyllum archeri* (Berk.) Pegler, D] *Oxyporus* sp. E] *Peniophora* sp. F] *Coprinellus radians* (Desm.) Vilgalys, Hopple & Jacq. Johnson G] *Auricularia cornea* Ehrenb. H] *Schizophyllum commune* Fr., I] *Flavodon flavus* (Klotzsch) Ryvarden, J] *Tryblidaria maharastrensis* Anahosur, K] *Phellinus gilvus* (Schwein.) Pat., L] *Pseudofavolus tenuis* (Fr.) G. Cunn. M] *Fomes* sp. N] *Leucocoprinus cepistipes* (Sowerby) Pat.

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CHAPTER**15****BOTANICAL FUNGICIDES: AN UPDATED REVIEW**

Dr. Asha Bhausaheb Kadam*

A

bstract

According to recent studies, there are ever more dangerous phytopathogenic fungi in the world, and this has a substantial negative impact on the global economy. There are numerous methods currently available for the quick identification and management of phytopathogenic fungi. Chemical and synthetic fungicides have been the mainstay for controlling phytopathogenic fungi since 1940. However, the adverse effects of synthetic fungicides are on the health of animals, people, and the environment. Also, the significant rise in the development of fungal resistance to these fungicides has led scientists from all over the world to investigate several novel approaches and environmentally friendly methods of controlling fungi. The creation of fresh strategies for combating fungal infections in plants is covered in this review. We contend that an effort should be made to reach farmers with these modern technologies.

Keywords: *Plant fungal diseases, Phytopathogenic fungi, Botanical fungicides.*

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Introduction

Plant extracts have been found to have potent antifungal effects *In Vitro*, according to various published research work. But, because of many the antifungal chemicals present in plant extracts are rather non-polar and do not diffuse well in agar, and agar diffusion experiments were not the best method for detecting these activities of plant extracts (Shuping, *et al.*, 2017). Due to the differences in the variables that affect agar diffusion, the results obtained from one laboratory to another were likewise very inconsistent (El-Baky *et al.*, 2021).

Various plant essential oils have the power to prevent fungal infections from starting, growing, and spreading during and after crop harvest, hence extending the shelf life of stored fruits and vegetables (Tripathi, *et al.*, 2004; Raut, *et al.*, 2014). Additionally, they can prevent some fungal species from producing mycotoxins, which leads to the post-harvest deterioration of stored fruits (Sivakumar, *et al.*, 2014). To defend themselves against many plants' fungal infections, some plants can create a variety of antimicrobial compounds (botanical fungicides) (Martínez, *et al.*, 2012).

The structure, molecular weight, purposes, and classification of these natural fungicides, such as phyto-anticipins and phytoalexins, vary (Ribera, *et al.*, 2012). The secondary plant metabolites phenolics, fatty acids, flavonoids, alkaloids, glycosides, terpenoids, and tannins can function as organic fungicides to inhibit phytopathogenic fungus. Plant extracts typically have the advantage of containing a variety of chemicals (antifungal compounds) combined and they may synergistically work against phytopathogenic fungi (Masoko *et al.*, 2005). Additionally, the various antifungal mechanisms used by these chemical combinations may reduce the resistance of fungal phytopathogens.

In addition, many metabolites produced by plants that shown effective antifungal actions against fungal phytopathogens *In Vivo*. The following are some examples of commercially available botanical fungicides.

Milsana

A natural fungicide called Milsana is made by extracting the *Reynoutria sachalinensis* plant's ethanol, and it is intended for use in reducing certain illnesses that damage plants cultivated in greenhouses, primarily powdery mildew (Konstantinidou *et al.*, 2006). Instead of being used as a therapy, it is frequently used as a preventative measure. The antifungal activity of Milsana and the induction of plant resistance are two of the control mechanisms. It uses to combat the powdery mildew disease of wheat. This botanical fungicide should be sprayed as a spray to run-off once, 48 hours before planting, to successfully decrease powdery mildew that affects young seedlings in glasshouses by roughly 97%. 48 hours before planting, this natural fungicide should be sprayed over run-off once (Bokshi, *et al.*, 2008; Reglinski, *et al.*, 2009). By functioning as a natural elicitor of phytoalexins, which are antimicrobial substances made and stored by plants in hypersensitive tissues as a response to pathogen infection, Milsana increases resistance and the natural immune system of the plant (Copping, *et al.*, 2004).

Jojoba Oil

The jojoba bean was used to obtain this vegetable oil. Powdery mildew is a fungal disease that can be efficiently controlled in ornamental plants and grapes (Copping, *et al.*, 2004, Abdu-Allah, *et al.*, 2017). Jojoba oil also shows itself as a broadly useful fungicide in nearly all climatic circumstances due to its stability, even at high temperatures (Copping, *et al.*, 2004, Abdu-Allah, *et al.*, 2017). The final concentration of this natural fungicide is sprayed at around 1% (Copping, *et al.*, 2004)

Essential Plant Oils

Plant volatile chemical components are condensed into liquids known as essential oils. The term "**volatile oils**" or "**just the oil of the plant**" might be used to describe them. The most common methods of extraction include resin tapping, solvent extraction, distillation, and expression. They are used in a wide range of products, including flavoring food and beverages, cosmetics, perfumes, soaps, and aromatherapy, an alternative kind of medicine in which aromatic chemicals are said to have therapeutic properties (Linskens, *et al.*, 1991).

The essential oils from peppermint and sweet basil, in addition to their main aroma components (linalool/eugenol in the case of the basil oil and menthol in the case of the peppermint oil), have been shown by Edris and Farrag (Edris, *et al.*, 2003) to have antifungal properties against some phytopathogenic fungi, such as *Rhizopus stolonifer* and *Sclerotinia sclerotiorum*. Additionally, *Calocedrus macrolepis* essential oil and its constituents have been shown to have an antifungal impact on *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Pestalotiopsis funerea*, and *Fusarium solani* (Chang, *et al.*, 2008)

Six important toxinogenic and pathogenic fungi species (*Fusarium verticillioides*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium brevicompactum*, and *Penicillium expansum*) were found to be inhibited by essential oils extracted from 25 different medicinal plant species in another study (Zabka, *et al.*, 2009)

Eucalyptus globulus Labill, *Nigella sativa* L., and *Allium cepa* L. essential oils have been shown by Elgorban *et al.* (2015) to have antifungal action against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Fusarium verticillioides*, and *Fusarium solani*.

Three essential oils derived from *Majorana hortensis*, *Verbena officinalis*, and *Salvia officinalis* were described by Elshafie *et al.* (2016) and their antifungal activity was demonstrated against *Colletotrichum acutatum* and *Botrytis cinerea*. All of the examined essential oils belong to the chemotype carvacrol/thymol, and monoterpene molecules make up the majority of their chemical structures.

The fungicide activity of the essential oil from *Solidago canadensis* L. was studied more recently by Elshafie *et al.* (2019) and its impact on some postharvest phytopathogenic fungi, including *Aspergillus niger*, *Botrytis cinerea*, *Monilinia fructicola*, and *Penicillium expansum*, was confirmed. *In Vitro*, antifungal activity against several postharvest

phytopathogens (*Aspergillus niger*, *Penicillium expansum*, *Botrytis cinerea*, and *Monilinia fructicola*) was observed for two essential oils extracted from *Origanum heracleoticum* L. and *O. majorana* L. (Della, *et al.*, 2019).

In a recent study, Perczak *et al.* (2019) employed certain essential oils as antifungal agents to inhibit the growth and formation of mycotoxins in wheat grain by *Fusarium culmorum* and *Fusarium graminearum*. Nevertheless, although essential oil's potent antifungal activity against a variety of phytopathogenic fungi has been repeatedly described in the scientific literature, their use in agriculture remains surprisingly limited. (Raveau, *et al.* 2020)

Cinnamaldehyde

Since its initial separation from the cinnamon essential oil in 1834, cinnamaldehyde has been chemically synthesized for use as a crop protection agent. *Sclerotinia homoeocarpa*, *Verticillium fungicola* and *Fusarium moniliforme* which cause pitch canker infection, a dry bubble on *Agaricus bisporus* and dollar spot on turfgrass, respectively, can all be efficiently controlled by it (Copping, *et al.*, 2004). According to a paper, cinnamon possesses antifungal properties against the fungus responsible for wood degradation (Wang, *et al.*, 2005). Cinnamaldehyde has also been demonstrated to have antifungal effects on the fungi that cause wood rot (Cheng, *et al.*, 2008)

Additionally, Khan *et al.* (2011) showed that among the important elements of essential oils (citral, eugenol, or geraniol), cinnamaldehyde had the most antifungal effects. Along with harming their virulence factors and hyphal ultrastructure, it demonstrated the most inhibitory effect on the fungal growth of *Trichophyton rubrum* and *Aspergillus fumigates*. Commercially, cinnamaldehyde that has been made into a wettable powder is offered and sold under the brand names VERTIGO and Proguard fungicides. This formulation is insoluble in water and quickly breaks down in soil without endangering the environment or harming non-target organisms.

Phenolic Substances

Numerous investigations indicated that phenolic compounds produced by medicinal plants have antifungal properties (Yoon *et al.*, 2013; Choi, *et al.*, 2008; Cho, *et al.*, 2006; Cho, *et al.*, 2006; Pizzolitto, *et al.*, 2015). These plant phenols fall under the categories of coumarins, flavonoids, anthraquinones, and simple phenolics. *In Vivo*, investigations have shown that pure curcuminoids (polyphenolic pigments) from *Curcuma longa* rhizomes have antifungal effects against some phytopathogenic fungi (Yoon *et al.*, 2013; Choi, *et al.*, 2008; Cho, *et al.*, 2006).

Three curcuminoids, primarily curcumin, bisdemethoxycurcumin, and demethoxycurcumin, are present in the *C. longa* rhizomes after being extracted with methanol. On late blight of tomato and blast of rice, demethoxycurcumin which is a curcuminoid, demonstrated the best antifungal activity. Furthermore, *Colletotrichum coccodes*, a phytopathogenic fungus that causes anthracnose on red pepper, could be effectively inhibited by *C. longa* curcuminoids both in terms of spore germination and mycelia growth. However, *In Vivo*, research demonstrated that the phytopathogenic fungi that cause wheat leaf rust, tomato grey mould,

rice sheath blight, and powdery mildew on barley did not have any or little antifungal effects from *C. longa* curcuminoids. The fruit rinds of *Myristica malabarica*, which are extracted using methanol, contain malabaricones (diarylnonanoids), which have antifungal properties against phytopathogenic fungi, according to (Choi, *et al.*, 2008; Cho, *et al.*, 2006)

Alkaloids Substances

These substances contain nitrogen in their rings and are categorized as isoquinoline alkaloids, pyridine alkaloids, and indole alkaloids based on their heterocyclic rings. They may help protect plants from herbivores and diseases. Some alkaloids have analgesic qualities, whereas others act as cardiac or respiratory stimulants. Numerous they also have local anesthetic properties. Alkaloids made by plants have been referred to as phytopathogenic fungi fungicides (Singh, *et al.*, 2007)

The discovery of a piperidine alkaloid from *Piper longum*, which possesses fungicidal activity against a few phytopathogenic fungi, serves as an illustration of this (Lee, *et al.*, 2001) Additionally, two alkaloids were discovered to exhibit considerable fungicidal activity against five phytopathogenic fungi after being extracted from the seeds of *Chimonanthus praecox* (Zhang, *et al.*, 2009)

Allosecurinine, a compound found in the roots of *Phyllanthus amarus*, was discovered to have *In Vitro* fungicidal activity (hinders the development of fungal spores) against five phytopathogenic fungi in a different study (Zhang, *et al.*, 2009) Also, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* are susceptible to the fungicidal effects of the dihydropyrrole which is derived from *Datura metel* (Abad, *et al.*, 2007)

A combination of quaternary alkaloids derived from *Argemone mexicana* displays antifungal action (inhibited spore germination) against ten phytopathogenic fungi, according to Singh *et al.* (2010). Plant alkaloids may be used by farmers in the field to manage various plant fungal infections as a result of their potent in vitro antifungal effectiveness.

Future Perspectives

Farmers and agriculturists should learn healthy, non-toxic, efficient, and eco-friendly methods (green techniques) for treating fungal diseases in plants to safeguard the well-being of people and animals as well as soil biodiversity. When using these methods to control plant fungal diseases, *In Vivo* conditions must be used. More emphasis in research needs to be placed on creating innovations.

Conclusions

An extensive review of the scientific literature identifies some botanical fungicides as recent, secure, and efficient alternative control methods (green strategies of fungal control). Even though these fungicides are still in the developmental stages, they nonetheless represent potential control methods that need to be properly introduced to field applications. They safeguard both human and animal health as well as soil biodiversity, which might revolutionize agriculture by eradicating the negative impacts of pesticides on both the environment and human and animal health.

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CHAPTER

16

NEW ANTIFUNGAL AGENTS AND THERAPEUTIC APPROACHES TO OVERCOME THE CRISIS IN ANTIFUNGAL THERAPY

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A**bstract**

Fungal infections pose a significant global burden, particularly among immunocompromised individuals, leading to high morbidity and mortality rates. The crisis in antifungal therapy, characterized by limited treatment options, emerging drug resistance, and the lack of effective diagnostics, demands urgent attention. This article explores recent advances and innovations in the development of new antifungal agents and therapeutic approaches to address these challenges. It highlights the importance of overcoming the crisis in antifungal therapy and the implications for improving patient outcomes and global health.

Keywords: *Fungal Infection, Therapeutics, Immunocompromised, Development, Global Burden.*

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Introduction

Fungal infections, also known as mycoses, have emerged as a growing global health concern, posing significant challenges to healthcare systems worldwide. Unlike bacterial and viral infections, fungal pathogens exhibit unique characteristics that make them particularly formidable adversaries. From mild superficial skin infections to life-threatening systemic diseases, fungal infections affect millions of people each year, cutting across geographic boundaries and impacting individuals of all ages and backgrounds.

The impact of fungal infections is particularly pronounced in immunocompromised individuals, such as those with weakened immune systems due to underlying medical conditions or medical treatments. This includes patients with HIV/AIDS, cancer patients undergoing chemotherapy, organ transplant recipients on immunosuppressive drugs, individuals with autoimmune diseases on immunosuppressive therapies, and those with severe malnutrition.

The chapter will provide an overview of the current state of antifungal therapy and the challenges faced in combatting fungal infections. It will also emphasize the critical importance of developing new antifungal agents to overcome the crisis in antifungal therapy. The chapter will delve into specific examples of recent advances, showcasing the mechanisms of action, spectrum of activity, pharmacokinetics, safety, and efficacy of these innovative agents.

Ultimately, by understanding and exploring the recent advances in new antifungal agents, this chapter seeks to underscore the significance of ongoing research and development in this field. The exploration of these novel therapeutic options will contribute to improved treatment strategies, better patient outcomes, and a more effective response to the escalating global burden of fungal infections.

Factors attributing for global burden of fungal infections

Opportunistic Infections: Fungi that are normally harmless in healthy individuals can become opportunistic pathogens in immunocompromised patients. These infections can be challenging to manage due to the compromised immune response, leading to prolonged illnesses and increased morbidity.

High Mortality Rates: Invasive fungal infections, such as invasive aspergillosis, candidiasis, and mucormycosis, have high mortality rates, especially in immunocompromised patients. Early diagnosis and appropriate treatment are crucial for improving patient outcomes.

Antifungal Resistance: Similar to antibiotic resistance, antifungal resistance has become a major concern in recent years. The overuse and misuse of antifungal drugs, particularly in healthcare settings, have contributed to the emergence of drug-resistant fungal strains. Fungi have demonstrated an ability to develop resistance to multiple classes of antifungal agents, reducing the effectiveness of available treatments.

Challenges in Diagnosis: Fungal infections can be challenging to diagnose due to their nonspecific symptoms, which may lead to delays in appropriate treatment. The lack of rapid and accurate diagnostic tools can further exacerbate the global burden of fungal infections.

Nosocomial Infections: Hospital-acquired fungal infections, like candidemia, can occur in immunocompromised patients during hospital stays. Nosocomial infections can further burden healthcare systems and increase treatment costs.

Endemic Mycoses: Some regions have a higher prevalence of specific endemic fungal infections. These infections are often associated with environmental exposures and can affect large populations, posing a considerable burden on local healthcare systems.

Immunocompromised Populations: The rise in the number of immunocompromised individuals, such as those living with HIV/AIDS, undergoing cancer chemotherapy, organ transplant recipients, and patients on immunosuppressive therapies for autoimmune diseases, has led to an increased susceptibility to fungal infections. The weakened immune systems of these individuals create an opportunistic environment for fungal pathogens to cause severe and invasive infections.

Changing Epidemiology: The epidemiology of fungal infections has shifted due to various factors, including globalization, travel, climate change, and increased agricultural practices. This has led to the emergence of previously rare or geographically localized fungal pathogens in new regions, impacting global health.

Invasive Healthcare-Associated Infections: Hospital-acquired or healthcare-associated fungal infections have become a significant challenge. These infections often occur in patients with underlying health conditions and invasive medical interventions, such as those using medical devices, catheters, or ventilators.

Fungal Outbreaks: Fungal outbreaks in various settings, such as hospitals, long-term care facilities, and communities, have been reported in recent years. These outbreaks can lead to severe infections, increased healthcare costs, and strain on healthcare resources.

Addressing the growing global burden of fungal infections requires a multifaceted approach, including

Infection Control Measures: Implementing strict infection control practices in healthcare facilities can help reduce the risk of nosocomial fungal infections.

Preventative Measures: Prophylactic antifungal treatments may be considered for certain high-risk immunocompromised patients to prevent opportunistic infections.

Increased Awareness: Enhanced education and awareness among healthcare professionals and the general public about the risk factors, symptoms, and appropriate diagnostic approaches for fungal infections can lead to earlier detection and treatment.

Improved Diagnostic Tools: Developing and implementing rapid and accurate diagnostic tests can aid in early identification and appropriate management of fungal infections.

New Antifungal Agents: Continued research and development of new antifungal agents with different mechanisms of action are crucial to combat drug-resistant strains and expand treatment options.

Antifungal Stewardship: Promoting responsible use of antifungal drugs in healthcare settings can help reduce the development of resistance and preserve the efficacy of available treatments.

Research and Surveillance: Continuous monitoring of fungal infections, antifungal resistance, and epidemiological trends is necessary to inform public health strategies and interventions.

By addressing these challenges and implementing appropriate measures, the global health community can work towards mitigating the impact of fungal infections and improving patient outcomes worldwide.

Challenges and limitations in current antifungal therapy

Current antifungal therapy faces several challenges and limitations that hinder its effectiveness in treating fungal infections. These challenges encompass various aspects of antifungal treatment, ranging from drug-related issues to diagnostic and clinical considerations. Some of the key challenges and limitations include:

Limited Drug Classes: Unlike antibiotics for bacterial infections, the number of available antifungal drug classes is relatively small. The majority of antifungal medications belong to a few classes, such as azoles, polyenes, echinocandins, and allylamines. This limited diversity makes it difficult to target a broad spectrum of fungal pathogens and contributes to the development of drug resistance.

Emergence of Drug-Resistant Strains: Fungal pathogens have demonstrated a remarkable ability to develop resistance to antifungal drugs. Overuse or inappropriate use of antifungals, especially in healthcare settings, can lead to the emergence of drug-resistant strains. Antifungal resistance can significantly reduce the effectiveness of current therapies and limit treatment options for patients with invasive or difficult-to-treat fungal infections.

Limited Spectrum of Activity: Some antifungal drugs have a narrow spectrum of activity and may be effective against only certain types of fungi. For instance, some antifungals are more suitable for treating yeasts like *Candida*, while others are more effective against molds like *Aspergillus*. This limitation can lead to challenges in selecting the most appropriate drug for a specific fungal infection.

Toxicity and Drug Interactions: Certain antifungal medications can be toxic to human cells, leading to adverse side effects. For example, some azole antifungals can interact with various medications metabolized through the cytochrome P450 system, potentially causing drug interactions and reducing the efficacy of other medications. These interactions can complicate treatment regimens, especially in patients with multiple medical conditions.

Biofilm Formation: Fungal pathogens can form biofilms, which are protective structures composed of microbial cells and extracellular matrix. Biofilms are highly resistant to antifungal treatment and are frequently associated with chronic or recurrent infections, particularly in medical devices like catheters or prosthetic implants.

Lack of Rapid and Accurate Diagnostics: Fungal infections often present with nonspecific symptoms, leading to delays in diagnosis and treatment initiation. The lack of specific and rapid diagnostic tools can result in disease progression, poorer outcomes, and the unnecessary use of broad-spectrum antibiotics, which can further contribute to the development of antifungal resistance.

Immunocompromised Patients: Immunocompromised individuals, such as those with HIV/AIDS, cancer patients undergoing chemotherapy, and organ transplant recipients on immunosuppressive drugs, are at higher risk of severe fungal infections. The compromised immune response in these patients can reduce the effectiveness of antifungal therapy and prolong the course of the infection.

Cost and Access: Some antifungal agents can be costly, making them less accessible to patients in low-resource settings. The affordability and availability of antifungal drugs may vary across different regions, affecting treatment options and patient outcomes.

Fungal Diversity: Fungi comprise a wide range of species with different pathogenic capabilities. Some fungal species may exhibit intrinsic resistance to certain antifungal drugs, limiting the choice of effective treatment options for infections caused by these species.

Addressing these challenges and limitations requires a concerted effort from the medical community, researchers, and policymakers. Research and development of new antifungal agents with novel mechanisms of action, along with improved diagnostic tools and infection control measures, are essential to enhance the management of fungal infections and improve patient outcomes. Additionally, promoting judicious use of antifungal drugs and implementing antifungal stewardship programs can help mitigate the development of resistance and preserve the effectiveness of available treatments.

Importance of developing new antifungal agents

The development of new antifungal agents and therapeutic approaches is of paramount importance for several reasons:

Diverse Mechanisms of Action: The development of new antifungal agents with diverse mechanisms of action is crucial in overcoming the crisis in antifungal therapy. Fungal pathogens have complex and varied biological structures, making them highly adaptable to different environments and treatment strategies. By targeting multiple cellular processes and structures, new antifungal agents can effectively combat various fungal species, including those with intrinsic resistance to existing drugs. Diverse mechanisms of action can also reduce the likelihood of cross-resistance, where resistance to one antifungal agent confers resistance to others within the same class.

Broader Spectrum of Activity: New antifungal agents with a broader spectrum of activity are essential for addressing infections caused by a wide range of fungal pathogens. Currently available antifungal classes have limited activity against certain fungal species or strains, leaving some infections difficult to treat. Broader spectrum agents can cover a wider array of pathogens, providing more treatment options and reducing delays in initiating appropriate therapy. This is particularly important in the context of emerging and rare fungal pathogens that may cause severe infections and have limited treatment options.

Overcoming Drug Resistance: Antifungal resistance poses a significant challenge in the treatment of fungal infections. Fungi can develop resistance through genetic mutations or the acquisition of resistance genes, rendering existing antifungal drugs less effective. Developing new antifungal agents that can overcome or circumvent resistance mechanisms is essential to restore treatment efficacy. By targeting different molecular pathways and cellular components, these agents can provide alternative treatment options for infections caused by drug-resistant fungal strains.

Targeted Therapies for Specific Pathogens: The ability to tailor therapies to specific pathogens is crucial for optimizing treatment outcomes. Fungal infections vary in their clinical presentations and susceptibilities to different antifungal agents. New antifungal agents can be designed to selectively target specific pathogens while minimizing collateral damage to beneficial microorganisms. This targeted approach reduces the risk of adverse effects and improves the overall efficacy of antifungal therapy.

Combination Therapy and Synergistic Effects: Combination therapy, involving the use of two or more antifungal agents with distinct mechanisms of action, has shown promising results in overcoming drug resistance and improving treatment outcomes. The synergistic effects of combining antifungal agents can enhance their potency and reduce the likelihood of resistance development. Additionally, combination therapy can broaden the spectrum of activity, allowing for more comprehensive coverage against different fungal species. Developing new antifungal agents that can be effectively used in combination therapy expands treatment options and offers innovative approaches to combat challenging fungal infections.

The importance of developing new antifungal agents cannot be overstated. Diverse mechanisms of action, broader spectrum of activity, and targeted therapies for specific pathogens are essential for improving the efficacy of antifungal treatment. Overcoming drug resistance and exploring combination therapies provide additional strategies to address the challenges in antifungal therapy effectively. The development of new antifungal agents holds the potential to revolutionize the field of antifungal therapy and significantly improve patient outcomes in the face of fungal infections.

New Antifungal Agents: Recent Advances

Below are some examples of new antifungal agents along with their key characteristics:

Isavuconazole: Isavuconazole is a second-generation triazole antifungal agent with a broad spectrum of activity against various fungal pathogens. It was developed to address the

limitations of older azole antifungals and offers a broad spectrum of activity against both yeasts and molds.

Mechanism of Action: Its mechanism of action involves inhibition of the fungal enzyme lanosterol 14 α -demethylase, which is a cytochrome P450-dependent enzyme involved in ergosterol biosynthesis. Ergosterol is a crucial component of the fungal cell membrane, and its disruption by isavuconazole leads to impaired membrane integrity, cell wall instability, and ultimately, fungal cell death. This mechanism is similar to other azole antifungal agents, but isavuconazole exhibits a higher affinity for the fungal enzyme, contributing to its potent antifungal activity.

Spectrum of Activity: Isavuconazole demonstrates broad-spectrum activity against various fungal pathogens, making it effective against both yeasts and molds. It is active against *Candida* species, including some fluconazole-resistant strains, and *Aspergillus* species, including certain azole-resistant strains. Additionally, isavuconazole has shown activity against less common and rare fungi, such as certain Mucorales and *Fusarium* species. This wide spectrum of activity makes isavuconazole a valuable option for treating a range of fungal infections.

Pharmacokinetics: Isavuconazole is available in both oral and intravenous formulations. It exhibits good oral bioavailability, allowing for a convenient switch from intravenous to oral administration once the patient's condition stabilizes. The drug is well-absorbed and has predictable pharmacokinetics, leading to steady plasma concentrations with once-daily dosing. It has a long half-life, enabling less frequent dosing, which is particularly advantageous in the treatment of chronic or prolonged infections.

Safety and Efficacy: Clinical trials have demonstrated the safety and efficacy of isavuconazole in the treatment of invasive fungal infections. It has been studied in both candidiasis and aspergillosis, and the results have shown non-inferiority to other standard antifungal therapies. Isavuconazole has been generally well-tolerated, with adverse effects comparable to other azole antifungal agents. The incidence of adverse events, including liver toxicity, has been relatively low, making it a viable option for patients who may be more susceptible to antifungal-related side effects.

Potential Advantages over Existing Antifungals: Isavuconazole possesses several advantages over existing antifungals:

Broad Spectrum: Its wide spectrum of activity allows for the treatment of various fungal infections with a single agent, streamlining therapy and reducing the need for multiple drugs.

Limited Drug Interactions: Unlike some other azole antifungals, isavuconazole has minimal inhibition of cytochrome P450 enzymes, leading to fewer drug interactions and a reduced risk of altered metabolism of co-administered medications.

Oral and Intravenous Formulations: The availability of both oral and intravenous formulations provides flexibility in dosing and route of administration, allowing for easy transition between the two depending on the patient's clinical status.

Reduced Toxicity: The relatively low incidence of adverse events, including hepatotoxicity, makes isavuconazole a safer option, especially in patients with compromised liver function.

Overall, isavuconazole's potent antifungal activity, broad spectrum, convenient dosing, and favorable safety profile position it as a promising agent in the management of invasive fungal infections, particularly in immunocompromised patients or those with difficult-to-treat fungal pathogens.

Ibrexafungerp (Formerly SCY-078)

Ibrexafungerp, formerly known as SCY-078, is a novel antifungal agent developed by SCYNEXIS Inc. It represents a new class of antifungals known as triterpenoid glucan synthase inhibitors. Ibrexafungerp has shown promising activity against a wide range of fungal pathogens, making it a potential therapeutic option for various invasive fungal infections.

Mechanism of Action: Ibrexafungerp exerts its antifungal activity by inhibiting the enzyme 1,3-beta-D-glucan synthase, which is responsible for the synthesis of beta-D-glucan in the fungal cell wall. Beta-D-glucan is a critical component of the cell wall, providing structural integrity and protection to the fungal cell. By inhibiting its synthesis, ibrexafungerp disrupts the cell wall formation, leading to osmotic instability and ultimately, fungal cell death. The unique mechanism of action of ibrexafungerp distinguishes it from other classes of antifungal drugs, providing an alternative approach to treat fungal infections.

Spectrum of Activity: Ibrexafungerp exhibits a broad spectrum of activity against various fungal pathogens, including both yeasts and molds. It is active against *Candida* species, such as *Candida albicans*, *Candida glabrata*, and *Candida auris*, including some drug-resistant strains. Moreover, it has demonstrated activity against molds, such as *Aspergillus* species and *Pneumocystis jirovecii*. The broad spectrum of activity makes ibrexafungerp a promising therapeutic option for a wide range of invasive fungal infections.

Pharmacokinetics: Ibrexafungerp is available in both oral and intravenous formulations, allowing for flexible dosing options. It is well-absorbed orally, with predictable pharmacokinetics, and its bioavailability is not affected by food. The drug has a relatively long half-life, enabling once-daily dosing in most patients. This convenient dosing schedule is particularly advantageous in the management of fungal infections, simplifying treatment and improving patient compliance.

Safety and Efficacy: Clinical trials have demonstrated the safety and efficacy of ibrexafungerp in the treatment of invasive fungal infections. It has been studied in both candidiasis and aspergillosis, showing promising results in terms of efficacy and tolerability. The drug has been generally well-tolerated, with adverse effects similar to those of other antifungal agents. Additionally, ibrexafungerp has shown a low potential for drug interactions, making it a safer option for patients requiring multiple medications.

Potential Advantages over Existing Antifungals: Ibrexafungerp offers several potential advantages over existing antifungal agents-

Novel Mechanism of Action: Its unique mechanism of action as a triterpenoid glucan synthase inhibitor provides an alternative approach to combat fungal infections, potentially reducing the risk of cross-resistance with other antifungal classes.

Broad Spectrum: Its wide spectrum of activity against both yeasts and molds allow for the treatment of various fungal infections with a single agent, streamlining therapy and potentially reducing the need for combination antifungal regimens.

Convenient Dosing: The availability of both oral and intravenous formulations and once-daily dosing make ibrexafungerp convenient and user-friendly for patients and healthcare providers.

Ongoing research and clinical trials will further elucidate its potential benefits and role in combating fungal infections, particularly in patients with limited treatment options due to drug resistance or intolerance to existing antifungals.

Rezafungin (Formerly CD101)

Rezafungin is a novel echinocandin antifungal agent developed by Cidara Therapeutics. It belongs to the same class of antifungals as caspofungin and micafungin. Rezafungin has been designed to address the limitations of existing echinocandins and offers potential advantages in the treatment of invasive fungal infections.

Mechanism of Action: Rezafungin exerts its antifungal activity by inhibiting the synthesis of 1,3-beta-D-glucan, an essential component of the fungal cell wall. It targets the enzyme 1,3-beta-D-glucan synthase, which is responsible for the production of beta-D-glucan. By inhibiting this enzyme, rezafungin disrupts the formation of the fungal cell wall, leading to osmotic instability and cell lysis, resulting in fungal cell death. This mechanism is similar to other echinocandin antifungals but rezafungin has been engineered to offer improved pharmacokinetics and extended half-life.

Spectrum of Activity: Rezafungin exhibits a broad spectrum of activity against *Candida* species, including both common and drug-resistant strains. It is also active against certain molds, such as *Aspergillus* species. The broad spectrum of activity makes rezafungin an effective option for the treatment of various invasive fungal infections caused by different fungal pathogens.

Pharmacokinetics: Rezafungin is administered intravenously and has been designed to have a long half-life, allowing for once-weekly dosing. This extended dosing interval is advantageous, particularly in the treatment of chronic or prolonged infections, as it reduces the frequency of administration and may improve patient compliance. The predictable pharmacokinetics of rezafungin contribute to its ease of use in clinical practice.

Safety and Efficacy: Clinical trials have demonstrated the safety and efficacy of rezafungin in the treatment of invasive fungal infections. The drug has been studied in candidemia and invasive candidiasis, showing promising results in terms of clinical response rates. Rezafungin has been well-tolerated in patients, with adverse effects similar to those of other echinocandin antifungals.

Potential Advantages over Existing Antifungals: Rezafungin offers several potential advantages over existing echinocandin antifungals:

Extended Half-life: Rezafungin's long half-life allows for once-weekly dosing, potentially reducing the frequency of drug administration and minimizing the risk of dosing errors or missed doses.

Broad Spectrum: Its wide spectrum of activity against both *Candida* species and certain molds makes rezafungin suitable for the treatment of a variety of invasive fungal infections, streamlining therapy and potentially reducing the need for combination antifungal regimens.

Convenience and Compliance: The once-weekly dosing schedule may improve patient compliance and simplify the treatment course, particularly in the outpatient setting or for patients on long-term therapy.

Rezafungin's unique pharmacokinetic profile, broad spectrum of activity, and favorable safety and efficacy make it a promising addition to the armamentarium of antifungal agents. Continued research and clinical trials will further elucidate its potential benefits and optimal role in the management of invasive fungal infections, especially in patients with limited treatment options due to drug resistance or intolerance to existing antifungals.

Olorofim (formerly F901318)

Olorofim is a novel antifungal agent developed by F2G Ltd. It belongs to the class of orotomides, a new group of antifungal drugs with a unique mechanism of action. Olorofim has shown promising activity against a broad range of fungal pathogens, including drug-resistant strains, making it a potential therapeutic option for various invasive fungal infections. Here are some key details about olorofim:

Mechanism of Action: Olorofim exerts its antifungal activity through a distinct mechanism of action. It targets the enzyme dihydroorotate dehydrogenase (DHODH), which is involved in the de novo pyrimidine biosynthesis pathway of fungi. By inhibiting DHODH, olorofim disrupts the production of pyrimidine nucleotides, essential building blocks for DNA and RNA synthesis in fungal cells. This disruption leads to the accumulation of toxic metabolites and ultimately, fungal cell death. The unique mechanism of action of olorofim sets it apart from other classes of antifungal drugs, making it a valuable addition to the antifungal armamentarium.

Spectrum of Activity: Olorofim exhibits a broad spectrum of activity against various fungal pathogens, including yeasts and molds. It is active against *Candida* species, including some fluconazole-resistant strains and emerging multidrug-resistant *Candida auris*. Olorofim has also shown activity against molds, such as *Aspergillus* species and the emerging mold *Lomentospora prolificans*. This wide spectrum of activity makes olorofim a promising option for the treatment of diverse and challenging invasive fungal infections.

Pharmacokinetics: Olorofim is administered orally, allowing for convenient and non-invasive drug delivery. It has good oral bioavailability and is well-absorbed in the gastrointestinal tract. The drug has demonstrated favorable pharmacokinetics, with steady

plasma concentrations achieved at therapeutic doses. Olorofim's oral administration and predictable pharmacokinetic profile contribute to its ease of use and potential advantages in outpatient settings.

Safety and Efficacy: Clinical trials have shown the safety and efficacy of olorofim in the treatment of invasive fungal infections. It has been studied in both candidiasis and aspergillosis, showing promising results in terms of clinical response rates. Olorofim has been generally well-tolerated in patients, with adverse effects comparable to those of other antifungal agents. Importantly, olorofim has demonstrated a low potential for drug interactions, making it a safer option for patients on multiple medications.

Potential Advantages over Existing Antifungals: Olorofim offers several potential advantages over existing antifungal agents:

Novel Mechanism of Action: Its unique mechanism of action targeting DHODH provides an alternative approach to combat fungal infections, potentially reducing the risk of cross-resistance with other antifungal classes.

Broad Spectrum: Its wide spectrum of activity against both yeasts and molds make olorofim suitable for the treatment of various invasive fungal infections, offering new possibilities in managing drug-resistant strains.

Oral Administration: Olorofim's oral formulation allows for convenient dosing and drug delivery, which can be particularly beneficial for outpatient treatment and patient compliance.

Olorofim's unique mechanism of action, broad spectrum of activity, and favorable safety and efficacy profile make it a promising antifungal agent. Ongoing research and clinical trials will further define its optimal role in the management of invasive fungal infections, especially in patients with limited treatment options due to drug resistance or intolerance to existing antifungals.

Fosmanogepix (APX001)

Fosmanogepix is a novel antifungal agent developed by Amplyx Pharmaceuticals. It belongs to the class of fosmanogepix manogepix (MGX) antifungals, which have a unique mechanism of action. Fosmanogepix has shown promising activity against a wide range of fungal pathogens, including drug-resistant strains, making it a potential therapeutic option for various invasive fungal infections. Here are some key details about fosmanogepix:

Mechanism of Action: Fosmanogepix exerts its antifungal activity through a multifaceted mechanism of action. It is a prodrug that is converted into the active compound manogepix (MGX) after intracellular uptake by fungal cells. Manogepix inhibits the enzyme Gwt1, which is involved in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway in fungi. The GPI anchor is essential for anchoring certain cell wall proteins, and its disruption by manogepix leads to impaired cell wall integrity and function, ultimately resulting in fungal cell death. The unique mechanism of action of fosmanogepix sets it apart from other classes of antifungal drugs, offering a new approach to treating invasive fungal infections.

Spectrum of Activity: Fosmanogepix exhibits a broad spectrum of activity against various fungal pathogens, including yeasts and molds. It is active against *Candida* species, including some fluconazole-resistant strains and emerging multidrug-resistant *Candida auris*. Fosmanogepix has also shown activity against molds, such as *Aspergillus* species and certain rare and difficult-to-treat fungal pathogens. This wide spectrum of activity makes fosmanogepix a promising option for the treatment of diverse and challenging invasive fungal infections.

Pharmacokinetics: Fosmanogepix is available in both intravenous and oral formulations, allowing for flexible dosing options. It has good bioavailability and is well-absorbed in the gastrointestinal tract after oral administration. The drug has demonstrated favorable pharmacokinetics, with steady plasma concentrations achieved at therapeutic doses. Fosmanogepix's intravenous and oral formulations and predictable pharmacokinetic profile contribute to its ease of use in clinical practice.

Safety and Efficacy: Clinical trials have shown the safety and efficacy of fosmanogepix in the treatment of invasive fungal infections. It has been studied in both candidiasis and aspergillosis, showing promising results in terms of clinical response rates. Fosmanogepix has been generally well-tolerated in patients, with adverse effects comparable to those of other antifungal agents. Importantly, it has demonstrated a low potential for drug interactions, making it a safer option for patients on multiple medications.

Potential Advantages over Existing Antifungals: Fosmanogepix offers several potential advantages over existing antifungal agents:

Unique Mechanism of Action: Its distinctive mechanism of action targeting the GPI anchor biosynthesis pathway provides a novel approach to treat fungal infections, potentially reducing the risk of cross-resistance with other antifungal classes.

Broad Spectrum: Its wide spectrum of activity against both yeasts and molds makes fosmanogepix suitable for the treatment of various invasive fungal infections, offering new possibilities in managing drug-resistant strains and difficult-to-treat infections.

Intravenous and Oral Formulations: Fosmanogepix's availability in both intravenous and oral formulations provide flexibility in dosing and administration options, allowing for easy transition based on the patient's clinical status.

Fosmanogepix's unique mechanism of action, broad spectrum of activity, and favorable safety and efficacy profile make it a promising antifungal agent. Ongoing research and clinical trials will further define its optimal role in the management of invasive fungal infections, especially in patients with limited treatment options due to drug resistance or intolerance to existing antifungals.

New Therapeutic Approaches: Recent Innovations

Advances in antifungal therapy have been accompanied by the development of innovative therapeutic approaches that aim to enhance the effectiveness of antifungal treatment and

overcome existing challenges. These novel approaches offer new strategies to combat drug-resistant fungi, improve treatment outcomes, and reduce the burden of fungal infections.

Antifungal drug repurposing

It is a strategy in which existing drugs, originally developed for other therapeutic purposes, are explored for their potential antifungal properties. This approach aims to identify new therapeutic uses for these drugs, expanding their applications beyond their original intended indications.

Drug repurposing offers several advantages in antifungal research and drug development:

Expedited Drug Development: Repurposing drugs for antifungal purposes can significantly expedite the drug development process. Since these drugs have already undergone extensive preclinical and clinical testing for their original indications, much of the safety and pharmacokinetic data are already available. This can save time and resources, as researchers can directly focus on evaluating their antifungal properties.

Established Safety Profiles: One of the major benefits of drug repurposing is that the safety profiles of these drugs have been well-established in humans. This reduces the risks associated with new drug development, as the adverse effects and potential drug interactions are often already known. It may also expedite regulatory approval for their use in treating fungal infections.

Overcoming Drug Resistance: Fungal drug resistance is a significant challenge in antifungal therapy. By repurposing drugs with different mechanisms of action, there is a potential to overcome or circumvent existing drug resistance. Combining repurposed drugs with conventional antifungals may lead to synergistic effects, enhancing their overall effectiveness.

Diverse Mechanisms of Action: Many drugs developed for other purposes may have inherent antifungal properties due to their mechanisms of action. For example, some antibiotics, antimalarials, and immunomodulators can interfere with fungal growth, cell wall synthesis, or virulence factors. Exploring these diverse mechanisms of action can offer new targets for combating fungal infections.

Cost-Effectiveness: Repurposing drugs can be a cost-effective approach compared to developing entirely new drugs from scratch. It reduces the financial burden and risks associated with early-stage drug development, making it an attractive option for academic and industry researchers.

Examples of Repurposed Drugs

Several drugs that were originally developed for non-antifungal indications have shown promising antifungal properties through repurposing:

Fluoxetine: An antidepressant that has demonstrated antifungal activity against *Candida* species by affecting fungal membrane integrity and disrupting cell growth.

Ivermectin: An antiparasitic drug that has shown antifungal activity against various fungal pathogens by affecting fungal cell membranes and interfering with essential cellular processes.

Chloroquine: An antimalarial drug that exhibits antifungal activity by disrupting lysosomal functions and affecting the fungal cell membrane.

Statins: Cholesterol-lowering drugs that have demonstrated antifungal effects by inhibiting ergosterol synthesis, a crucial component of the fungal cell membrane.

Miltefosine: An anticancer drug that has been explored for its antifungal properties against *Candida* and other fungal species.

Feasibility: Antifungal drug repurposing is a promising strategy with the potential to expedite the development of new antifungal therapies. The feasibility of this approach lies in the following aspects:

Existing Drug Libraries: The availability of extensive drug libraries containing compounds that have been previously developed for various medical conditions provides a vast resource for screening potential antifungal candidates.

Known Safety Profiles: Repurposed drugs have a well-established safety profile, as they have already undergone clinical trials for their original indications. This can streamline the safety assessment process for antifungal use.

Multiple Mechanisms of Action: Many non-antifungal drugs may have inherent antifungal properties due to their mechanisms of action. Exploring these diverse mechanisms allows researchers to identify potential new targets for fungal infections.

Challenges: However, despite its potential, antifungal drug repurposing also comes with several challenges:

Limited Specificity: Repurposed drugs may not be as specific to fungal pathogens as conventional antifungals. This could result in off-target effects and potential toxicity to human cells.

Lack of Clinical Data: Although repurposed drugs have undergone clinical trials for their original indications, their efficacy and safety in treating fungal infections may not be well-documented. Further clinical trials are required to establish their antifungal efficacy.

Drug Interactions: Repurposed drugs may interact with other medications that the patient is taking, leading to potential adverse effects or reduced efficacy of either drug.

Resistance Development: The use of repurposed drugs in antifungal therapy may also lead to the development of drug resistance in fungal pathogens, similar to what is observed with conventional antifungals.

Benefits: Despite the challenges, antifungal drug repurposing offers several benefits:

Cost-Effectiveness: Drug repurposing is a cost-effective approach compared to developing new drugs from scratch, as it bypasses much of the early-stage drug development costs and timelines.

Rapid Development: The availability of preclinical and clinical data for repurposed drugs allows for faster translation into antifungal therapy, potentially addressing urgent medical needs.

Combination Therapy: Repurposed drugs can be used in combination with existing antifungals, offering synergistic effects and potentially reducing the development of drug resistance.

Overcoming Drug Resistance: Repurposed drugs with novel mechanisms of action can help overcome existing drug-resistant fungal strains, providing new treatment options for difficult-to-treat infections.

Wider Therapeutic Options: Antifungal drug repurposing can expand the range of therapeutic options available to clinicians, improving patient outcomes and reducing the burden of fungal infections.

In conclusion, antifungal drug repurposing holds significant promise as an innovative approach to address the challenges of antifungal therapy. While feasibility and benefits are evident, researchers must also carefully navigate the challenges and ensure thorough evaluation through preclinical and clinical studies before repurposed drugs can be fully integrated into antifungal treatment guidelines. The potential to enhance the antifungal armamentarium and provide effective treatments against drug-resistant fungi makes antifungal drug repurposing a valuable and worthwhile pursuit in the fight against fungal infections.

Combination Antifungal Therapy

Combination antifungal therapy involves the simultaneous or sequential use of two or more antifungal agents with different mechanisms of action to treat fungal infections. This approach aims to enhance the effectiveness of treatment, broaden the spectrum of activity, and reduce the development of drug resistance. Combination therapy has become an important strategy in the management of invasive fungal infections, especially in patients with severe or refractory infections, immunocompromised individuals, or those infected with drug-resistant fungal strains.

Here are some key aspects of combination antifungal therapy-

Synergistic Effects: The rationale behind combination therapy is that the combined action of different antifungal agents may produce synergistic effects, leading to improved fungal eradication and enhanced clinical outcomes. Synergy refers to the effect when the combined action of drugs is greater than the sum of their individual effects. Synergistic drug combinations can allow for lower doses of each drug, reducing the risk of adverse effects associated with high-dose monotherapy.

Broad Spectrum of Activity: By using antifungal agents with different mechanisms of action, combination therapy can target a broader range of fungal species. This is particularly important when dealing with polymicrobial infections or infections caused by multiple species, including those that are inherently resistant to certain antifungal classes.

Overcoming Drug Resistance: Combining antifungal agents with different modes of action can help overcome existing drug resistance. The simultaneous use of drugs that inhibit different fungal targets makes it more difficult for the fungus to develop resistance to both agents simultaneously, reducing the likelihood of treatment failure due to drug resistance.

Management of Severe Infections: In severe or life-threatening fungal infections, combination therapy may be employed to rapidly control the infection and improve patient outcomes. Combining antifungals with different pharmacokinetic profiles can help achieve higher drug concentrations at the site of infection, ensuring more effective fungal eradication.

Reducing Treatment Duration: In some cases, combination therapy may allow for shorter treatment durations compared to monotherapy. This can be particularly advantageous for patients at risk of prolonged exposure to antifungal drugs, reducing the potential for drug-related adverse effects or the development of drug resistance.

Clinical Considerations: While combination antifungal therapy offers many potential benefits, it is essential to consider several factors before implementation:

Selection of appropriate antifungals based on the specific fungal pathogen and its susceptibility pattern.

Monitoring for drug interactions, as certain antifungal combinations may affect the metabolism of other medications or lead to adverse effects.

Adjusting dosages and treatment durations based on patient factors such as renal or hepatic function.

Cost implications, as combination therapy may be more expensive than monotherapy, depending on the selected antifungal agents.

Examples of Combination Antifungal Therapy: Some examples of combination antifungal therapy include-

Amphotericin B and Flucytosine: Used for the treatment of cryptococcal meningitis, this combination has shown synergistic activity against *Cryptococcus neoformans* and *Cryptococcus gattii*.

Echinocandin plus Azole: Combining an echinocandin (e.g., caspofungin, micafungin) with an azole (e.g., fluconazole, voriconazole) has been explored in the treatment of invasive candidiasis and has shown improved outcomes in certain patient populations.

Echinocandin plus Amphotericin B: This combination may be employed for refractory aspergillosis or other invasive mold infections.

Feasibility: The feasibility of combination antifungal therapy lies in the availability of multiple antifungal agents with different mechanisms of action. Many classes of antifungals are currently in use, including azoles, echinocandins, polyenes, and others. This diversity of available antifungal drugs provides numerous options for designing combination regimens to target specific fungal pathogens and infections.

Challenges of Combination Antifungal Therapy:

Drug Interactions: When combining antifungal agents, there is a risk of drug interactions that could lead to reduced efficacy or increased toxicity. Some antifungal combinations may alter the metabolism of other drugs, requiring careful monitoring and dose adjustments.

Cost and Accessibility: Combination antifungal therapy may be more expensive than monotherapy due to the higher cost of using multiple drugs. In certain regions or healthcare settings, access to specific antifungal agents may be limited, affecting the feasibility of combination therapy.

Adverse Effects: The use of multiple antifungal agents can increase the risk of adverse effects, especially if both drugs have overlapping toxicities. Balancing the benefits and risks is essential to avoid compromising patient safety.

Complexity of Treatment: Combination antifungal therapy can be more complex to manage than monotherapy, especially in critically ill patients or those with multiple comorbidities. This may require closer monitoring and expertise in drug administration.

Benefits of Combination Antifungal Therapy:

Synergistic Effects: Combining antifungal agents with different mechanisms of action can lead to synergistic effects, where the overall effectiveness of the treatment is greater than that of individual drugs. This can enhance the antifungal activity and improve treatment outcomes.

Broad Spectrum of Activity: Combination therapy can target a wider range of fungal pathogens, including those with intrinsic or acquired resistance to individual antifungal drugs. This is particularly beneficial in the treatment of polymicrobial or mixed fungal infections.

Overcoming Drug Resistance: By using drugs with distinct modes of action, combination therapy reduces the risk of developing drug resistance, as it becomes more challenging for fungi to acquire resistance against multiple drugs simultaneously.

Improved Clinical Outcomes: Combination antifungal therapy may lead to more rapid fungal eradication, shortened treatment durations, and reduced rates of treatment failure, especially in severe or refractory fungal infections.

Tailored Treatment: Combination therapy allows clinicians to tailor treatment regimens based on the specific characteristics of the fungal infection, patient factors, and susceptibility patterns, optimizing treatment efficacy.

Reduced Dosing and Toxicity: In some cases, combining antifungal agents may allow for lower individual drug dosages, reducing the risk of drug-related toxicity while maintaining therapeutic efficacy.

Combination antifungal therapy is a valuable approach to address the challenges of invasive fungal infections. The feasibility of this approach is supported by the availability of diverse antifungal drugs. However, it also presents challenges, such as drug interactions, cost implications, and potential adverse effects. Despite these challenges, the benefits of combination therapy, including synergistic effects, broader spectrum of activity, and potential to overcome drug resistance, make it a valuable tool in the management of fungal infections, especially in complex or refractory cases. Careful consideration of patient-specific factors, clinical evidence, and drug interactions is essential to ensure safe and effective treatment outcomes.

Antifungal Biomolecules

Antifungal biomolecules are naturally occurring compounds with potent antifungal properties that have been identified in various living organisms, including plants, animals, and microorganisms. These biomolecules have evolved as part of the host's defense mechanisms to combat fungal infections. In recent years, there has been increasing interest in exploring these natural antimicrobial agents as potential therapeutic options to address the challenges posed by fungal infections, including drug resistance and limited treatment options.

Key Aspects of Antifungal Biomolecules

Diverse Sources: Antifungal biomolecules are derived from a wide range of sources, such as antimicrobial peptides, plant extracts, fungal secondary metabolites, and animal-derived peptides. Each source offers a unique set of bioactive compounds with specific mechanisms of action against fungal pathogens.

Mechanisms of Action: Antifungal biomolecules exert their antifungal activity through diverse mechanisms, including disrupting the fungal cell membrane, inhibiting cell wall synthesis, interfering with intracellular processes, and modulating the host immune response. These multifaceted mechanisms make them effective against a broad spectrum of fungal pathogens.

Synergy and Combinations: Some antifungal biomolecules may exhibit synergistic effects when used in combination with conventional antifungal drugs, leading to enhanced antifungal activity and improved treatment outcomes. Combining antifungal biomolecules with existing therapies offers a potential strategy to overcome drug resistance and enhance the overall efficacy of antifungal treatment.

Low Likelihood of Resistance: Antifungal biomolecules often target essential components of fungal cells, making the development of resistance less likely compared to conventional antifungal drugs that may target specific enzymatic pathways.

Some examples of antifungal biomolecules-

Polyenes: Polyene antifungal biomolecules are a class of natural compounds that disrupt fungal cell membranes by binding to ergosterol, a crucial component of fungal cell membranes. This binding leads to membrane permeability and cell death. Amphotericin B and Nystatin are examples of polyene antifungals used to treat severe fungal infections.

Azoles: Azoles are synthetic and naturally occurring antifungal agents that inhibit the synthesis of ergosterol, leading to impaired cell membrane function. Examples include fluconazole, itraconazole, and voriconazole, which are commonly used to treat various fungal infections.

Echinocandins: Echinocandins are a class of antifungal biomolecules that inhibit the synthesis of β -glucan, an essential component of the fungal cell wall. Caspofungin, micafungin, and anidulafungin are examples of echinocandins used to treat invasive fungal infections.

Terpenoids: Terpenoids are a diverse group of natural antifungal compounds found in plants and some microorganisms. They exhibit antifungal activity by disrupting fungal membranes or interfering with metabolic pathways. Examples include carvacrol from oregano oil and tea tree oil compounds (terpinen-4-ol).

Griseofulvin: Griseofulvin is an antifungal biomolecule produced by the fungus *Penicillium griseofulvum*. It interferes with the formation of microtubules during fungal cell division, preventing fungal growth. Griseofulvin is commonly used to treat fungal skin infections like ringworm.

Ciclopirox: Ciclopirox is an antifungal agent with broad-spectrum activity against various fungi. It works by inhibiting fungal enzymes involved in nucleic acid and protein synthesis. Ciclopirox is used in topical formulations for nail and skin fungal infections.

Plant Defensins: Plant defensins are small cysteine-rich peptides found in various plants. They possess antifungal activity by disrupting fungal membranes and interfering with fungal cell wall synthesis. Some plant defensins have been explored for their potential as antifungal therapeutics.

Streptovaricins: Streptovaricins are antifungal antibiotics produced by certain *Streptomyces* bacteria. They inhibit fungal protein synthesis and are effective against a range of fungi.

Feasibility: The feasibility of using antifungal biomolecules as therapeutic agents lies in the abundance and diversity of these natural compounds in various organisms. Many antimicrobial peptides and secondary metabolites with antifungal properties have been isolated and characterized from plants, animals, and microorganisms. The availability of advanced bioinformatics and genetic engineering techniques allows researchers to study the structure and function of these biomolecules, leading to the development of synthetic analogs with improved efficacy and stability.

Challenges: Stability and Formulation: Some antifungal biomolecules may be sensitive to environmental conditions or prone to degradation, making formulation and storage

challenging. Overcoming these limitations while preserving their activity is crucial for their clinical application.

Toxicity and Immunogenicity: Some antifungal biomolecules may exhibit cytotoxicity or immunogenicity, which could limit their clinical use. Ensuring safety profiles and minimizing potential adverse effects is essential during the development process.

Bioavailability and Pharmacokinetics: The bioavailability and pharmacokinetic properties of antifungal biomolecules need to be carefully studied to determine the appropriate dosage regimens and administration routes for optimal therapeutic efficacy.

Benefits

Novel Therapeutic Options: Antifungal biomolecules offer novel therapeutic options for treating fungal infections, especially in cases of drug-resistant strains where conventional antifungals may be less effective.

Reduced Drug Resistance: The diverse mechanisms of action of antifungal biomolecules reduce the risk of developing drug resistance in fungal pathogens.

Enhanced Antifungal Activity: Some antifungal biomolecules may exhibit higher potency than conventional antifungal drugs, allowing for lower doses and potentially reducing adverse effects.

Potential for Combination Therapy: Antifungal biomolecules can be used in combination with existing antifungal drugs to achieve synergistic effects and improve treatment outcomes.

Antifungal biomolecules represent a promising avenue for the development of novel antifungal therapies. The diverse sources, mechanisms of action, and potential for synergy with conventional antifungal drugs offer new strategies to combat fungal infections. While challenges such as stability, toxicity, and pharmacokinetics need to be addressed, the benefits of using antifungal biomolecules, including reduced drug resistance and enhanced antifungal activity, make them valuable candidates in the ongoing efforts to overcome the global burden of fungal infections. Continued research and clinical studies are essential to fully explore the potential of these natural antimicrobial agents and their integration into antifungal therapy.

Anti-Infective Agents

Anti-infective agents are substances or medications used to prevent, treat, or eliminate infectious diseases caused by various pathogens, such as bacteria, viruses, fungi, and parasites. These agents play a crucial role in modern medicine, helping to control infections and reduce their impact on human health. They can be administered through various routes, including oral, topical, intravenous, and inhalation, depending on the type and severity of the infection.

General Aspects of Anti-Infective Agents-

Types of Anti-Infective Agents: Anti-infective agents encompass a wide range of drugs, including antibiotics (bacterial infections), antivirals (viral infections), antifungals (fungal infections), and antiparasitic drugs (parasitic infections).

Mechanisms of Action: Different anti-infective agents target specific components or functions of pathogens. For example, antibiotics may inhibit bacterial cell wall synthesis or disrupt protein synthesis, while antivirals might interfere with viral replication processes.

Drug Resistance: One of the significant challenges in anti-infective therapy is the development of drug-resistant strains of pathogens. Misuse and overuse of these agents can lead to the emergence of resistant strains, reducing the effectiveness of existing treatments.

Combination Therapy: In some cases, combining two or more anti-infective agents can improve treatment outcomes, prevent resistance, and broaden the spectrum of action against various pathogens.

Feasibility

The development and implementation of anti-infective agents are feasible due to several factors:

Scientific Research: Continuous advancements in biomedical research and technology contribute to the identification of new drug targets and the development of novel compounds.

Drug Development Pipeline: Pharmaceutical companies and research institutions have active pipelines for discovering and testing potential anti-infective agents.

Regulatory Framework: Health authorities worldwide have established regulatory processes to ensure the safety and efficacy of new anti-infective agents before they reach the market.

Challenges

Drug Resistance: As mentioned earlier, the emergence of drug-resistant pathogens poses a significant challenge, making some previously effective treatments less viable.

Toxicity and Side Effects: Anti-infective agents can have adverse effects on the human body, and balancing their effectiveness with tolerable side effects can be challenging.

Broad-Spectrum vs. Targeted Therapy: Deciding whether to use broad-spectrum agents that act against various pathogens or targeted agents that focus on specific organisms requires careful consideration based on the infection's nature and severity.

Access and Affordability: Ensuring widespread access to anti-infective agents, especially in developing countries, can be an obstacle due to cost and distribution issues.

Benefits

Controlling Infectious Diseases: Anti-infective agents have been instrumental in controlling and eradicating various infectious diseases, thus saving millions of lives worldwide.

Improving Healthcare Outcomes: Effective treatment of infections leads to better patient outcomes, shorter hospital stays, and reduced healthcare costs.

Prevention of Outbreaks: In certain situations, anti-infective agents can be used prophylactically to prevent the spread of infectious diseases in high-risk populations.

Advancing Medical Progress: The study and development of anti-infective agents contribute to our understanding of pathogens and host-pathogen interactions, driving further advancements in medical science.

In conclusion, anti-infective agents play a vital role in modern medicine by preventing, treating, and controlling infectious diseases. While their development and implementation are feasible, challenges such as drug resistance and side effects persist. Nonetheless, the benefits of these agents in improving healthcare outcomes and advancing medical progress are significant.

Biologics and Vaccines

Biologics and vaccines have emerged as promising therapeutic approaches in the field of antifungal therapy. Biologics encompass a wide range of biological agents, including monoclonal antibodies, peptides, and proteins, that target specific fungal pathogens or modulate the host immune response to combat fungal infections. On the other hand, vaccines aim to prevent fungal infections by stimulating the immune system to produce a protective response against specific fungal pathogens. Both biologics and vaccines offer novel strategies to enhance the management and prevention of fungal infections, especially in the context of drug resistance and limited treatment options.

Key Aspects

Targeted Therapies: Biologics are designed to target specific components of fungal pathogens or host immune responses. Monoclonal antibodies, for example, can bind to specific fungal antigens, blocking their virulence and aiding in the clearance of the infection. Vaccines, on the other hand, induce the production of specific antibodies or T-cell responses to prevent infection or reduce the severity of disease upon exposure.

Host Immune Modulation: Some biologics can modulate the host immune response to enhance antifungal defenses. By boosting the immune system's ability to recognize and eliminate fungal pathogens, these agents can complement traditional antifungal drugs and reduce the risk of recurrent infections.

Preventive Approach: Vaccines offer a preventive approach to combat fungal infections. By priming the immune system to recognize and respond to specific fungal pathogens, vaccines can reduce the incidence and severity of infections, especially in vulnerable populations.

Feasibility

The feasibility of using biologics and vaccines in antifungal therapy is supported by advances in biotechnology and immunology. Techniques in genetic engineering and monoclonal antibody production have paved the way for the development of targeted biologic therapies.

Additionally, the success of vaccines in preventing viral and bacterial infections has inspired the exploration of vaccine strategies against fungal pathogens.

Challenges

High Costs: The production and development of biologics and vaccines can be costly, potentially limiting their accessibility, especially in resource-limited settings.

Specificity and Cross-Reactivity: Biologics must be carefully designed to target specific fungal antigens without causing cross-reactivity or adverse effects on host tissues.

Vaccine Design Complexity: Developing effective vaccines against fungal pathogens can be complex due to the diverse nature of fungal antigens and the challenges in generating long-lasting protective immune responses.

Risk of Immune Evasion: Fungal pathogens have evolved various strategies to evade the host immune response. Developing biologics or vaccines that can effectively overcome these evasion mechanisms is a significant challenge.

Benefits

Targeted Therapy: Biologics offer targeted therapy, minimizing off-target effects and reducing the risk of adverse reactions in comparison to conventional systemic antifungal agents.

Complementary Approach: Biologics can be used in combination with traditional antifungal drugs, providing a complementary approach to enhance the overall antifungal response.

Prevention and Long-Term Protection: Vaccines offer a proactive approach to preventing fungal infections and can provide long-term protection, reducing the need for repeated antifungal treatments.

Reduced Drug Resistance: Biologics and vaccines can help reduce the development of drug resistance by supporting the host's immune response in combating fungal pathogens.

Biologics and vaccines have shown great promise as innovative approaches in antifungal therapy. Targeted therapies, host immune modulation, and preventive strategies offered by these approaches can address challenges associated with drug resistance and limited treatment options. However, challenges related to costs, specificity, and vaccine design complexity need to be overcome. The potential benefits of biologics and vaccines, such as targeted therapy, complementary approaches, and prevention of infections, make them valuable candidates in advancing the fight against fungal infections. Continued research and clinical trials are essential to fully realize the potential of biologics and vaccines in improving the management and prevention of fungal diseases.

Nanotechnology-based Therapies

Nanotechnology-based therapies represent a cutting-edge approach in the field of antifungal treatment. These therapies involve the application of nanoscale materials and structures to

target fungal pathogens and enhance the delivery and efficacy of antifungal agents. Nanotechnology offers unique advantages, such as increased drug stability, improved bioavailability, targeted drug delivery, and the potential to overcome drug resistance. In recent years, nanotechnology has shown great promise in revolutionizing antifungal therapy, addressing challenges associated with conventional treatments, and opening new avenues for combating fungal infections.

Key Aspects

Nanomaterials and Nanoparticles: Nanotechnology utilizes various nanomaterials and nanoparticles, such as liposomes, micelles, dendrimers, and nanocrystals, to encapsulate and deliver antifungal drugs. These nanocarriers protect the drugs from degradation, improve their solubility, and enable targeted delivery to the site of infection.

Targeted Drug Delivery: Nanotechnology enables targeted drug delivery by functionalizing nanoparticles with specific ligands or antibodies that recognize fungal cell surface markers. This selective delivery enhances drug accumulation at the infection site, reducing systemic toxicity and improving treatment outcomes.

Enhanced Drug Efficacy: The small size and unique properties of nanoparticles can facilitate better drug penetration into fungal cells and biofilms, increasing drug efficacy against resistant or hard-to-reach fungal populations.

Combination Therapy: Nanotechnology allows for the combination of multiple antifungal agents within a single nanocarrier, enabling synergistic effects and the simultaneous targeting of different fungal components.

Feasibility

The feasibility of nanotechnology-based therapies is supported by advancements in nanomaterial synthesis, surface engineering, and drug encapsulation techniques. Many nanotechnology-based formulations have already demonstrated promising results in preclinical studies and some have progressed to clinical trials. Moreover, nanomaterials used in these therapies are biocompatible and biodegradable, enhancing their safety profiles.

Challenges

Regulatory Approval: The regulatory approval process for nanotechnology-based therapies can be complex, as safety assessments and standardized guidelines specific to nanomedicine need to be established.

Costs and Scalability: The manufacturing of nanotechnology-based formulations may involve additional costs and complexities, potentially affecting their scalability and commercial viability.

Long-Term Safety: Long-term safety of nanoparticles in the human body requires thorough investigation, especially regarding potential accumulation and unintended side effects.

Nanotoxicity: Some nanoparticles may exhibit nanotoxicity, leading to cellular damage or inflammation. Ensuring the biocompatibility and safety of nanomaterials is essential.

Benefits

Enhanced Drug Delivery: Nanoparticles enable enhanced drug delivery, allowing for higher drug concentrations at the infection site, improving therapeutic efficacy.

Reduced Drug Resistance: Nanotechnology-based therapies may help overcome drug resistance by delivering drugs directly to resistant fungal cells or using synergistic drug combinations.

Site-Specific Targeting: Targeted drug delivery reduces off-target effects, minimizing damage to healthy tissues and reducing systemic toxicity.

Improvement of Conventional Drugs: Nanotechnology can improve the properties of conventional antifungal drugs, such as solubility and stability, without the need for significant chemical modifications.

Nanotechnology-based therapies offer exciting opportunities to revolutionize antifungal treatment. The use of nanomaterials and nanoparticles for targeted drug delivery, enhanced drug efficacy, and combination therapy holds great potential in addressing challenges associated with conventional antifungal treatments. While feasibility is supported by advances in nanotechnology, regulatory approval, safety, and cost considerations need to be carefully addressed. The benefits of nanotechnology-based therapies, such as improved drug delivery, reduced drug resistance, and site-specific targeting, make them promising candidates to enhance the management and treatment of fungal infections in the future. Continued research and development in this field are vital to fully harness the potential of nanotechnology for antifungal therapy and improve patient outcomes.

Implications and Future Directions

Importance of Ongoing Research and Development of New Antifungal Agents:

The ongoing research and development of new antifungal agents are crucial to address the growing global burden of fungal infections. As antifungal resistance continues to emerge, and the limitations of current therapies become apparent, the discovery and optimization of novel antifungal compounds become imperative. Investing in research to identify new drug targets, innovative drug classes, and combination therapies will help expand the antifungal armamentarium and ensure effective treatment options for patients, especially those with compromised immune systems or difficult-to-treat infections.

Advancing Studies on Combination Therapy for Optimal Treatment Outcomes:

Combination antifungal therapy has shown promise in enhancing treatment outcomes and reducing drug resistance. Further research into the synergistic effects of antifungal drug combinations, optimal dosing regimens, and the use of combination therapy in specific patient populations will be essential. Investigating the safety, efficacy, and long-term

outcomes of combination regimens will guide clinicians in effectively managing severe or refractory fungal infections.

Exploring Immunotherapeutic Approaches to Enhance Host Immunity Against Fungal Infections:

Immunotherapeutic approaches, such as the use of biologics and vaccines, offer innovative strategies to enhance host immunity against fungal pathogens. Continued research into the development of effective vaccines, the identification of potent antifungal biomolecules, and the modulation of host immune responses will contribute to preventing fungal infections, reducing disease severity, and supporting the host's ability to clear fungal pathogens.

Research on Nanotechnology-based Delivery Systems for Targeted Drug Delivery:

Nanotechnology-based therapies have shown great potential in targeted drug delivery and improving antifungal treatment efficacy. Advancing research on nanomaterials, their biocompatibility, and the development of efficient drug delivery systems will lead to optimized antifungal therapies. Efforts to address regulatory challenges, scale-up production, and assess long-term safety will be essential to translate these promising approaches into clinical practice.

Monitoring and Understanding Antifungal Resistance Mechanisms:

With the emergence of antifungal resistance, continuous monitoring and understanding of the underlying resistance mechanisms are crucial. Surveillance of resistance patterns, analysis of genetic mutations in resistant strains, and investigation of factors contributing to resistance will help guide the development of new antifungal agents and treatment strategies to effectively manage resistant fungal infections.

Importance of Rapid and Accurate Diagnostics for Early Detection and Targeted Treatment:

Rapid and accurate diagnostics play a pivotal role in identifying fungal infections and determining their susceptibility to specific antifungal agents. Advancements in diagnostic technologies, such as molecular diagnostics, point-of-care testing, and biomarker-based assays, will aid in early detection, prompt initiation of appropriate therapy, and better patient outcomes. Moreover, research efforts should focus on developing affordable and accessible diagnostic tools, particularly for resource-limited settings.

Conclusion

The growing global burden of fungal infections, particularly among immunocompromised individuals, highlights the urgent need to develop new antifungal agents and therapeutic approaches. The limitations of current antifungal therapies, including drug resistance, toxicity, and lack of effective diagnostics, underscore the importance of continuous research and innovation. New antifungal agents with diverse mechanisms of action can overcome drug resistance, broaden the spectrum of activity, and improve treatment options for difficult-to-treat infections. Combination therapy, immunotherapeutic approaches, and nanotechnology-

based delivery systems offer promising strategies to enhance treatment outcomes and combat fungal infections effectively.

Addressing the crisis in antifungal therapy requires collaborative efforts from researchers, healthcare providers, policymakers, and pharmaceutical industries. Collaborative research and development initiatives can accelerate the discovery and optimization of new antifungal agents, combination therapies, and immunotherapeutic approaches. Sharing data, knowledge, and experiences related to antifungal resistance and treatment outcomes can facilitate evidence-based practices and inform clinical decision-making. By working together, we can address the challenges of drug resistance, improve patient access to innovative therapies, and strengthen the global response to fungal infections.

Developing new antifungal agents and therapeutic approaches has significant implications for improving patient outcomes and global health. Timely diagnosis and targeted treatment through rapid and accurate diagnostics can prevent disease progression and reduce the impact of fungal infections on patients' well-being. Enhanced host immunity through immunotherapeutic approaches can provide added protection, especially for vulnerable populations, and potentially prevent infections altogether. Additionally, advancements in nanotechnology-based drug delivery systems can optimize treatment efficacy, minimize adverse effects, and expand treatment options. These developments, coupled with monitoring and understanding antifungal resistance mechanisms, are crucial for preserving the effectiveness of antifungal drugs and ensuring successful disease management.

In conclusion, the significance of developing new antifungal agents and therapeutic approaches cannot be overstated. Collaborative efforts in research, clinical practice, and public health initiatives are essential to overcome the crisis in antifungal therapy. By embracing innovative approaches, fostering research, and prioritizing patient care, we can improve outcomes for individuals affected by fungal infections and advance global health in the face of this growing medical challenge.

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CHAPTER

17

FUNGI AND FUNGICIDES

Dr. Kirankumar Khandare*

A**bstract**

The fungi are achlorophyllous and heterotrophic thallophytes. Their plant body consists mostly of a thallus, made up of hyphae, which together constitute mycelium. A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes, saprobes). Some fungi infect a living host but kill host cells to obtain their nutrients; these are called necrotrophy.

Keywords: *Fungi, Fungicides, Mycoflora.*

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Introduction

The fungi are achlorophyllous and heterotrophic thallophytes. Their plant body consists mostly of a thallus, made up of hyphae, which together constitute mycelium. A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes, saprobes). Some fungi infect a living host but kill host cells to obtain their nutrients; these are called necrotrophy.

As fungi are eukaryote organisms, they absorb nutrients directly through their cell walls and digest food externally most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are achlorophilic, heterotrophs and, like animals, obtain their food and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes, saprobes). Some fungi infect a living host but kill host cells to obtain their nutrients; these are called necrotrophs.

Fungi, considered organisms like plants, are more closely related to animals than plants. Some fungal species multiply only asexually, whereas others undergo both asexual and sexual reproduction with alternation of generations. Most fungi produce many spores, which are haploid cells that can undergo mitosis to form multicellular, haploid individuals. Fungi play an essential role in ecosystems because they are decomposers and participate in the cycling of nutrients by breaking down organic materials into simple molecules.

Fungal cell

Fungal cells are eukaryotic cells, all cell organelles are present in the fungal cell as a normal eukaryotic cell. Fungal nuclei are usually small ($< 2 \mu\text{m}$ diameter) and can compress and stretch to move through septal pores and into developing spores. Many fungi (Ascomycota) have a life cycle that is predominantly haploid, while others (Basidiomycota) have a long dikaryotic phase. Fungi have been found to possess between 6 and 21 chromosomes coding for 6,000 to nearly 18,000 genes. Genome sizes range from 8.5 megabase pairs (Mb) to just over 400 Mb in filamentous fungi (Zolan 1995; Spanu *et al.* 2010; Duplessis *et al.* 2011), making fungal genomes among the smallest of eukaryotic organisms on average approximately 1% the size of mammalian genomes and only 1.3 times the size of the largest known bacterial genome (Stover *et al.* 2000).

Fungi are involved in a wide range of activities some fungi are decomposers, parasites, or pathogens of other organisms, and others are beneficial partners in symbiosis with animals, plants, or algae.

Fungi associated with animals

Fungi have the ability to grow on and in both invertebrate and vertebrate animals. Many fungi can attack insects and nematodes, for example, and may play an important role in keeping populations of these animals under control. Insect-attacking fungi, called entomopathogens, include a wide range of fungi in phyla Ascomycota, Zygomycota, and Chytridiomycota. Some of the best-known and most spectacular entomopathogens belong to the Ascomycota genus *Ophiocordyceps* and related genera. These fungi infect and consume insects such as caterpillars and ants, and then form conspicuous stromata that emerge from their victim's body in a most dramatic manner. These fungi can also alter the insect's behavior. Zombie-ant fungi from Brazil infect insect brains, directing the victim to climb up plants and bite into the plant tissue in a death grip (Evans *et al.* 2011; Hughes *et al.* 2011). Paradoxically, humans have been using one of these entomopathogens, *Ophiocordyceps sinensis*, for thousands of years to treat a wide range of ailments. This fungus is an important component of traditional Asian medicine and is commonly called winter worm, summer grass.

Fungi and plants

The association of fungi and plants has a historical background, it is ancient and involves many different fungi. Fungi are an important group of plant pathogens most plant diseases are caused by fungi but fewer than 10% of all known fungi can colonize living plants (Knogge, 1996). Plant pathogenic fungi represent a relatively small subset of those fungi that are associated with plants. Most fungi are decomposers, utilizing the remains of plants and other organisms as their food source. Other types of associations that will be discussed here include the role of fungi as decomposers, beneficial symbionts, and cryptic plant colonizers called endophytes.

Most fungi are associated with plants as saprotrophs and decomposers. These fungi break down organic matter of all kinds, including wood and other types of plant material. Wood is composed primarily of cellulose, hemicellulose, and lignin. Lignin is a complex polymer that is highly resistant to degradation, and it encrusts the more readily degradable cellulose and hemicellulose. Fungi are among the few organisms that can effectively break down wood, and fall into two main types brown and white rot fungi. Brown rot fungi selectively degrade the cellulose and hemicellulose in wood, leaving behind the more recalcitrant lignin. The decayed wood is brown in color and tends to form cubical cracks due to the brittle nature of the remaining lignin. Only ~10% of the wood decay fungi cause brown rot, and most of these fungi (80%) occur on conifer wood. Brown rot residues make up humus in temperate forest soils and are important for mycorrhizal formation, moisture retention, and sequestering carbon. Brown rot residues are highly resistant to decomposition and can remain in the soil for up to 300 years. White rot fungi are more common than brown rot fungi; these fungi degrade cellulose, hemicellulose, and lignin at approximately equal rates. The decayed wood is pale in colour, light in weight, and has a stringy texture. White rot fungi are the only organisms that can completely degrade lignin. Lignin is one of the most abundant organic polymers, accounting for 30% of the organic carbon on the planet only cellulose is more abundant (Boerjan *et al.* 2003).

A fungicide is nothing but any poisonous agent used to kill or inhibit the growth of the fungus. Fungicides are used to manage pathogenic fungi that cause economic harm to crops and endangering the health of domestic animals and even humans. Mostly fungicides used in agriculture and horticulture are administered as sprays or dust. Before germination, seed fungicides are administered as a protective layer. Systemic fungicides, also known as chemotherapeutics, are sprayed on plants, where they are disseminated throughout the tissue and work to eliminate or prevent disease.

Fungicides are substances that are used to eliminate or prevent the growth of fungi or their spores. Fungi include but are not limited to mold, mildew, blights, and rust. Fungi cannot produce their own carbohydrate food as they lack chlorophyll and feed on living plants or dead organic matter. Fungi are a big concern in agriculture as most crop diseases are caused by fungi. The effect of fungi and fungal plant diseases has been recorded even in early history but their cause was not identified. It was only in 1861 that Anton de Bary, the father of plant pathology, identified a fungus that was causing rot in potatoes, and in 1885 a fungicide was developed to inhibit its growth. The major boost to fungicide development was in the 20th century. Many new types of fungicides were developed and widespread use was adopted, providing protection from many plant diseases for the first time in history. As an alternative to conventional fungicides, bio fungicides, which are formulations of living microorganisms, are also being adopted as a method to control the growth of fungi.

Fungi can be difficult to control. They produce a massive quantity of spores which can spread via rain or wind over long distances to settle in favourable conditions such as surfaces of leaves, stems, or fruit. Fungi can grow through and between plant cells, withdraw nutrients, and even in some cases produce toxins that kill the plants. Fungi can also thrive in crop debris and infect the next cycle of crops.

Fungicides for plants can prevent the fungi spores from growing and eliminate the fungi that might already exist to protect the health of the crops. Modern fungicides have been developed to specifically target fungi and do a lot more. Synthetic chemical fungicides can provide protection without being absorbed or translocated by the plant. Fungicides also make it possible to provide protection against a wide variety of fungi. Some systemic fungicides have also been developed that can be absorbed by the plant and have a therapeutic effect by curing plant diseases. Apart from synthetic fungicides, bio fungicides can also be used to control certain types of fungi and are especially useful for organic production. Fungicides are a necessary part of today's agricultural sector as without them production would be severely impacted as there would be no protection from diseases.

Fungicides are agents that are used to prevent or eradicate fungal infections from plants or plant parts. Most of the fungicides have low to moderate toxicity. However, several fungicides, such as alkyl dithiocarbamic acid (manganese, zinc, and ammonium salts), halogenated substituted monocyclic aromatics (dinocap), carbamic acid derivatives, ferbam, mancozeb and maneb metabolites, HCB, benzimidazoles (benomyl and carbendazim), bis(tri-*n*-butyltin) oxide, chloro-alkyl-thio-dicarboximides (captafol I and folpet), and tridemorph, are known to cause developmental toxicity and oncogenesis. More than 80% of all oncogenic risk from the use of pesticides derives from a few fungicides; only

a small number of pesticide-related deaths from fungicides have been reported. Some fungicides are known to disrupt the endocrine system and may lead to reproductive and developmental abnormalities. Based on the teratogenicity, several fungicides including cycloheximide have been deregistered or banned in many countries but are still used in other, less regulated areas of the world.

Fungicides are chemical-based pesticides used to inhibit parasitic fungi or their spores or mycelium. Fungicides are most commonly chemical compounds but may include biocontrol's and fungistatic. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections in animals and even humans. Fungicides are also used to control oomycetes, which are not taxonomically/genetically fungi, despite their large differences in method of plant invasion. Fungicides can either be contact, translaminar or systemic. Contact fungicides are not taken up into the plant tissue and protect only the plant where the spray is deposited. Translaminar fungicides redistribute the fungicide from the upper, sprayed leaf surface to the lower, unsprayed surface. Systemic fungicides are taken up and redistributed through the xylem vessels. Few fungicides move to all parts of a plant. Some are locally systemic, and some move upward.

Most fungicides that can be bought retail are sold in liquid form. A very common active ingredient is sulfur, present at 0.08% in weaker concentrates, and as high as 0.5% for more potent fungicides. Fungicides in powdered form are usually around 90% sulfur and are very toxic. Other active ingredients in fungicides include neem oil, rosemary oil, jojoba oil, the bacterium *Bacillus subtilis*, and the beneficial fungus *Ulocladium oudemansii*.

Fungicide residues have been found on food for human consumption, mostly from post-harvest treatments. Some fungicides are dangerous to human health, such as vinclozolin, which has now been removed from use. Ziram is also a fungicide that is toxic to humans with long-term exposure, and fatal if ingested. A number of fungicides are also used in human health care.

Fungal diseases on crops have been increasing in severity and scale since the mid-20th Century and now pose a serious threat to global food security (overview in Fisher *et al.*, 2012, Savary *et al.*, 2019). Today, we lose an estimated 10–23% of our crop's pre-harvest, despite disease interventions, and a further 10–20% post-harvest (Fisher *et al.*, 2012). Fungi destroy both our essential calorie crops, such as rice, wheat, maize and soybean and decimate our commodity crops, such as bananas, coffee and barley. Indeed, the economic stability of several nations has become reliant upon export revenues, generated by the global trading of these commodity or cash crops, to buy and import food grown elsewhere in the world (Fones *et al.*, 2020).

Modern agricultural intensification has heightened the challenge of fungal disease. Here, the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and partially protected from disease by the use of single target site antifungals, has hastened emergence of new virulent and fungicide-resistant strains (Fisher *et al.*, 2018). Such monoculture cropping practices have, quite literally, become ideal feeding

and breeding grounds for the fast emergence of new fungal variants. Indeed, their rapid life-cycles generate prolific numbers of spores and their plastic genomes, prone to mutations as well as gene acquisitions or hybridisations, generate considerable strain diversity. These pathogens are realising their true evolutionary potential, thanks to the hand of man. The race has become skewed – it is no longer between the plant and the pathogen but between the pathogen and man (Fones *et al.*, 2020).

Diseases are a major source of crop and plant damage that can be caused by a number of plant pathogenic (disease-causing) organisms. Fungi are the number one cause of crop loss worldwide. Viruses, nematodes, and bacteria also cause diseases in plants. Symptoms resembling those caused by pathogens can be caused by abiotic (non-living) factors, such as nutrient deficiency and air pollution and also insects.

Fungicides, herbicides and insecticides are all pesticides used in plant protection. A fungicide is a specific type of pesticide that controls fungal disease by specifically inhibiting or killing the fungus causing the disease. Not all diseases caused by fungi can be adequately controlled by fungicides. These include the vascular diseases Fusarium and Verticillium wilt. Diseases caused by other types of organisms, disorders caused by abiotic factors, and insect damage are not controlled by fungicides. Thus, it is essential to first determine the cause of symptoms before applying a fungicide.

Why are Fungicides Needed?

Diseases are a common occurrence on plants, often having a significant economic impact on yield and quality, thus managing diseases is an essential component of production for most crops. Broadly, there are three main reasons fungicides are used: (a) To control a disease during the establishment and development of a crop. (b) To increase productivity of a crop and to reduce blemishes. Diseased food crops may produce less because their leaves, which are needed for photosynthesis, are affected by the disease. Blemishes can affect the edible part of the crop or, in the case of ornamentals, their attractiveness, which both can affect the market value of the crop. (c) To improve the storage life and quality of harvested plants and produce. Some of the greatest disease losses occur post-harvest. Fungi often spoil (render unusable) stored fruits, vegetables, tubers, and seeds. A few which infect grains produce toxins (mycotoxins) capable of causing severe illness or even death in humans and animals when consumed. Fungicides have been used to reduce mycotoxin contamination in wheat affected by Fusarium head blight, but most fungicides developed so far have not been sufficiently effective to be useful for managing mycotoxins associated with other diseases.

Application Methods

Fungicides are applied as dust, granules, gas, and, most commonly, liquid. They are applied to:

(a) Seed, bulbs, roots of transplants, and other propagative organs. These treatments are usually done by the seed company. Some treatments need to be done by the grower on-site at the time of planting. The goal is to kill pathogens that are on the planting material or to protect the young plant from pathogens in the soil.

- (b) Soil either in-furrow at planting, after planting as a soil drench (including through drip irrigation), or as a directed spray around the base of the plant.
- (c) Foliage and other aboveground parts of plants by means of a sprayer.
- (d) Inside of trees via trunk injection. (f) Harvested produce, as a dip or spray in the packinghouse.

Fungicides are used as a formulated product consisting of an active ingredient plus inert ingredients that improve the performance of the product. Fungicides are typically mixed with water then applied by spraying. Application equipment ranges from small hand-held and back-pack sprayers to large spray units carried by tractors or aircraft. A few fungicides are applied as dusts. Fungicides can also be applied in greenhouses as smoke, mist, fog or aerosol. Coverage of all parts of the plant susceptible to the disease is critical because very few fungicides can move adequately throughout a plant. Advancements are continually being made to nozzles and sprayers to improve coverage.

For many diseases, effective control necessitates multiple applications of fungicides, sometimes as frequently as every 5 days. Repeated applications are needed to protect new growth and to replace fungicide lost from the plant by chemical decomposition, UV-light degradation, and erosion by wind and water.

Fungicide Resistance

Fungicide resistance is a stable, heritable trait that results in a reduction in sensitivity to a fungicide by an individual fungus. This ability is obtained through evolutionary processes. Fungicides with single-site mode of action are at relatively high risk for resistance development compared to those with multi-site mode of action. Most fungicides being developed today have a single-site mode of action because this is associated with lower potential for negative impact on the environment, including non-target organisms.

When fungicide resistance results from modification of a single major gene, pathogen subpopulations are either sensitive or highly resistant to the pesticide. Resistance in this case is seen as complete loss of disease control that cannot be regained by using higher rates or more frequent fungicide applications. This type of resistance is commonly referred to as qualitative resistance.

Variation in sensitivity within the population is continuous, when fungicide resistance results from modification of several interacting genes, pathogen isolates exhibit a range in sensitivity to the fungicide depending on the number of gene changes. Resistance in this case is seen as an erosion of disease control that can be regained by using higher rates or more frequent applications. Long-term selection for resistance in the pathogen by repeated applications may eventually result in the highest labelled rates or/ and shortest application intervals not being able to adequately control the disease. This kind of fungicide resistance is commonly known as quantitative resistance.

Fungal isolates that are resistant to one fungicide are often also resistant to other closely-related fungicides, even when they have not been exposed to these other fungicides, because

these fungicides all have similar mode of action. This is known as cross resistance. Fungicides with the same Group Code are likely to exhibit cross resistance. Sometimes negative cross resistance occurs between unrelated fungicides because the genetic change that confers resistance to one fungicide makes the resistant isolate more sensitive to another fungicide.

Managing fungicide resistance is critically important to extend the period of time that an at-risk fungicide is effective. The primary goal of resistance management is to delay its development rather than to manage resistant fungal strains after they have been selected. Therefore, resistance management programs need to be implemented when at-risk fungicides first become available for commercial use. The objective of resistance management is to minimize use of the at-risk fungicide without sacrificing disease control. This is accomplished by using the at-risk fungicide with other fungicides and with non-chemical control measures, such as disease resistant cultivars, in an integrated disease management program.

To delay the build-up of resistant strains, it is critical to use an effective disease management program. At-risk fungicides should be used at the manufacturer's recommended rate (full rate) and application interval. Using full rates is expected to minimize selection of strains with intermediate fungicide sensitivity when resistance involves several genes (quantitative resistance). At-risk fungicides should be used in alternation with other at-risk fungicides with different modes of action or different chemical groups, and they should be combined or alternated with fungicides that have a low resistance risk.

When one crop could serve as a source of inoculum for a subsequent crop, the alternation scheme among at-risk fungicides should be continued between successive crops such that the first at-risk fungicide applied to a crop belongs to a different cross-resistance group than the last at-risk fungicide applied to the previous crop. Some at-risk fungicides are formulated as premix products with other fungicides to manage resistance. At-risk fungicides should be used only when needed most. The most critical time to use them for resistance management is early in an epidemic when the pathogen population is small. Multi-site contact fungicides should be used alone late in the growing season, where they have been shown to provide sufficient disease control to protect yield. Another important component of resistance management is assessing disease control and reporting any loss of efficacy potentially due to resistance.

To promote resistance management, companies registering fungicides are voluntarily putting on the labels guidelines developed recently by EPA through a joint effort with the Canadian Pest Management Regulatory Agency (PMRA) under the North American Free Trade Agreement (NAFTA). These are described in Pesticide Registration (PR) Notice 2001-5 (www.epa.gov/opppmsd1/PRNotices/pr2001-5.pdf). Group codes for designating chemical groups were developed as part of these guidelines.

Many fungicides remain on the surface of plant tissues and do not spread throughout the plant. Others penetrate the cuticle and circulate through plant tissues. Pruning shears and other tools can carry plant diseases from one plant to another. Learn about garden sanitation

to prevent spreading fungal pathogens yourself. Although they can slow or stop the development of new symptoms, many fungicides are designed only to prevent disease. These are not highly effective after symptoms have developed.

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CHAPTER**18****ENDOPHYTES A BOON FOR
SUSTAINABLE CROP PRODUCTION:
AN INCLUSIVE REVIEW**

Dr. Femina Sobin*

Abstract

Any organism that grows within plant tissues are termed as endophytes. Fungi and bacteria are the most commonly reported endophytes. The other microbial endophytes being actinomycetes and mycoplasma, as endophytes of plants. The most frequently isolated endophytes are the fungi. Almost all the vascular plants and grasses on earth are thought to harbor endophytes. Most of the microbes that inhabit plants play a major role in the plant's health and development. They play significant role as producers of plant growth promoting hormones and metabolite, insect and pest repellents, possess antimicrobial activity against plant pathogens and protectors in stress conditions. Excessive use of chemical fertilizers, pesticides and modern agricultural practices has several environmental hazards. Increased soil pollution and reduced microbial diversity in soil are among few of such concerns. Endophytes are being used and can be more explored to be used as biofertilizers and biological control agents (biopesticides) for sustainable crop production, thus, may be a potential solution to resolve the above stated concerns. They can even be exploited for their novel secondary metabolites of great industrial values.

Keywords: Biofertilizers, Biopesticides, Crop, Endophytes, Fungi, Sustainability.

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Introduction

The term endophyte was coined by De Bary (1866). The first definition of an endophyte, as “Any organism that grows within plant tissues are termed as endophytes,” however, the definition continues to change as per various researchers.

Endophytes characteristically exhibit a prolonged, inconspicuous period in which growth and colonization cease temporarily, resuming after a physical or maturational change in the host (Petrini, 1986). Include competent, facultative, obligate, opportunistic and passenger endophytes (Hardoim *et.al.* 2008).

According to Fisher and Petrini (1987); Fadiji and Babalola (2020), endophyte are microbes residing within the plant tissues i.e., the internal parts of a plant, without showing apparent harm to the host plant. The word endophyte is derived from Greek words, ‘endo’ or ‘endon’ meaning within and ‘phyte’ or ‘phyton’ meaning plant.

Gouda *et. al.* (2016) informs that endophytes are an endosymbiotic group of microbes that colonize plants. They further state, these microbes can be easily isolated from microbial or plant growth medium. Fungi, bacteria, actinomycetes and mycoplasma were reported as endophytes of plants by Bandara *et. al.* (2006). The most frequently isolated endophytes are the fungi. It turns out that the vast majority of plants have not been studied for their endophytes. Thus, enormous opportunities exist for the recovery of novel fungal forms, taxa and biotypes (Hawksworth and Rossman, 1987). Endophytic fungi comprise primarily of members of Ascomycetes and occasionally Basidiomycetes and Zygomycetes (Sun and Guo, 2012).

Among these endophytic microbes, endophytic fungi have fascinated much research interest as they have provided novel sources of cytotoxic compounds, like anticarcinogenic molecules and antibacterial substances; biostimulants for essential oil synthesis; enhance nutrient solubilization in the plant rhizosphere; promote plant growth; act as biological control agents; resistances to biotic or abiotic stresses in plants (Alam *et. al.*, 2021).

Endophyte association with plants

Azevedo *et. al.*, (2001) reported that endophytes gain entrance into the seed (Yadav, 2018), dwell within the plants specifically in leaves, stems, roots, petioles, inflorescences of weeds, fruits, buds, seeds and also dead and hollow hyaline cells of plants (Jalgaonwala *et. al.*, 2011; Hata and Sone, 2008; Specian *et al.*, 2012; Stepniewska and Kuzniar, 2013). Zang *et. al.*, (2006) states that more or less all the vascular plants and grasses studied so far were found to host endophytic organisms in them. **Table-1** states the examples of some vascular plants, grasses and endophytes associated with them.

Endophytes display complex interactions with their host which comprises of mutualism, antagonism and infrequently parasitism (Gouda *et. al.*, 2016).

The population of endophytes in a plant species is highly variable and depends on various components such as – host species, host development stage, inoculum density and environment conditions ((Dudeja and Giri, 2014).). It is considered that a single plant species

could possess thousands of microbes categorized as epiphytes (microbial inhabitants of the rhizosphere and phyllosphere; those near or on plant tissue) or endophytes (microbes residing within plant tissues in leaves, roots or stems), depending on their area of colonization of plant species (**Figure-1**) (Oldroyd *et al.*, 2011; Turner *et. al.*, 2013; Andreote *et. al.*, 2014).

Table-1: Endophytes in some vascular plants and grasses.

Common name of plant	Scientific name	Endophyte	Plant description	Type of plant
	<i>Caralluma acutangula</i>	<i>Phoma</i> sp., <i>Alternaria alternata</i>	Apocynaceae family, perennial succulent bushes (Amrati <i>et. al.</i> , 2020; https://www.giromagicactusandsucculents.com/caralluma-acutangola-giromagi-cactus-succulents/)	Medicinal plant (Khan <i>et. al.</i> , 2017)
Harmal	<i>Rhazya stricta</i>	<i>Phoma</i> sp., <i>Alternaria</i> sp., <i>Bipolaris sorokiniana</i>	Apocynaceae family, Evergreen miniature shrub (Albeshri <i>et. al.</i> , 2021; Ayman <i>et. al.</i> , 2014)	
Habb El Pan	<i>Moringa peregrina</i>	<i>Phoma</i> sp., <i>Cladosporium sphaerospermum</i>	Moringaceae family, a fast-growing tree or shrub (Hussein <i>et. al.</i> , 2017)	
Orchard grass	<i>Dactylis glomerata</i>	<i>Epichloë typhina</i>	Poaceae, a large taxonomic family of grasses (Lee <i>et. al.</i> , 2021)	Grasses
Tall fescue	<i>Festuca arundinacea</i>	<i>E. coenophiala</i>		
Perennial ryegrass	<i>Lolium perenne</i>	<i>E. festucae</i> var. <i>lolii</i>		
Annual ryegrass	<i>Lolium multiflorum</i>	<i>E. occultans</i>		

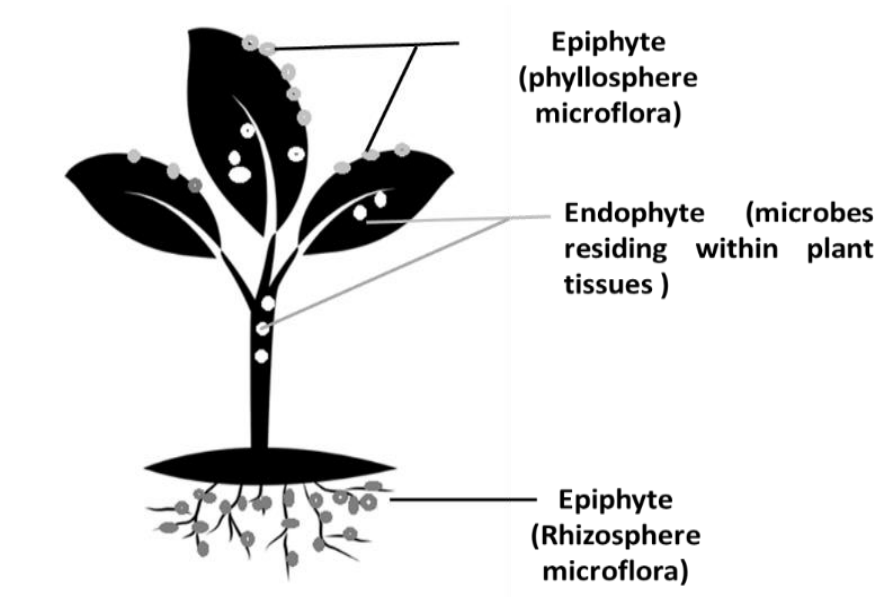


Figure-1: Microbes categorized depending on their area of colonization in plants.

Types of Endophytes

Endophytes are classified on the basis of mode of entry to the host plant and their relationship with host plants. On this basis endophytes are of two types -

- a) **Obligate endophytes:** Such endophytes depend on the metabolism of plant for survival. They are spread amid plants by the activity of different types of vectors i.e. by vertical transmission.
- b) **Facultative endophytes:** They are those that live outside the host body during a certain stage of their life cycle and are mostly associated with plants from its neighboring soil environment and atmosphere (Hardoim *et. al.*, 2008).

There exists a third type of endophyte as explained by Hardoim *et.al.* (2008) -

- c) **Passenger endophytes:** A passenger endophyte enters the plant by accident, in the absence of selective forces, maintaining it in the internal tissue of the plant.

Plant-endophyte relationships

The relationship between plants and endophytes can be commensal or mutualistic (Freeman and Rodriguez, 1993; Redman *et. al.*, 2011):

- a) **Commensal relationship:** It means the endophyte benefits, while the plant host is unharmed.
- b) **Mutualistic relationship:** It means that both, the endophyte and the plant host benefit from each-other.

Most of the microbes that inhabit plants play a major role in the plant's health and development, although, sometimes they are neutral. Some of the microbe dwelling within plant are disease causing. However, this observation continued to be unexplored until the definition of endophytes came into existence toward the end of last century.

Plant growth promoting activity of endophytes

Ten research papers spanning from the year 2015 to 2022 has been reviewed in **Table-2**. As per the research articles studied, bacterial and fungal endophytes have been isolated from plants namely - Xerophytes, Tea Cultivars, *Teucrium polium*, *Pistacia atlantica*, *Eupatorium odoratum*, *Pulicaria incisa*, *Camellia sinensis*, *Vanda cristata*, and *Oryza sativa*. The study was conducted in following places – Egypt, Iran, India, Saudi Arabia, China, Nepal and Singapore. According to these papers reviewed following plant growth promoting (PGPR) activity has been attributed to the endophytic isolates –

The endophyte apart from producers of growth promoting metabolites are also insect and pest repellents, possess antimicrobial activity against plant pathogens and protectors in stress conditions (Rya *et al.* 2007; Staniek *et. al.* 2008; Rai *et al.* 2014 a, b; Passari, *et. al.*, 2015) **Figure-2**.

Production of -	Other PGPR attributes
• Indole acetic acid • Ammonia • Protease • Chitinase • Hydrogen cyanide • Phytohormone - auxin and gibberellins • Siderophore	• Atmospheric nitrogen fixation • High potential in phosphate solubilization • Positive effects on plant growth and shoot P and N contents • Hormone modulation

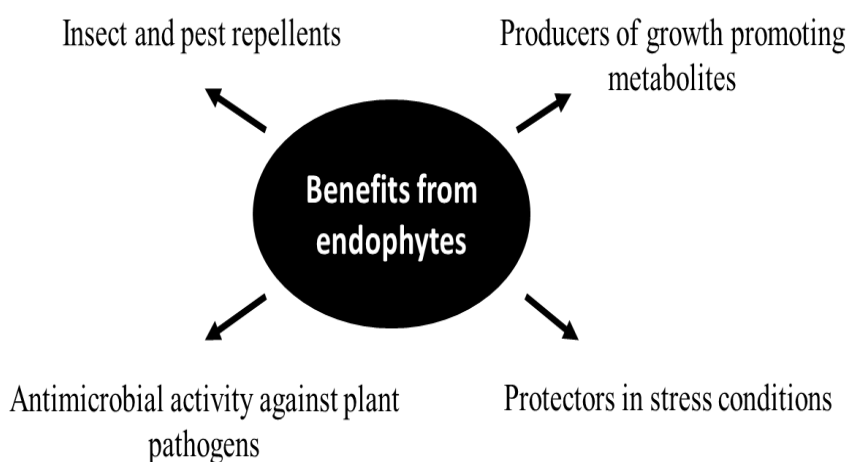


Figure-2: Benefits to host plants from endophyte.

Table-2: Growth promoting activity of some of the endophytes.

Year	Author	Plant	Place	Plant Growth Promoting Activity	Endophyte
2017	Hassan E. S.	<i>Teucrium Polium</i> L.	Egypt	Indole acetic acid, ammonia	Bacteria <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> Fungi <i>Penicillium chrysogenum</i> , <i>Penicillium crustosum</i> .
2018	Etminani F. and Harighi B.	<i>Pistacia atlantica</i> L.	Iran	Production of protease and hydrogen cyanide, phytohormone such as- auxin and gibberellins, siderophore. Atmospheric nitrogen fixation	Bacteria <i>Pseudomonas</i> , <i>Stenotrophomonas</i> , <i>Pantoea</i> and <i>Serratia</i> genus.
2015	Passari, et. al.	<i>Eupatorium odoratum</i> , <i>Musa superb</i> , <i>Mirabilis</i>	Mizoram, India	Production of Chitinase, ammonia and siderophore.	Bacteria <i>Streptomyces</i> sp., <i>Microbacterium</i> ,

		<i>jalapa</i>			<i>Actinomycetes sp.</i> , <i>Leifsonia</i> and <i>Brevibacterium</i>
2021	Fouda, <i>et. al.</i>	<i>Pulicaria incisa</i>	Egypt	Showed high efficiency in IAA, High potential in phosphate solubilization and ammonia production	Bacteria <i>Agrobacterium fabrum</i> , <i>Acinetobacter radioristant</i> , <i>Brevibacillus bervis</i> .
2022	Singh <i>et. al.</i>	<i>Musa acuminata</i>	Satna, India	Production of IAA, siderophore, hydrogen cyanide and ammonia. Nitrogen fixation, phosphate solubilization.	Fungi <i>Fausarium oxysporum</i> , <i>Macrophomia phaseolina</i> Bacteria <i>Bacillus cereus</i> , <i>Enterobacter hormaechei</i> , <i>Enterobacter hormaechei</i>
2020	ALKahtani <i>et. al.</i>	<i>Fagonia mollis</i> <i>Delile</i> and <i>Achillea fragrantissima</i>	Saudi Arabia	Production of IAA. Positive effects on plant growth and shoot P and N contents.	Bacteria <i>Bacillus</i> and <i>Bervibacillus</i> sp.
2018	X Yan	Tea Cultivars	China	Atmospheric nitrogen fixation, indole acetic acid production	Bacteria <i>Methylobacterium</i> spp. <i>Herbaspirillum</i> spp.
2021	Hazarika <i>et. al.</i>	<i>Camellia sinensis</i>	Guwahati , India	Phosphate solubilization, IAA production, Nitrogen fixation, Siderophore.	Bacteria <i>Pseudomonas</i> sp., <i>Stenotrophomonas</i> sp.
2021	Shah, <i>et. al.</i>	<i>Vanda Cristata</i>	Nepal	Promote growth through secretion of IAA	Bacteria <i>Bacillus</i> sp.
2017	Walitang, <i>et. al.</i>	<i>Oryza sativa</i> L.ssp. indica	Singapore	Hormone modulation, Siderophore,nitrogen fixation.	Bacteria <i>Xanthomonas</i> sp., <i>Microbacterium</i> sp.

Endophytes for sustainable crop production

Use of agricultural equipment, high-yielding crop varieties, intensive tillage, irrigation, fertilizers, pesticides, and other manufactured inputs were main agricultural activities in the 20th century (Hegazy *et. al.*, 2017). Excessive use of chemical fertilizers has several environmental hazards, such as increased soil pollution, reduced microbial diversity in soil, decreased food safety, and the leaching of minerals into the groundwater (Elkelish *et.al.*, 2020; Rafi *et.al.*, 2019). These applications can harmfully affect the quality of the soil and add to environmental pollution (Azab *et. al.*, 2016). Microbial inoculations like biofertilizers are gaining more attention to lessen these negative effects due to conventional farming techniques. The symbiotic relationship formed between microorganisms and plants, is useful for both the partners. The cohabitation of the microbe in the plant, affects the health and growth performance of the plant, which effectively improves the agricultural properties, such as the root length and the fresh and dry weight of the shoot and root, and improves the yield productions, quality of the soils, and the nutrient cycling (Habib *et. al.*, 2020; Soliman *et. al.*, 2019; Elkelish *et. al.*, 2020). Attention towards endophytes has increased recently because of its diversity and multiple functions (Paul *et. al.*, 2012). Endophytes are being used and can be more explored to be used as biofertilizers for sustainable crop production.

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CHAPTER

19

STUDY OF IMPACT OF SEED BORNE MYCOFLORA OF *C. arietinum* ISOLATED FROM JABALPUR DISTRICT (M.P.) ON POST HARVEST LOSSES

Mrs. Harleen Kaur* and Mrs. Nidhi Narula

A**bstract**

Cicer arietinum generally known as chickpea, belongs to pulse family, “*Fabaceae*”. Also called Leguminosae. Chickpeas is an important food plant in India, and in other countries and widely grown for its nutritious value. The seeds are high in fibre and protein and is a good source of iron, phosphorus, and folic acid. The present study dealt with the isolation and study of the role of isolated mycoflora in post-harvest loss. Blotter paper method was used for the isolation of seed mycoflora collected from local market. A total of 21 fungal isolates were collected from Jabalpur district which comprises of *Aspergillus sp.* (11 colonies), *Mucor* (4 colonies), *Rhizopus* (4 colonies) and *Alternaria sp.* (2 colonies). All these fungal isolates are having a significant role in the deterioration of the seed quality. It has also been observed by other scientists that seed-borne fungi are responsible for the deterioration of health of seeds and cause reduction in plant growth and productivity of crops.

Keywords: Chickpea, Mycoflora, Blotter Paper Method, Seed Borne Disease, Productivity.

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Introduction

Chickpea is the common name for an annual plant, *Cicer arietinum*, of the Fabaceae (or Leguminosae) family. Two types of chickpeas are recognized, the white-seeded “Kabuli” and the brown coloured “Desi” types. Kabuli chickpeas are creamy-white relatively bigger in size having a thicker seed coat. The brown chickpea contributes to around 80% and the white chickpea around 20% of the total production. In general, Chickpea seed has 38-59% carbohydrate, 3% fiber, 4.8-5.5% oil, 3% ash, 0.2% calcium, and 0.3% phosphorus (Hulse, 1991, Huisman and van der poel, 1994). Pulses also contain a higher amount of an essential amino acid i.e. lysine which is either absent or present in lesser amount in cereals. Thus, pulses provide two to three times more protein than other cereals and help in overcoming the deficiency of essential amino acids.

Measurable qualitative and quantitative loss of the product from harvesting to its consumption is known as Post harvest loss. The post-harvest system works in seven states; harvesting, threshing, drying, storage, processing, packaging and transportation. The extent of losses in pulses differ from grain to grain and the stage of post-harvest loss. The extent of loss also depends upon location, weather condition, type, processing and storage techniques used for the particular grain. Among these, in case of pulses, the major loss occurs during storage. Pulses are good source of protein so easily attracted by storage insects and if amount of moisture is good then a wide range of mycoflora can be seen.

Materials and Methods

Isolation of Seed Mycoflora

The isolation of seed mycoflora from kabuli chana was done by using Standard blotter paper method. This method is under the recommended list by International Seed Testing Association ISTA (1966) and Agarwal (1976). Total percent incidence of individual fungus was calculated by the following formula -

Incidence of individual fungus = (Number of seeds infected with particular species/Total number of isolated genera) × 100

Standard blotter paper method

This is simple and most convenient method adopted by Doyer (1938) and De Temp (1953) in seed health management. In this method, Sterile white blotter papers were soaked in sterile distilled water and placed in pre-sterilized Petri-plates. 5-6 chana seeds were then placed on moist blotter paper. The plates were incubated at 28°C.

Results and Discussion

A total of 10 samples were collected from 5 different markets of Jabalpur. The selected samples which were placed in petriplates look apparently clean and healthy but a total of 32 fungal colonies were isolated from these samples. The isolated colonies were *Mucor sp.*, *Rhizopus sp.*, *Aspergillus sp.*, *Alternaria sp.*, *Fusarium sp.*, and *Penicillium sp.*

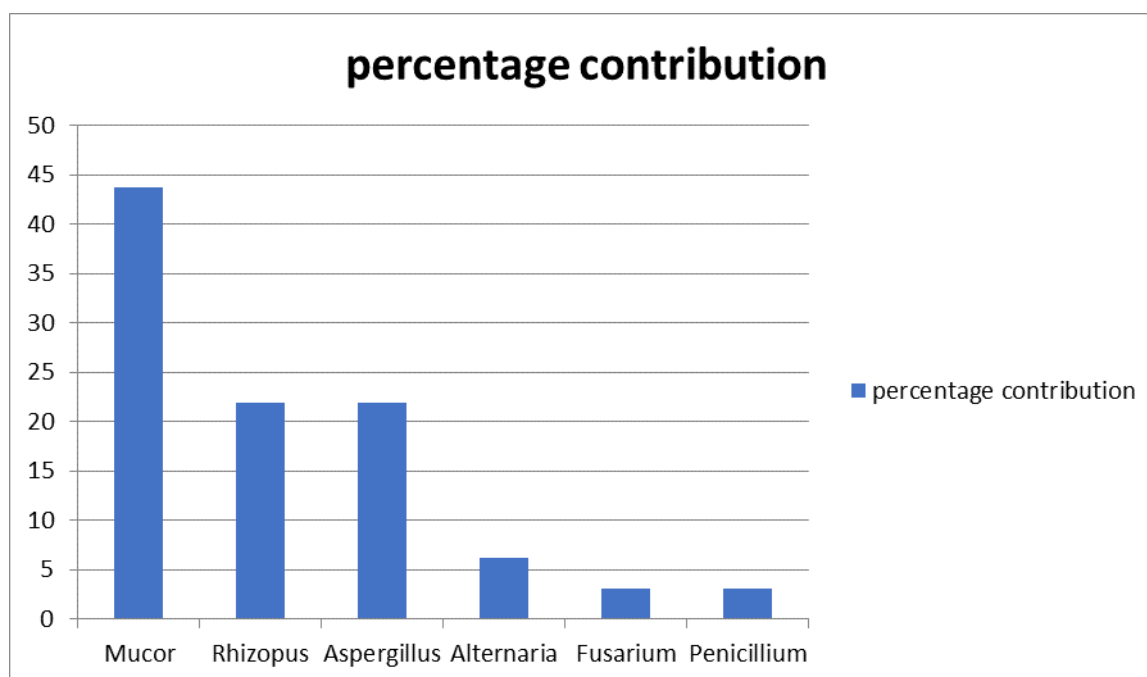


Fig-1: Percentage contribution of isolated genera from Kabuli Chana.

Among these isolated genera many of them comes under the dominant fungal flora during storing stage of post-harvest system.

Conclusion:

The impact of fungal genera in seeds are considerable as they are responsible for many physical and chemical changes in seeds. The fungal pathogens are dominant in grain storage. Mashilla, (2004) talked about principal storage fungal genera as *Aspergillus*, *Penicillium*, *Rhizopus*, *Sclerotium* and *Fusarium*. The seed mycoflora can also be responsible for large losses when the stored seeds become damp. The common mycoflora grow in stored grains, belonging to filamentous fungi, may result in the production of mycotoxins. These mycotoxins can cause little to major allergic responses to humans depending upon the immunity of an individual.

The other effects of seed borne fungi are reduction in crop yield, loss in germination, development in crop diseases, alteration in physical properties in seed, biochemical changes in seeds, economic loss and high impact in society. The management of seeds are important as they are the basic unit for food production. Proper storage conditions should be maintained after harvesting to maintain high quality seeds.

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CHAPTER

20

DISTRIBUTION OF AQUATIC FUNGI IN FRESH WATER HABITAT

Dr. Mehtab Yasmeen*

A**bstract**

Fungi are omnipresent and therefore associated with almost every organism, often as parasites, sometimes as symbionts and of course as decomposers. Parallel to fungi in soil, aquatic fungi act as prominent decomposers of particulate organic matter (POM), foremost coarse particulate organic matter (CPOM) including plant and animal debris. The aquatic fungi which typically decompose leaf litter and wood with a hyphal network are the polyphyletic group known as “aquatic hyphomycetes”. Taxonomically, they are mainly associated with the Ascomycota and only a small percentage is affiliated other groups. Another category is “aero-aquatic hyphomycetes” which colonizes submerged plant debris in stagnant and slow flowing waters, such as shallow ponds and water-filled depressions. Taxonomically, most aero-aquatic fungi are classified as Ascomycota, although some species have been classified as Basidiomycota also. So far, more than 640 species of freshwater fungi mostly from temperate, as compared to tropical regions are recorded. These include about 340 ascomycetes, 300 deuteromycetes, and a number of lower fungi. *Aniptodera*, *Annulatascus*, *Massarina*, *Ophioceras* and *Pseudohalonectria* are common freshwater ascomycetes. *Cancellidium*, *Dactylaria*, *Dictyosporium* and *Helicomycetes* are common deuteromycetes.

Keywords: Fungi, Aquatic Hyphomycetes, Aero-Aquatic Hyphomycetes, Ascomycetes, Deuteromycetes.

Introduction

Water is uniform and specialized habitat with a characteristic flora of adapted forms. Though in constant motion, water supports the vegetative growth of organisms so that their distribution is controlled primarily by edaphic or nutritional factors, and to some extent by temperature, especially where the amount of water is small. The ecology of fungi as the basis for study of geographical distribution, unlike that of phanerogams, has not been widely investigated. Hyde et al (2007) have estimated that there are approximately 1.5 million fungal species on earth. Of these, only around 3000 species are known to be associated with aquatic habitats and only 640 species in fresh water (Goh and Hayde, 1996) and 465 species occur in marine waters (Shearer et al., 2007). This small proportion of aquatic fungal taxa is surprising because the aquatic environment is a potentially good habitat for many species.

Freshwater Fungi

Aquatic fungi are generally microscopic organisms and do not form visible fruiting bodies but reproduce asexually. Many aquatic fungi are saprophytes and grow on dead organic matter (Dodds, 2002), but they may also found to be parasites or symbionts. In aquatic ecosystems, the fungal groups fluctuate depending on the type of substrates (Shearer and Webster, 1985; Findlay et al., 1990; Mille-Lindblom et al., 2006), physico-chemical nature of the habitat like flow (Pattee & Chergui, 1995; Baldy et al., 2002), dissolved oxygen concentration (Field & Webster, 1983; Medeiros et al., 2009), nutrient concentrations (Gulis & Suberkropp, 2004; Rankovic, 2005), salt concentrations (Hyde & Lee, 1995; Roache et al., 2006), temperature (Bärlocher et al., 2008) turbidity of water and light penetration (Wurzbacher et al., 2010). Hence, fungal groups show lot of diversity in different types of fresh water bodies like streams, shallow lakes and wetlands, deep lakes and other habitats also such as salt lakes and estuaries.

Most freshwater fungi are phycomycetes and saprobic members of the Chytridiales, Leptomitales, Saprolegniales which are able to utilize a variety of substrata such as dead plant and animal remains. While few are the facultative parasitic members of the Chytridiales on aquatic algae, fungi and animals' others like members of Plasmodiophorales are obligate parasites of aquatic and semiaquatic angiosperms.

Freshwater bodies due to their relatively small size are under the influence of temperature changes and this effect the distribution of fungi. When we consider the global scenario, body of information regarding the fungal distribution is lacking, still with the limited data one can see that number and frequency of occurrence of fungal species decreases as one progress northward to equator. *Monoblepharis* and *Allomyces* colonize similar substrata line

submerged plant debris, but species of *Monoblepharis* tend to prevail in the cooler waters of the temperate zones, while *Allomyces* is confined to tropical and subtropical regions (Emerson, 1941).



Fig.-1: *Monoblepharis*



Fig.-2: *Allomyces*

Diversity of fresh water aquatic fungi

Freshwater fungi are diverse group of organisms. They include representatives from different groups of fungi namely the zoosporic fungi [Fuller and Jaworski, 1987], hyphomycetes [Goh, 1996; Ingold, 1975; Nawawi, 1973, 1985, 1987], ascomycetes [Hyde et al., 1996; Jones, 1976], basidiomycetes [Nawawi, 1985; Nawawi and Khutbutheen, 1988], coelomycetes [Crib and Crib, 1993; Hyde, 1993], zygomycetes [Descals and Webster, 1984] and trichomycetes [Lichtwardt and Williams, 1992.]. Among these, the freshwater hyphomycetes (Moniliales, Deuteromycotina) are well documented.

Ascomycetes: Shearer [1993] listed 288 species of ascomycetes in her review paper on freshwater fungi. This included a wide variety of ascomycetes, encompassing discomycetes, plectomycetes, pyrenomycetes and loculoascomycetes. When Shearer published her list in 1993 only 11 of the records were from tropical locations. Several tropical freshwater ascomycetes have since been described and a total of 50 species is now known to occur in the tropics, with 16 from water cooling towers, two from high altitude ponds and 32 from tropical streams [Hyde, 1996; Hyde et al., 1996]. Three new freshwater ascomycetes have also been described recently from temperate regions [Read et al., 1992] and the number of ascomycetes known to occur in freshwater habitats now stands about 340 where most species listed are temperate.

The most common freshwater fungi belong to the genera viz., *Aniptodera*/ *Halosarpheia*, *Chaetomium* (Ando and Kawamoto, 1986), *Hymenoscyphus* (Ando and Tubaki, 1984a), *Mollisia* (Ando and Tubaki, 1985), *Ophioceras*/*Pseudohalonestria* (Ando and Tubaki, 1984b), *Phaeosphaeria* and *Vibrissia* (Au DWT, 1993). In tropical regions, the most specious genera are *Aniptodera*/*Halosarpheia*, *Annulatascus* (Ando and Tubaki, 1984a) and *Savoryella* (Ando, 1992). However, the most frequently recorded genera in 250 collections made in

Australia, Brunei, Ecuador, Malaysia and the Philippines were *Annulatascus*, *Massarina*, *Ophioceras*, *Savoryella* and *Aniptodera* [Hyde et al., 1996].

Hyphomycetes: In the early 1940s it was found [Hyde et al., 1996] that hyphomycetes group of fungi occurs regularly on submerged decaying leaves, twigs and wood of dicotyledonous trees and shrubs. These fungi were never reported from the needles of conifers. Over 30 years now, the freshwater hyphomycetes were mainly reported from cold and temperate regions. However, tropical countries having rich fungal diversity may certainly also rich in diversity of freshwater hyphomycetes. It is now reported that the freshwater hyphomycetes have a worldwide distribution (Sridhar et al., 1992; Subramanian, 1983; Tan and Koh, 1995). Examples of species which are worldwide in distribution include *Anguillospora crassa*, *Campylospora chaetoclada*, *Flagellospora penicillioides*, *Jaculispora submersa*, *Lunulospora curvula*, *Tetrachaetum elegans*, *Tetracladium setigerum*, *Tricladium angulatum*, *Triscelophorus monosporus* and *Varicosporium elodeae* [Wood-Eggenschwiler and Bairlocher, 1985]. Nevertheless, some species appear to be localized in a given climatic zone. *Alatospora acuminata*, *Flagellospora curvula*, *Heliscella lugdunensis*, *Lemonniera aquatica* are some of the dominant species in the high North, whereas *Angulospora aquatica*, *Brachiosphaera tropicalis*, *Campylospora filicladia*, *Ingoldiella hamata*, *Isthmotricladia gombakiensis*, *Lunulospora cymbiformis*, *Phalangispora constricta*, *Pyramidospora casuarinae*, *Speiropsis pedatospora*, *Tricladomyces malaysianum*, *Triscelophorus acuminatus* are some of the tropical species. According to the latest data, more than 300 species of freshwater hyphomycetes are known and the number is still counting. Many new taxa have been discovered like *Canalisporium* [Nawawi and Kuthubutheen, 1989a], *Quadricladium* (Nawawi and Kuthubutheen, 1989b), *Crucella* (Marvanova and Suberkropp, 1990), *Nidulispora* (Nawawi and Kuthubutheen, 1990a), *Obeliospora* (Nawawi and Kuthubutheen, 1990b), *Candelosynnema* (Hyde and Seifert, 1992), *Isthmophragmospora* (Kuthubutheen and Nawawi, 1992) and *Paracryptophiale* (Kuthubutheen and Nawawi, 1994). However, there are many more aquatic fungi awaiting isolation and identification.

Nutritional diversity of freshwater aquatic fungi

Saprophytic fungi: Aquatic fungi, being heterotrophs, are reliant upon photosynthetically produced organic matter. In order of decreasing biodegradability, the fungal community consumes microscopic algae, macroscopic aquatic macrophytes and terrestrial plant litter. Resources are either derived from animals like dead fish, fish eggs, carcasses, feathers and hair, or/and plant-derived resources include pollen, spores, seeds and fruits (Cole et al., 1990). Strangely, there is not a single natural organic source that cannot be utilized by aquatic fungi (Sparrow, 1960). Another interesting feature of aquatic habitats is the coupling of aquatic systems to terrestrial environments via animals, mainly insects, which are able to export nutrients from the aquatic ecosystem (Vander Zanden & Gratton, 2011). Fungi are often closely associated with insects, which can be key organisms in aquatic freshwater systems. Although often overlooked, fungi represent a common and important component of almost every trophic level of any aquatic ecosystem.

Endophytic fungi: An important group of endophytic fungi, which is clearly beneficial for the plant, consists of mycorrhiza forming symbionts present in the roots of several aquatic macrophytes. Many mycorrhiza forming symbionts belong to a phylum of the “lower fungi” called Glomeromycota. Certain orders of the Glomeromycota are obligate root symbionts characterized by a vesicular arbuscular mycorrhiza (VAM) that supply their hosts with nutrients. In return, the host plant provides the fungus with sugars rich in energy, amongst other things. VAM fungi were formerly believed to be purely terrestrial, but today it is known that they are particularly important in nutrient poor clear waters. For example, in oligotrophic waters, VAM fungi allow macrophytes to grow under nutrient limiting conditions by supplying the plant with solid-phase bound nutrients (Baar et al., 2011). Freshwater algae, e.g. *Dunaliella* and the autotrophic protozoan *Euglena* can establish a mutual relationship with the fungus *Bispora* or the yeast *Cryptococcus*, respectively (Gimmler, 2001). Moreover, fungi belonging to the Kickxellomycotina are endosymbionts of invertebrates, especially of aquatic arthropods and together with specialized protozoans and grouped under the term trichomycetes (Lichtwardt, 2004; Hibbett et al., 2007).



Fig.-3: VAM Fungi

Parasitic fungi: In few cases, fungi can cause severe damage to larger aquatic organisms, especially infect fishes or fish eggs (Noga, 1993; Chukanhom & Hatai, 2004) and thereby exert strong population pressure. This is of great importance for aquaculture since it necessitates antifungal treatments, but even in natural systems, fungi have the potential to severely harm the indigenous fish population. *Aphanomyces astaci* (Oomycetales) causing the crayfish plague has driven the European crayfish (family Astacidea) population to the edge of extinction (Reynolds, 1988). In contrast, *Coelomomyces* (Blastocladiomycota) effectively infecting several mosquito species (Sparrow, 1960) has been discussed as a biological control for malaria mosquitoes (Whisler et al., 1975). The most infamous fungal parasite is *Batrachochytrium dendrobatidis* (Chytridiomycetales), which contributes to worldwide extinction of several known and unknown amphibian species (Berger et al., 1998; Skerratt et al., 2007).



Fig.-4: *Batrachochytrium dendrobatidis*

Aquatic plants are also greatly affected by fungal pathogens. A recently discovered plant parasite is *Pythium phragmites*, causing reed decline (Nechwatal et al., 2005). Some human pathogens may also be found amongst the aquatic fungi. Common freshwater yeasts belonging to *Candida* and *Cryptococcus* are both potentially harmful to humans (e.g. *C. albicans* and *C. tropicalis*). These fungi are frequently found along bathing sites (Vogel et al., 2007). Several typical dermatophytes and keratinophilic fungi are transferred via water and can also occur in aquatic ecosystems (Ali-Shtayeh et al., 2002). Chytridiomycetes and “Microsporidia”, however, are rarely pathogenic and only infect immune-deficient patients.

Fungal diversity in different freshwater aquatic habitats

Streams (Lotic Environment): Running waters such as stream or river support a variety of aquatic fungi. Fungi like *Vibrissia* and *Apostermidium* are found to be common on submerged twigs (Sanchez and Korf, 1966). According to the investigations of Nikolcheva & Bärlocher (2004) it was observed that the structure of fungal communities on leaves submerged in an upland stream, community was dominated by Ascomycota, whereas Basidiomycota comprised a small but consistent fraction of aquatic fungi. Chytridiomycota represented a substantial proportion of the fungal community in winter, while Oomycetes were only present in summer. Glomeromycota, however, were of minor importance in the stream environment. *Saprolegnia monoica*, *Achlya racemose*, *A. flagellate*, *Leptomit* were found to be flourishing during winters (Waterhouse, 1942).

When fungal species in an Australian upland stream were studied, the most common species found to be *Tetrachaetum elegans*, *Lunulospora cymbiformis*, *Flagellospora penicillioides* and *Alatospora acuminata* (Thomas et al., 1992). When lowland and upland streams or rivers are compared, in the low land rivers, flow remains heavy but water quality and the source of primary production are substantially different from those in upland streams. Wider channels lead to a proportional reduction in litter from riparian plants, and production from phytoplankton is of increased significance (Vannote et al., 1980). Nutrient concentrations and dissolved organic carbon may also be higher, leading to lower or fluctuating concentrations of

dissolved oxygen. Thus, while aquatic hyphomycetes still dominate submerged litter in these streams (Baldy et al., 2002), fungal community composition differs from upland streams (Shearer & Webster, 1985) and biomass accumulation may be limited by competition with other microorganisms, substrate burial and lower oxygen availability (Bärlocher, 1992; Medeiros et al., 2009).



Fig.-5: *Achlya racemose*



Fig.-6: *Saprolegnia monoica* growing on fish

Shallow lakes and wetlands (Lentic Environment): Small temporary pools which take many years to reappear, present hard conditions for living creatures in them but aquatic fungi known to inhabit them. Dayal and Tandon (1962) found certain temporary pools in India had two thirds to one half the varieties of species that more permanent bodies of water supported. The dominant fungi colonizing submerged plant litter in shallow, stagnant habitats common in wetlands and shallow lakes are the aero-aquatic hyphomycetes (Glen-Bott, 1951; Shearer et al., 2007). On occasion, aero-aquatic hyphomycetes may be found in streams and aquatic hyphomycetes in wetlands (Bärlocher & Kendrick, 1974; Fisher et al., 1983; Bärlocher, 1992), but aero-aquatic hyphomycetes are capable of out-competing aquatic hyphomycetes when colonising substrates in water with lower oxygen or higher nutrient concentrations (Voronin, 1997). Oomycetes and terrestrial fungi can also be found in wetlands (Bärlocher, 1992).

Fungal genera commonly found in wetlands include *Alternaria*, *Cylindrocarpon*, *Cladosporium*, *Penicillium*, *Fusarium*, *Trichoderma* and aquatic hyphomycetes like *Alatospora*, *Tetracladium*, *Helicodendron*, *Helicoon* (Kaushik & Hynes, 1971; Kjoller & Struwe, 1980; Ford, 1993).

Aquatic lichens (a symbiotic partnership between a fungus and an alga) are possibly present in the littoral zone of wetlands, lakes and streams (McCarthy & Johnson, 1997), in particular in temperate or boreal regions (Hawksworth, 2000). There are about 200 species of lichenised fungi known from freshwater systems (Hawksworth, 2000). The main orders of Oomycetes found in aquatic environments are the Leptomitales, Saprolegniales and Peronosporales. Their requirement for dissolved oxygen varies widely among species, and many are intolerant of high salinity (Dick & Newby, 1961; Dick 1962; 1963; 1969; 1972).

Deep lakes and reservoirs: In deep lakes and reservoirs, distribution and type of aquatic fungi found to vary with the zones. While fungi are found abundantly and also in great diversity in both the littoral and profundal zone (Rankovic, 2005), the pelagic zone found to support only few specialized fungal species and serve as a medium for spore dispersal (Wurzbacher et al., 2010). Fungi from the littoral zone can be are saprobes, parasites, predators, endosymbionts or occasionally lichens. These organisms colonize substrates ranging from submerged plants and litter to the carapaces of dead microcrustaceans (Czeczuga et al., 2002; 2004; 2007). In the pelagic zone, fungi consist mainly of parasitic fungal species on phytoplankton, zooplankton and fish.

Taxonomically, the fungal community comprising of rivers consists of Ascomycete, Basidiomycete and “zoosporic fungi” (Chytridiomycota and Oomycetes) (Rankovic 2005; Lefevre et al., 2007). Zoosporic fungi and their propagules in the pelagic zone play important role in food web dynamics as they keep in check the phytoplankton blooms associated with diatoms (Kagami et al., 2004) and cyanobacteria (*Microcystis* spp) (Chen et al., 2010).

The profundal zone and lake sediments only serve as a propagule bank as all resting spores are deposited. Both aquatic and terrestrial species are frequently isolated from deep lake sediments. Moreover, that yeasts on plant litter and fungi belonging to Mucoromycotina (*Mucor* and *Rhizopus* sp.) which were isolated from various Serbian reservoirs are also found to be terrestrial in origin (Rankovic, 2005).

Conclusion

Presently, fungal diversity in the world greatly outnumbered any other group of microorganisms. Main function of fungal groups is nutrient cycling and to maintain ecosystem health which is of global importance. Fungal hyphae network interconnects a whole habitat and trigger the transport of macro- and micronutrients over large distances to other heterotrophic microbes (Harms et al., 2011). Aquatic habitats are also very important for the fungal diversity and any loss of fungi severely affects aquatic food web topology and effects its functioning (Lafferty et al., 2008). The loss of fungal diversity may cause aggradation of aquatic ecosystems via the accumulation of CPOM and polymers, decline in macroinvertebrate food sources, reduction in the rate and range of decontamination of industrial toxins, diminished total diversity in planktonic communities and the development of fungal monocultures that would potentially impact on total biodiversity. Since, fungal biodiversity is representative of ecosystem functioning and thus of ecosystem health, it is in the interests of human society to explore the fungal biodiversity present in natural environments, especially aquatic habitats.

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CHAPTER

21

DISTRIBUTIONAL RECORDS OF SOME NOTEWORTHY MEMBERS BELONGING TO ORDER *BOLETALES* OF CORTICIOID FUNGI FROM DISTRICT CHAMBA OF HIMACHAL PRADESH

Dr. Poonam Sabrwal*

Aabstract

The present chapter deals with 6 taxa belonging to 3 genera (*Coniophora*, *Leucogyrophana* and *Serpula*) under 3 families (*Coniophoraceae*, *Hygrophoropsidaceae* and *Serpulaceae*) of order *Boletales* based on the specimens collected from various localities of Chamba district (Himachal Pradesh). This chapter describes *Coniophora arida*, *C. cordensis*, *C. puteana*, *Leucogyrophana mollusca*, *L. pseudomollusca* and *Serpula himantioides*. Of the taxa recorded, *Leucogyrophana pseudomollusca* (Parmasto) Parmasto have been described for first time from India while *Coniophora cordensis* S.S. Rattan and *Leucogyrophana mollusca* (Fr.) Pouzar are the new additions to the fungal diversity from Chamba district of Himachal Pradesh.

Keywords: *Agaricomycetes*, *Boletales*, *Basidiomycota*, Corticoid Fungi, Western Himalayas.

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Introduction

Order *Boletales* is a unique assemblage of corticioid fungi and is characterised by many peculiar features. Sporophores are usually resupinate, effused, loosely adnate; hymenium is mostly smooth. Hyphal system monomitic or dimitic. Generative hyphae without clamps, sometimes single or multiple clamps present on the basal hyphae, thin- to thick-walled, smooth to encrusted. Skeletal hyphae thick-walled, aseptate. Ancillary elements present or absent. Basidia varies from clavate to subclavate to subutriform, somewhat sinuous, without clamp at the base, 4-sterigmate. Basidiospores are smooth, ellipsoid to broadly ellipsoid to pip-shaped to ovoid, thick-walled, with or without oily contents, The basidiospore wall is stained in Cotton blue but not stained in Melzer's reagent.

Fungal forays were conducted in various sub divisions of Chamba district in the years 2013-2018 and quite a good number of specimens of Order *Boletales* were collected. These were identified and described as 6 taxa belonging to 3 genera of Order *Boletales* on the basis of macromorphological as well micromorphological features and comparison with the literature (Rattan, 1977; Dhingra, 1992; Singh, 2007; Dhingra et al. 2011 & 2014; Sharma, 2012; Kaur, 2012; Prasher and Ashok, 2013; Ranadive, 2013; Samita, 2014; Sharma, 2017; Poonam et al., 2017; Kaur, 2018). The present chapter aims to provide an account of Order *Boletales* from Chamba district (Himachal Pradesh) based on six species spread over three genera. These 6 taxa include *C. arida*, *C. cordensis*, *C. puteana*, *Leucogyrophana mollusca*, *L. pseudomollusca* and *Serpula himantioides*. Of the six taxa described *Leucogyrophana pseudomollusca* have been described for first time from India while *Coniophora cordensis* and *Leucogyrophana mollusca* are the new additions to the fungal diversity from Chamba district of Himachal Pradesh.

Materials and Methods

Present studies comprise of collections made from different localities of district Chamba (Himachal Pradesh) during fungal forays conducted in different localities of district Chamba in the years 2012-2018. The sporophores along with a portion of the host substrate were collected with the help of a hammer and a chisel. The details pertaining to type of hymenium, colour, margins etc., were noted attentively with the help of a hand lens. A wet piece of the sporophore was used to get the spore print on a glass slide. These specimens were dried either in sun or using an electric drier. The dried sporophores were packed in bond paper envelopes carrying a standard herbarium label with required information. All the specimens have been deposited at the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The micromorphological details of the collected specimens were observed by making crush mounts/vertical sections of the sporophores in water, 3% KOH solution, 1% phloxine, 1% cotton blue, 1% congo red and Melzer's reagent (0.5gm Iodine + 1.5gm KI + 20gm Chloral hydrate + 20ml Distilled water). The outline of the microscopic structures was drawn with the help of a camera lucida mounted on compound microscope at 100X, 400X and 1000X magnifications. The colour citations are as per Kerner and Wanscher (1978).

Family – Coniophoraceae Ulbr., Krypt.-Fl. Anfäng. 1 (3): 120, (1928).

Sporophores resupinate, adnate, effused; hymenial surface smooth. Hyphal system monomitic or dimitic. Generative hyphae without clamps, sometimes single or multiple clamps present on the basal hyphae. Skeletal hyphae thick-walled, aseptate. Ancillary elements present or absent. Basidia clavate to subclavate to subcylindrical to subutriform, 4–sterigmate, without clamp at the base. Basidiospores broadly ellipsoid to ovoid to pip shaped, thick-walled, smooth, positive to Cotton Blue and negative to Melzer's reagent.

Coniophora DC., Flore française 6: 34, (1815).

Sporophores resupinate, effused, loosely adnate; hymenial surface smooth. Hyphal system monomitic or dimitic. Generative hyphae without clamps, sometimes single or multiple clamps present on the basal hyphae, thin- to thick-walled, smooth to encrusted. Skeletal hyphae thick-walled, aseptate. Ancillary elements present or absent. Basidia clavate to subclavate to subutriform, somewhat sinuous, without clamp at the base, 4–sterigmate. Basidiospores ellipsoid to broadly ellipsoid to ovoid to pip-shaped, smooth, thick-walled, with or without oily contents, positive to Cotton Blue and negative to Melzer's reagent.

1. Coniophora arida (Fr.) P. Karst., Not. Sällsk. Fauna et Fl. Fenn. Förh. 9: 370, (1868). - *Thelephora arida* Fr., Elench. fung. 1: 197, (1828).

Sporophore annual, resupinate, adnate, effused that can reach a thickness of 300 µm thick in section; hymenium smooth when fresh as well as on drying, yellowish white to pale yellow to olivaceous brown when fresh and grayish yellow to light orange to brownish orange after drying; margins pruinose; yellowish white to pale yellow. Generative hyphae simple septate; dark brown to golden brown, horizontal, ≤ 5.5 µm wide, less branched, thick-walled, smooth in the subicular zone; subhyaline to pale yellow, vertical, ≤ 3.5 µm wide, richly branched, thin-walled, with crystalline encrustation in the subhymenial zone. Basidia varies from subclavate to subcylindrical, sinuous, $36\text{--}52 \times 5.6\text{--}6.7$ µm; sterigma ≤ 5 µm long. Basidiospores ranges from $10\text{--}12.2 \times 7.2\text{--}8.3$ µm, broadly ellipsoid to ovoid thin-walled, smooth, cyanophilous, inamyloid.

Collection examined – India, Himachal Pradesh: Chamba; Holi, on burnt stump of *Cedrus deodara*, Poonam 10498 (PUN), August 23, 2015.

Remarks – This species is a rereported species from district Chamba (H.P.). Previously it has been described by Rattan (1977) from districts Chamba, Kullu and Shimla of Himachal Pradesh and Jammu & Kashmir; Dhingra (1992) and Dhingra et al. (2011) from Eastern Himalaya; Singh (2007), Kaur (2012) and Kaur (2018) from district Shimla (H.P.); Prasher and Ashok (2013), Dhingra et al. (2014) from districts Chamba, Kullu and Shimla (Himachal Pradesh); Ranadive (2013) from Maharashtra; Sharma (2017) from Jammu & Kashmir.

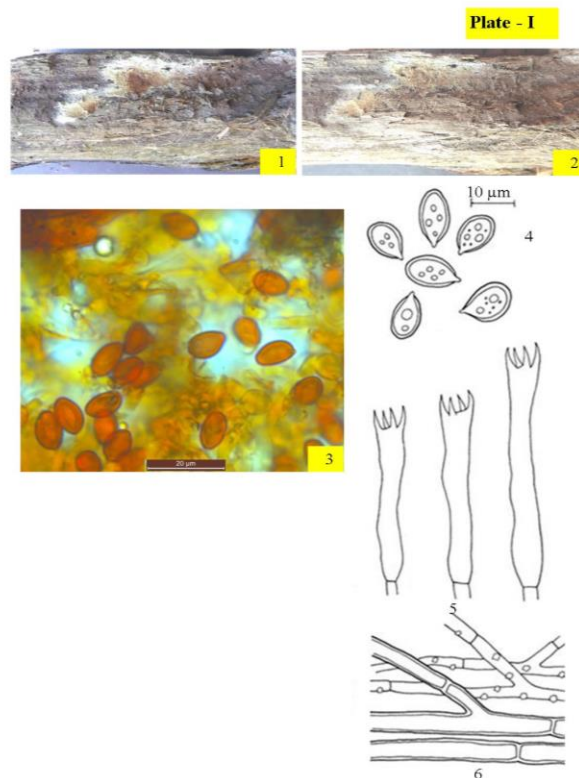


Fig. 1– 6. *Coniophora arida* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3. Photomicrograph showing Basidiospores; 4-6. Line diagrams [4. Basidiospores; 5. Basidia, 6. Generative hyphae].

2. *Coniophora cordensis* S.S. Rattan, Biblthca Mycol. 60: 78, (1977).

Sporophore annual, resupinate, adnate, effused that can reach a thickness of 280 μm thick in section; hymenium smooth both when fresh as well as on drying, pale orange to grayish orange when fresh and pale yellow to grayish orange to brownish orange on drying; margins fibrillose, paler concolorous when determinate; Hyphal strands present in the subiculum and margins. Generative hyphae simple septate; dark brown to golden brown, horizontal, ≤ 5.5 μm wide, less branched, thick-walled, smooth in the subicular zone; subhyaline to pale yellow, vertical, ≤ 3.5 μm wide, richly branched, thin-walled, with crystalline encrustation in the subhymenial zone. Basidia varies from clavate to subclavate, sinuous, $29\text{--}40 \times 7.7\text{--}9$ μm ; sterigma ≤ 6.6 μm long. Basidiospores ranges from $9\text{--}11 \times 5.6\text{--}6.5$ μm , broadly ellipsoid to ovoid thin-walled, smooth, cyanophilous, inamyloid.

Collections examined – India, Himachal Pradesh: Chamba; Holi, Nyagran, on stump of *Cedrus deodara*, Poonam 10500 (PUN), August 29, 2015; Churah, Bhandal, on stump of *C. deodara*, Poonam 10501 (PUN), August 15, 2014; Pangi, Saichu, on stump of *Pinus roxburghii*, Poonam 10502 (PUN), September, 11, 2016; Saichu, on stump of *P. roxburghii*, Poonam 10503 (PUN), September, 11, 2016.

Remarks – *C. cordensis* has earlier been reported Rattan (1977), Singh (2007), Kaur (2012), Prasher and Ashok (2013), Dhingra et al. (2014) and Kaur (2018) from district Shimla (H.P.) However, this is the first report from district Chamba.

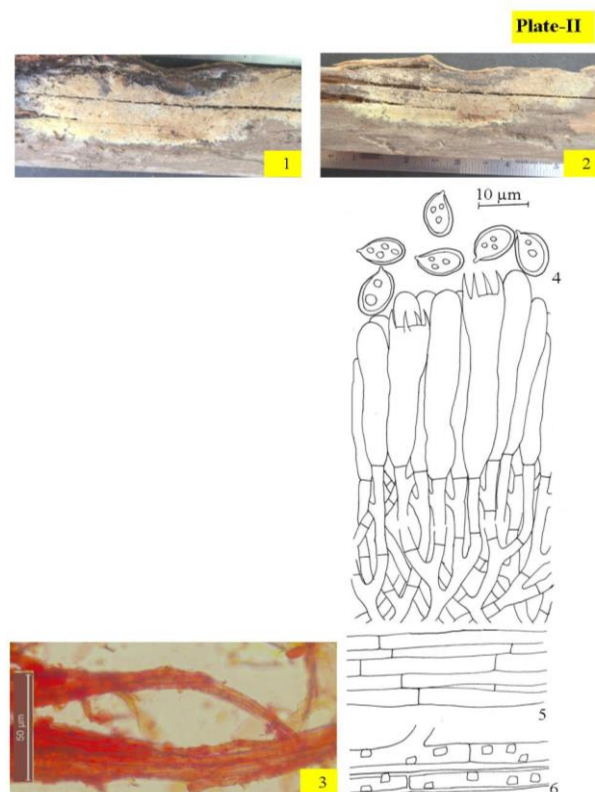


Fig. 1– 6. *Coniophora cordensis* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3. Photomicrograph showing Hyphal strands; 4-6. Line. diagrams [4. Basidiospores; 5. Reconstruction showing a portion of hymenium and subhymenium, 6. Generative hyphae].

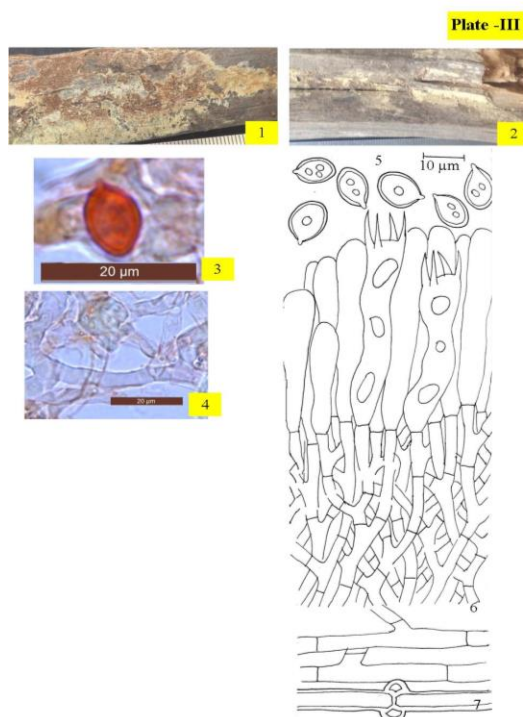
3. *Coniophora puteana* (Sahumach.) P. Karst., Not. Sällsk. Fauna et Fl. Fenn. Förh., II 6(6): 370, (1868). - *Thelophora puteana* Schumach., Enum. pl. (Kjbenhavn). 2: 397, (1803).

Sporophore annual, resupinate, adnate, effused that can reach a thickness of 300 μm thick in section; hymenium smooth both when fresh as well as on drying, pale yellow to light orange to olivaceous brown and grayish yellow to light orange to light brown on drying; margins fibrillose, paler concolorous. Generative hyphae smooth; brown to golden brown, horizontal, occasionally clamped (simple or multiple), $\leq 4.4 \mu\text{m}$ wide, less branched, thin- to thick-walled in the subicular zone; subhyaline to pale yellow, vertical, simple septate, $\leq 3.3 \mu\text{m}$ wide, richly branched, thin-walled in the subhymenial zone. Basidia varies from subclavate to subcylindrical, sinuous, without basal clamp, with oily contents, $40\text{--}47 \times 8.3\text{--}8.6 \mu\text{m}$; sterigma $\leq 7.2 \mu\text{m}$ long. Basidiospores ranges from $10\text{--}16 \times 7.2\text{--}8.8 \mu\text{m}$, broadly ellipsoid to ovoid thin-walled, smooth, cyanophilous, inamyloid.

Collections examined – India, Himachal Pradesh: Chamba; Churah, Langer, on stump of *Cedrus deodara*, Poonam 10170 (PUN), August 17, 2014; Langer, on stump of *C. deodara*, Poonam 10171 (PUN), August 17, 2014.

Remarks – This species differs from other described species in having single or multiple clamps on basal hyphae. It has earlier been described by Rattan (1977) from districts Chamba and Shimla (Himachal Pradesh); Dhingra & Singh (2009) from districts Kullu (Himachal Pradesh); Prasher and Ashok (2013); Dhingra et al. (2014) from Districts Chamba, Kullu and

Shimla (Himachal Pradesh); Sharma (2017) from Jammu & Kashmir and Devi (2019) from district Kangra (Himachal Pradesh).



Figs. 1–7. *Coniophora puteana* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidiospores, 4. Generative hyphae); 5-7. Line diagrams [5. Basidiospores; 6. Reconstruction showing a portion of hymenium and subhymenium, 7. Generative hyphae].

Family – *Hygrophoropsidaceae* Kühner, Bull. mens. Soc. linn. Lyon 49: 900, (1980).

Sporophores resupinate, loosely adnate, effused, pellicular; hymenial surface tuberculate to meruloid. Hyphal system monomitic. Generative hyphae with clamped septa. Ancillary elements absent. Hyphal strands present or absent. Basidia clavate to subclavate to subcylindrical, 4–sterigmate, with clamped septa at the base. Basidiospores ellipsoid to ovoid, smooth, positive to Cotton Blue and negative to Melzer’s reagent.

Leucogyrophana Pouzar, Česká Mykologie 12: 32, (1958).

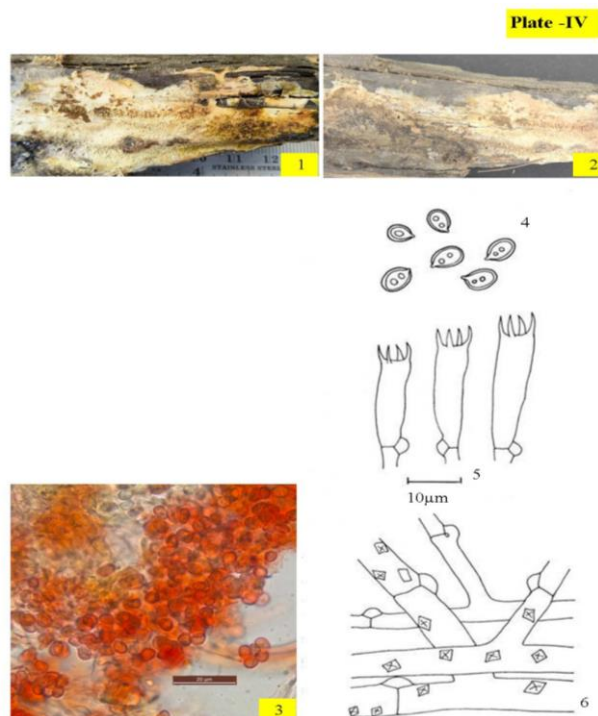
Sporophores resupinate, loosely adnate, effused, membranous to pellicular; hymenial surface tuberculate to meruloid. Hyphal system monomitic. Generative hyphae with clamped septa, thin- to thick-walled. Ancillary elements present or absent. Hyphal strands present or absent. Basidia clavate to narrowly clavate, with clamped septa at the base, 4–sterigmate. Basidiospores ellipsoid to ovoid, smooth, thick-walled, positive to Cotton Blue and negative to Melzer’s reagent.

1. ***Leucogyrophana mollusca*** (Fr.) Pouzar, Česká Mykol. 12: 33, (1958). - *Merulius molluscus* Fr., Systema Mycologicum 1: 329 (1821).

Sporophore annual, resupinate, adnate, effused, pellicular that can reach a thickness of 400 μm thick in section; hymenium merulioid when collected, reticulate on drying, orange white to pale orange to grayish orange to brownish orange when fresh and no major change on drying; margins fibrillose, paler concolorous when determinate Generative hyphae subhyaline, septate, clamped; horizontal, $\leq 7.2 \mu\text{m}$ wide, less branched, thin-walled, encrusted with double pyramidic crystals in the subicular zone; vertical, up to $\leq 4 \mu\text{m}$ wide, branched, thin-walled, smooth in the subhymenial zone. Basidia usually clavate, $20\text{--}27 \times 5.5\text{--}6.6 \mu\text{m}$; sterigma $\leq 5 \mu\text{m}$ long. Basidiospores ranges from $4.9\text{--}6.6 \times 3.8\text{--}4.9 \mu\text{m}$, ellipsoid to ovoid, thick-walled, smooth, cyanophilous, inamyloid.

Collections examined – India, Himachal Pradesh: Chamba, Pangi, Saichu, on stump of *Pinus roxburghii*, Poonam 8843 (PUN), September 11, 2016; Pangi, Saichu, on stump of *P. roxburghii*, Poonam 10347 (PUN), September 11, 2016; Saichu, on stump of *P. roxburghii*, Poonam 10348 (PUN), September 11, 2016; Saichu, on stump of *P. roxburghii*, Poonam 10349 (PUN), September 11, 2016.

Remarks – It is first report of this species from district Chamba. Previously it was reported by Rattan (1977) from district Kullu (Himachal Pradesh); Kaur (2012) from district Solan (Himachal Pradesh); Prasher and Ashok (2013) from district Shimla (Himachal Pradesh); Dhingra et al. (2014) from districts Kullu and Solan (Himachal Pradesh); Sharma (2012) from Uttarakhand and Sharma (2017) from Jammu & Kashmir.



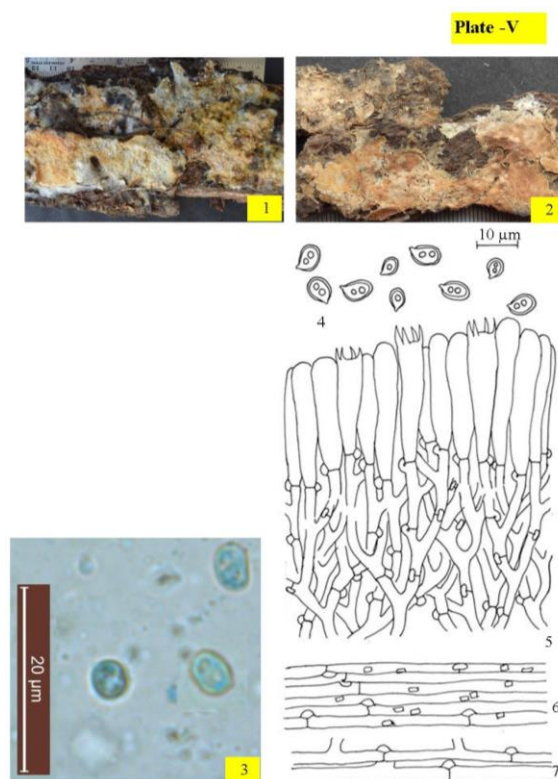
Figs. 1– 6. *Leucogyrophana mollusca* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3. Photomicrograph (Basidiospores); 4-6. Line diagrams (4. Basidiospores, 5. Basidium, 6. Generative hyphae)

2. *Leucogyrophana pseudomollusca* (Parmasto) Parmasto, Eesti NSV Teaduste Akadeemia Toimetised. 16: 386 (1967). - *Merulius pseudomolluscus* Parmasto, Bot. issled. II: 212(1962).

Sporophore annual, resupinate, loosely adnate, pellicular that can reach a thickness of 480 μm thick in section; hymenium merulioid when collected, reticulately folded while collected, smoothening to reticulate after; orange white to pale orange to grayish orange when collected grayish orange to brownish orange on drying; margins fibrillose, paler concolorous. Hyphal strands present in the subiculum and margins. Generative hyphae subhyaline, septate, clamped, thin-walled, smooth; horizontal, $\leq 7.2\ \mu\text{m}$ wide, less branched in the subicular zone; vertical, up to $\leq 2.8\ \mu\text{m}$ wide, branched in the subhymenial zone. Hyphal strands usually unbranched, $\leq 27\ \mu\text{m}$ wide, with individual hyphae $\leq 5\ \mu\text{m}$ wide, encrusted. Basidia usually narrowly clavate, $27\text{--}34 \times 6\text{--}6.6\ \mu\text{m}$; sterigma $\leq 5.5\ \mu\text{m}$ long. Basidiospores ranges from $5.5\text{--}7.7 \times 3.8\text{--}4.9\ \mu\text{m}$, ellipsoid to ovoid, thick-walled, smooth, cyanophilous, inamyloid.

Collections examined – India, Himachal Pradesh: Bharmour, Tosh ka got, on stump of *Pinus roxburghii*, Poonam 8847 (PUN), September 11, 2016; Chamba, Pangi, Saichu, on stump of *P. roxburghii*, Poonam 10350 (PUN); Bharmour, Tosh ka got, on stump of *P. roxburghii*, Poonam 10351 (PUN), September 11, 2016.

Remarks – This species differs from *Leucogyrophana mollusca* on the basis of presence of hyphal strands and larger basidiospores. It was described for the first time from India by Poonam et.al (2017).



Figs. 1– 7. *Leucogyrophana pseudomollusca* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3. Photomicrograph showing Basidiospores; 4. Basidiospores; 5. Reconstruction showing a portion of hymenium and subhymenium; 6. Hyphal strands, 7. Generative hyphae.

c. Family – *Serpulaceae* Jarosch & Bresinsky, in Jarosch, Bibliotheca Mycol. 191: 90, (2001).

Sporophores resupinate, loosely adnate, effused, reflexed to pileate, easily detachable; hymenial surface smooth to meruloid. Hyphal system dimitic. Generative hyphae with clamped septa. Skeletal hyphae thick-walled, aseptate, smooth. Ancillary elements absent. Basidia clavate to subclavate, somewhat sinuous, 4–sterigmate, with clamped septa at the base. Basidiospores ellipsoid to ovoid, smooth thick-walled, positive to Cotton Blue and negative to Melzer's reagent

Serpula (Pers.) Gray, A natural arrangement of British plants 1: 637, (1821).

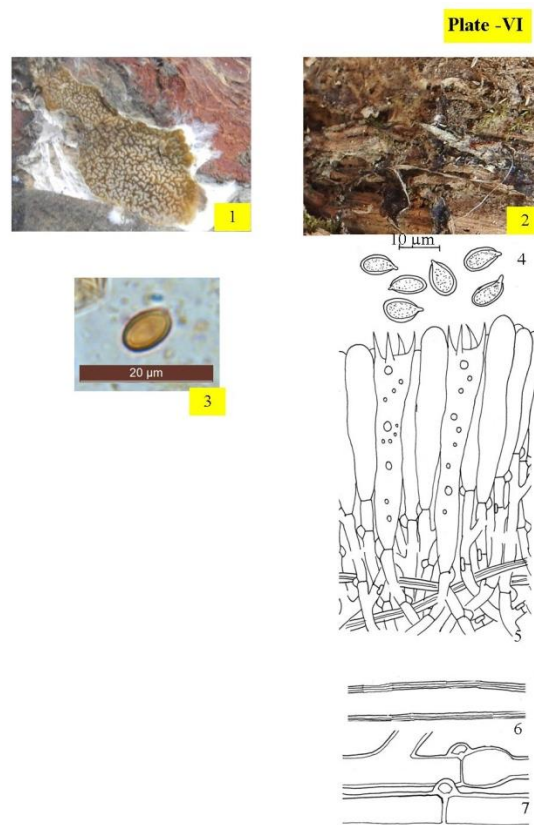
Sporophores resupinate, loosely adnate, effused, reflexed to pileate, soft, easily detachable; hymenial surface smooth to meruloid. Hyphal system dimitic. Generative hyphae with clamped septa, thin- to thick-walled, smooth. Skeletal hyphae thick-walled, aseptate, smooth. Ancillary elements absent. Basidia clavate to subclavate, somewhat sinuous, 4–sterigmate, with clamped septa at the base. Basidiospores ellipsoid to ovoid, smooth, thick-walled, positive to Cotton Blue and negative to Melzer's reagent.

Serpula himantioides (Fr.) P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 11: 137 (1884). - *Merulius himantioides* Fr., Observationes mycologicae 2: 238 (1818).

Sporophore annual, resupinate, adnate, effused that can reach a thickness of 300 µm thick in section; hymenium meruloid when collected, reticulately on drying, smoothening to reticulate after; orange white to pale orange to grayish orange when collected grayish orange to brownish orange on drying; margins fibrillose, whitish when collected, shrinking, less prominent after drying. Hyphal strands present in the subiculum and margins. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤ 9 µm wide, less branched, thick-walled, sometimes with ampullate septa in the subicular zone; vertical, ≤ 5 µm wide, richly branched, thin-walled in the subhymenial zone. Skeletal hyphae ≤ 4 µm wide, thick-walled, aseptate. Basidia usually clavate to subclavate, sinuous, 43–50 × 8–9 µm; sterigma ≤ 9 µm long. Basidiospores ranges from 8.8–9 × 4.9– 5.5 µm, ellipsoid to broadly ellipsoid, thick-walled, smooth, cyanophilous, inamyloid.

Collections examined – India, Himachal Pradesh: Chamba; Churah, Bhandal, on stump of *Picea smithiana*, Poonam 10109 (PUN), August 17, 2014; Dalhousie, Khajjiar, on stump of *Cedrus deodara*, Poonam 10467 (PUN), August 15, 2016.

Remarks – It is being described for the first time from tehsil Churah in the study area. Rattan (1977) described it from districts Chamba, Kullu and Shimla (Himachal Pradesh), Dhingra (1992) from Arunachal Pradesh; Lalji (2003) and Samita (2014) from Uttarakhand; Dhingra and Singh (2009) from districts Chamba and Sirmaur (Himachal Pradesh); Prashar et al. (2013) from districts Chamba Kullu and Shimla (Himachal Pradesh); Dhingra et al. (2014) from districts Chamba, Kullu, Sirmaur and Shimla (Himachal Pradesh) and Ranadive (2013) from Maharashtra.



Figs. 1– 7. *Serpula himantioides* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3. Photomicrograph showing basidiospore; 4-7. Line diagrams [4. Basidiospores; 5. Reconstruction showing a portion of hymenium and subhymenium; 6. Skeletal hyphae, 7. Generative hyphae]

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CHAPTER**22****EXPLORING ENDOPHYTIC FUNGI
ASSOCIATED WITH *PRUNUS*
SPECIES: AN OVERVIEW**

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A

bstract

The exploration of fungal interactions with diverse plant host species has gained significant traction in recent research, prominently featured in mycological literature. These investigations delve into the internal mycota residing within living plants. Due to the advantageous contributions offered by fungal endophytes in both agricultural and medical domains, extensive research efforts are being dedicated to isolating promising strains. In comparison to forest trees, there has been relatively limited inquiry into the fungal endophytes associated with fruit trees. This gap in knowledge prompts an increased focus on studying and understanding fungal endophytes within the genus *Prunus*. This comprehensive review article compiles a thorough examination of the various endophytic fungal species that have been isolated from different *Prunus* species.

Keywords: *Endophytic fungi, Prunus, tissue specificity.*

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Introduction

Endophytes, microorganisms including fungi and bacteria, reside within a host plant's intra or intercellular regions (Pan *et al.*, 2009; Bernardi-Wenzel *et al.*, 2010). Remarkably, they coexist with the host without causing apparent harm. The dynamics of the host-endophyte relationship are intricate and can range from simple mutualism to antagonism based on environmental conditions (Alvarez-Loayza *et al.*, 2011). Fungal endophytes enter host plants through damaged tissue, natural openings (such as stomata and lenticels), or by penetrating the cuticle. Subsequently, they invade various host tissues (Arnold *et al.*, 2003; Bernardi-Wenzel *et al.*, 2010).

Typically, the endophytic community within a host consists of a few dominant species alongside a diverse residual population. Some of these species display host specificity, while others have a broader range of hosts they can inhabit (Bernardi-Wenzel *et al.*, 2010; Rosa *et al.*, 2010; Pinto *et al.*, 2011). Over time, fungal endophytes adapt to their host plants, providing protection against both biotic and abiotic stresses. Certain endophytes act as potential antagonists against host pathogens, effectively mitigating, suppressing, or even inducing resistance to them (Larrán *et al.*, 2010). Furthermore, these endophytes possess the capacity to regulate plant growth and enhance productivity (Zabalgogea *et al.*, 2008; Rodríguez *et al.*, 2009). In addition, they synthesize bioactive compounds with biotechnological significance, such as antibiotics and antitumoral agents (Museti *et al.*, 2007).

In recent times, there has been a growing interest in the exploration of endophytic fungi associated with tree species. However, much of the research concerning fungal endophytes in fruit trees has predominantly focused on the above-ground components, including leaves, branches, and fruits (Novotný, 2007). Notably fewer investigations have been conducted regarding endophytic fungi found within *Prunus* species. The *Prunus* genus, which belongs to the Rosaceae family, encompasses numerous species of both horticultural and ornamental significance. It's noteworthy that the Rosaceae family ranks as the 19th largest plant family (Judd *et al.*, 1999; Hummer *et al.*, 2009; Hafiz *et al.*, 2015). The objective of this review is to accentuate the documented instances of fungal endophytes associated with various *Prunus* species. These species include *P. africana*, *P. avium*, *P. cerasus*, *P. domestica*, *P. grayana*, and *P. mandshurica*.

Fungal endophytes derived from *Prunus* species and subsequently identified

In a study conducted by Hortova and Novotny (2011), they investigated fungal endophytes present in the branches of sour cherry (*P. cerasus* L.) gathered from two locations (Těšetice and Lestkov) within the Czech Republic. They successfully isolated a total of fifteen species, including those with sterile mycelia. Notably, thirteen of these species were found in samples collected from both Těšetice and Lestkov. The findings revealed distinct prevalence patterns: Těšetice exhibited a population dominated by *Alternaria alternata* and *Coelomycete* sp. 1, whereas *Aureobasidium pullulans* and *Cladosporium herbarum* were prominent in Lestkov. Additionally, less frequently isolated species from both locations encompassed *Botrytis*

cinerea, *Cladosporium cladosporioides*, two other *Coelomycete* species, *Epicocum nigrum*, *Pezicula* sp., *Sarcinomyces* sp., as well as a sterile mycelium. Interestingly, *Coelomycete* sp. 3 and sterile dark mycelium were exclusively isolated from Těšetice, while *Pezicula* sp. was solely obtained from Lestkov. The study underscored that despite potential similarities in endophytic mycobiota composition between the study sites, noteworthy differences could arise in terms of their frequency.

Dugan and Roberts conducted a study in 1994 to explore the mycobiota present in *P. avium* L. fruits. Their investigation revealed the most frequently isolated fungi to be *Alternaria*, *Cladosporium*, and *Aureobasidium*. In 2016, Neda *et al.* identified 25 species of endophytic fungi from various tissues (root, twig, and leaf) of *P. avium* L. These species were classified into genera such as *Acremonium*, *Alternaria*, *Botryotinia*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Embellisia*, *Epicoccum*, *Fusarium*, *Glomerella*, *Macrophomina*, *Neonectria*, *Phoma*, *Diaporthe/Phomopsis*, *Pyronema*, *Rhizoctonia*, *Rhizopycnis*, *Rosellinia*, and *Xylaria*. Among these, *Alternaria* species constituted the majority with 51.7% of the total isolates, demonstrating their dominance. Following closely were *Fusarium* species (14.5%) and *Epicoccum* species (8.2%). Interestingly, these findings from Neda *et al.* (2016) displayed similarities with earlier research by Hortova and Novotny in 2011, suggesting a shared endophytic mycobiota between sour and sweet cherry species. Another study conducted by Aghdam *et al.* (2016), focused on fungal endophytes isolated from healthy twigs and leaves of both *P. avium* and *P. cerasus* trees in Iran. In addition to confirming previous findings, this study unveiled four new fungal species: *Coniothyrium olivaceum*, *Collophora paarla*, *Sarocladium strictum*, and *Quambalaria cyanescens*.

Pimenta *et al.* (2012) conducted a study on foliar fungal endophytes associated with *P. domestica* L. In their research, they identified 29 distinct morphotypes that were isolated from groups of leaves at different stages of maturity. These morphotypes encompassed 14 different species, out of which 9 species were genetically characterized. These identified species included *Phaeosphaeria nodorum*, *Sordaria macrospora*, *Chaetomium globosum*, *Gibberella moniliformis*, *Gibberella zeae*, *Alternaria brassicicola*, *Botryotinia fuckeliana*, *Glomerella graminicola*, and *Pyrenophora teres*.

The dominant species among these was *Phaeosphaeria nodorum*, accounting for 86.5% of the total isolates. Following this, *Sordaria macrospora* was the next most prevalent species, constituting 4.3% of the isolates. The distribution of isolated fungi varied across different leaf maturity stages. For mature leaves, 59 fungi representing five species were isolated. Medium-age leaves yielded 57 fungi representing six species, while the youngest leaves produced 47 fungi representing eight species. The research revealed an interesting pattern: the frequency of isolated fungi increased with the age of the leaves, suggesting a preference for older foliage. However, the number of species present was highest in younger leaves and decreased as the leaves matured.

Kato *et al.* (2016). conducted a study focusing on the foliar endophytic fungi present in four-month-old seedlings of *P. grayana* Maxim., cultivated within a cool temperate deciduous forest in northeastern Japan. The investigation revealed the isolation of 22 distinct fungal taxa from these seedlings. Notably, among these, two species of *Colletotrichum* were identified

and found to be consistently prevalent. Additionally, various other species were isolated, yet their exact identities remained unidentified. The research further highlighted that beyond abiotic factors like light intensity, temperature, and precipitation, as previously noted in studies by Elamo *et al.* (1999), Osono and Mori (2004), Bahnweg *et al.* (2005), Unterseher *et al.* (2007), Hashizume *et al.* (2010), Alvarez-Loayza *et al.* (2011), and Lau *et al.* (2013), other elements such as the specific canopy tree species, the degree of canopy openness, and the time elapsed after germination could significantly influence the composition of endophytic fungal communities in tree seedlings positioned beneath the canopy tree.

Ntuba-Jua *et al.* (2017) conducted a study on the diversity of endophytic fungi within various sources of *P. africana* (Hook. F.) Kalkman. All examined leaf segments exhibited fungal colonies, resulting in a 100% colonization rate. The isolated fungal species totalled 56, with 44 belonging to 27 distinct genera, while 12 species consisted of non-sporulating mycelia. The identified genera encompassed *Acremonium*, *Alternaria*, *Aschochyta*, *Aspergillus*, *Bispora*, *Cephalosporium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Gloecephalis*, *Gloeosporium*, *Glomerella*, *Lasiodiplodia*, *Mycogone*, *Nigrospora*, *Oidium*, *Penicillium*, *Pestalotia*, *Phomopsis*, *Phoma*, *Paecilomyces*, *Phyllosticta*, and *Trichoderma*. Among these, *Lasiodiplodia* sp. stood out as the most frequently encountered species, whereas *Penicillium* sp. and *Gloeosporium* sp. exhibited the lowest occurrence rates. Other notable dominant genera included *Colletotrichum*, *Aspergillus*, *Trichoderma*, *Curvularia*, *Fusarium*, and *Pestalotia*.

In their investigation, Nekrasov *et al.* (2022) delved into the endophytic fungal interactions present within the young shoots of *P. mandshurica* (Maxim.) Koehne. The study yielded a total of 12 distinct fungal species: *Alternaria alternata*, *A. tenuissima*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. herbarum*, *Diaporthe eres*, *Epicoccum nigrum*, *Fusarium graminearum*, *F. oxysporum*, *Nothophoma quercina*, *Sarocladium strictum*, *Tripaspermum myrti*, and *Gliocladium* sp.

Prominently, *A. alternata*, *N. quercina*, and *D. eres* emerged as the most frequently encountered species within the shoots. These were closely followed by *A. tenuissima* and *F. oxysporum* in terms of isolation frequency. Conversely, the remaining species exhibited a lower occurrence rate, ranging from rare to occasional within the studied samples.

Localization of endophytic fungi in specific tissues

Several plant species have been documented in the literature to exhibit a tissue-specific correlation with endophytic fungi, as highlighted in the work by Berg *et al.* (2015). In the research carried out by Neda *et al.* (2016) involving *P. avium*, the roots, shoots, and leaves were found to harbour *Alternaria*, *Fusarium*, and *Epicoccum*. *Cladosporium* was identified in both roots and leaves, while various other endophytes displayed specificity to certain tissues. Notably, *Acremonium*, *Davidiella*, and *Paraphoma* were exclusive to the roots, *Macrophomina* and *Chetomium* were isolated from the shoots, and *Rosellinia*, *Xylaria*, and *Pyronema* were uniquely present in the leaves. In the case of *P. africana*, a considerable number of endophytic fungal species were primarily associated with the leaves. These included *Colletotrichum*, *Phomopsis* sp., *Phoma* sp., *Paecilomyces* sp., *Acremonium* sp.,

Geotrichum sp., *Gloeosporium* sp., *Alternaria* sp., *Cephalosporium* sp., *Gloeosporium* sp., *Nigrospora* sp., and *Odium* sp. Conversely, *Bispora* sp. and *Cladosporium* sp. were exclusively confined to the seeds, while *Penicillium* sp. was solely isolated from the bark, according to the findings of Ntuba-Jua *et al.* (2017).

Conclusion

In conclusion, existing research has focused on the endophytic fungal connections with a limited number of *Prunus* species. These studies reveal that most of the identified fungal species are shared among the examined plant species, albeit with varying isolation rates. This observation finds support in the discoveries made by Dugan and Roberts (1994), who noted that the composition of endophytic mycobiota in fruit trees can differ widely based on the tree species under investigation. Nevertheless, the extent of diversity in endophytic genera and species among fruit trees remains consistent. The comprehensive review sheds light on the diverse array of fungal endophytes linked with different *Prunus* species, as well as their preference for specific tissues.

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CHAPTER

23

A COMPREHENSIVE REVIEW ON AMAZING AND BENEFICIAL APPLICATIONS OF LICHEN

Ishika Singh and Dr. Yashwant Kumar*

A Abstract

Lichens are fascinating but less understood life forms; they are diverse, attractive, beautiful, and can thrive in most unfavorable environments where fungi and algae alone cannot survive. Lichen accounts as pioneer species as they colonize environments and play a significant role in establishing and functioning of natural ecosystems. They contribute greatly for environment as they can alert us when ecosystems are at trouble. Lichens are wonderful organisms which have inspired researchers to investigate about various current and potential applications in different fields. Examination of lichen's secondary metabolites; their potential health benefits in terms of medicine and pharmaceuticals; lichen sensitivity towards polluted environments and their role as bio-indicators of air pollution and heavy metal accumulators; examination of consumable lichens for food and fodder; use of lichens in textile industry cosmetic industry; extraction of essential oils, perfumes and incense from lichens are some of the current uses of lichens discussed in this review. However, still future possibilities for lichen research might always be the point of interest.

Keywords: Lichen, Pollution, Heavy Metals, Sulfur Dioxide, Nitrogen, Environment.

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Introduction

Lichens are estimated to cover about 7% of the Earth's geographical area, which are known to be composite life forms accounting as miniature ecosystem showing symbiotic association between and a fungus (mycobiont) and a green alga or blue-green alga/cyanobacteria (phycobiont/photobiont) living in a mutualistic relationship. The fungal component is a dominant partner which absorbs water nutrients and minerals, providing shelter and majority of the characteristics to lichens like shape of thallus, fruiting bodies, etc. while algal component is a chlorophyll-containing partner which sustains inside the fungus, providing constant food and nutrition to them (Elkhateeb et al., 2022). The estimated number of lichen species by the year 2000 ranged from 13,500 to 30,000 which may rise slightly with the expansion of research (Calcott et al, 2018). Lichen plays a significant role in the establishment and evolution of ecosystems as they can thrive in both favorable (plateau regions, seashores, grasslands, rain forests, etc.) and unfavorable (alpine zone, polar region, tundra, deserts, etc.) biological environments (Molnar & Farkas, 2010).

Lichens can be frequently found on tree barks, exposed rocks, soil crusts, etc., due to their capability of colonizing a wide range of surfaces (Zhao et al., 2021). Lichens have various forms and structures which are broadly categorized into three main categories based on their growth forms and body types including: Fruticose lichen resembles like hanging fruits or pendant and can be hair or cup-like (*Usnea sp.*); Foliose lichens resembles flat, little, leafy plants (*Umbilicaria* and *Lobaria sp.*); Crustose lichens creates colorful crusts on rock and tree surfaces (*Rhizocarpon sp.*); while some of them are even between these above three types (*Claodnia sp.*) (Elkhateeb et al., 2022).

Lichen as bio-indicator of air pollution and heavy metals

Lichens Receive all their nutrients directly from the atmosphere as they do not have roots, hence cannot filter what they absorb, thus pollutants are able to penetrate and affect their community which makes them toxic. Lichen shows sensitivity towards air pollution which makes them great air quality indicators as they have a remarkable capacity to absorb nutrients from extremely dilute sources and take in a variety of harmful compounds from the atmosphere, such as heavy metals, nitrogen, and sulfur dioxide. Growth and activity of lichens can be affected by two main air pollutants; nitrogen and sulfur dioxide. Nitrogen is important for the growth and survival of lichens as it helps them create essential proteins and organic acids which they need. Every form of nitrogen is utilized by lichen, i.e., nitrogen dioxide (NO₂), nitric oxide (NO), and gaseous nitrogen (N₂). In addition, cyanobacteria perform nitrogen fixation which changes nitrogen into ammonia (NH₃) which is a more stable and usable form, but lichen species also show ammonia sensitivity. Studies have mentioned that several lichen species become over fertilized in presence of excessive nitrogen causing chlorophyll present in alga to die; depriving the fungi of essential carbohydrates which they need for survival. The presence of sulfur dioxide can also trigger sensitivity in lichens. High level of SO₂ prevents cyanobacteria from fixing nitrogen and damage algae's chlorophyll, which inhibits photosynthesis, lichen reproduction and spore germination or causes death of phycobiont. As the fungal component cannot survive on its own, this results in the death of

the entire system. A large number of lichen species are extremely vulnerable to air pollution and are known to be the bio-indicators of air quality and can be used as “permanent control systems for assessment of air pollution”. According to some research studies, crustose lichens are more resistant to air pollution than fruticose lichens. In the presence of nitrogen, certain lichens will perish while others will flourish. For example: Ammonia-rich environments are suitable for *Xanthoria parietina* (golden shield lichen), frequently occurs on trees and structures close to farms. *Usnea* sp. (old man's beard lichen) does not flourish in locations where SO₂ is present. (Breeze Technologies; National Parl Services; Natural History Museum; U.S. Forest Services).

Not all lichen species exhibit sensitivity towards heavy metal pollutants, but some of them are efficient bio-accumulators of elements and trace elements, also pollution-tolerant lichens accumulate heavy metals in significant amounts. By measuring the quantity of trace elements that have been accumulated within lichens over time, they can be utilized as bio-monitors of various pollutants and heavy metals. The ability of several lichen species to gather airborne metals like Cu, Cd, Pb, Ni, and Zn in their thallus has also been discovered. Lichen species *Lecanora conizaeoides* Nyl. is acknowledged as a typical pollution-tolerant for conducting studies on air toxicity in Europe. *Pyxine cocola*(Sw.)Nyl. in tropical Asian nations, is regarded as an efficient monitor and accumulator of pollutants. *Pyxine cocola* have also been utilized by employing the transplant procedure in order to analyze the accumulation of heavy metals in the commercial, industrial, and residential regions of Lucknow, India. This species also demonstrates its capability of accumulating heavy elements like arsenic and fluoride as well as Al, As, Cu, Fe, and Zn (Elkhateeb et al., 2022; Zhao et al., 2021).

Lichen used as food and nutritional source for humans

Even though there are numerous lichen species worldwide but only some of them are known to be both equally edible and nutritious. The presence of mildly poisonous secondary chemicals and polysaccharides in lichens which are indigestible to humans are two issues with lichen consumption. Still there is a comprehensive list of numerous species of lichen that have been used as food source by humans as well as also used as energy source during famines, as they are also a great source of certain enzymes, vitamins, and polysaccharides.

Cetraria islandica (Iceland moss) was once a staple diet for people in northern Europe and Scandinavian nations like Iceland, Sweden, Norway; which could be prepared in various types, including salad, soup, bread, porridge or pudding. Various lichen species can be used as spices or spice mixes for flavouring various dishes like some *Parmelia* sp. are used as curry powder in India. *Parmotrema perlatum* is used as a prominent ingredient in spices blends or mixes in potli masala from Uttar Pradesh; bhojwar masala, kaala masala, garam masala, and goda masala from Hyderabad, India. Some lichens species like *Everniastrum cirrhatum*, *Rimelia reticulate*, *Ramalina conduplicans*, *Parmotrema tinctorum*, and *Platismatia glauca* are also used as spices in India, Niger and the Middle East. *Lecanora esculenta* is used as food source by people living in Japan, Israel and Middle Eastern deserts. Some lichen species are also used as an ingredient in the confectionary industry to make pastries and chocolates in France (). *Umbilicaria esculenta* (Iwa-take) which is an expensive lichen, collected from the edges of cliffs and used as an ingredient in numerous traditional Japanese and Korean dishes. In some regions of North America, *Bryoria fremontii* was used as a staple dish that was

typically cooked in a pit. Lichen species, *Lethariella cladonioides*, *Thamnolia subuliformis*, and *Thamnolia subuliformis* can be used to prepare tea, drinks or beverages. A powder of lichen, *Cetraria islandica* is used to prepare alcohol by fermentation process. Lichen species which were processed as ingredients to make special bread to counter food shortages during certain times in Estonia, Iceland, Norway, and Czech Republic are *Cetraria islandica*, *Cladonia rangiferina*, and *Evernia prunastri*. A common emergency food source in North America is *Lasalia spp.* and *Umbilicaria spp.* (rock tripe) (Zhao et al., 2021; Elkhateeb et al., 2022).

Lichen used as food and fodder for animals

Many species of lichen also serve as important food source for invertebrates and for numerous creatures like caterpillars, mites, snails, termites, slugs, etc., which sustain entirely or mostly on lichen; and as fodder for many animals. In arctic regions, lichen which accounts as the main food source for caribou and reindeers is *Cladonia rangiferina* (Reindeer moss); *Cetraria islandica* (Iceland moss) is often served as fodder for horses. Various lichen species like *Evernia*, *Lecanora*, *Parmelia*, and *Stereocaulon* are utilized as fodder for animals (Elkhateeb et al., 2022).

Metabolites and antibiotics from lichen used as medicines in pharma industry

The primary metabolites of lichen serve a vital role in structural processes as well as cellular metabolism and most of these metabolites are water-soluble intracellular byproducts. It includes: polysaccharides, amino acids, vitamins, pigments, polyols, and chitin. Over 1000 secondary metabolites have so far been identified in lichen which is unique to lichen-forming fungi. The majority of these are categorized as: phenolic compounds, dibenzofurans (usnic acid), depsides (barbatic acid), depsidones (salazinic acid), depsones (picrolichenic acid), lactones (nephrosterinic acid, protolichesterinic acid), quinones (parietin), and pulvinic acid derivatives (vulpinic acid). The lichen's secondary metabolites are crucial for self-defense (including properties like: antioxidant, anti-microbial, photo-protectiveness, herbivore resistance, etc.), niche competitiveness as well as pollution and metal resistance (Moln'ar & Farkas, 2010). It also exhibits a wide range of biological properties which includes anti-inflammatory, antibacterial, analgesic, enzyme inhibition, anti-proliferation, plant growth inhibition, and cytotoxicity (Zambare & Christopher, 2012). The presence of secondary metabolites in lichens has great therapeutic value and exhibits wide range of health-promoting properties like antibiotic, antioxidant, anti-inflammatory, analgesic, antiviral, anti-cancerous, antipyretic, anti-mycobacterial, anti-proliferative, and others have been reported (Boustie & Grube, 2005).

Lichens have been employed in numerous traditional medicine systems of China and India, homeopathic as well as western medicine systems. Many medical conditions and diseases, like leprosy, constipation, arthritis, kidney problems, pharyngitis rabies, worm infestation, alopecia, infections, etc., can be treated using lichens. In traditional medicine system, lichen species have been used to treat skin conditions, heal wounds, digestive & respiratory problems, gynecological and obstetrical issues, etc. For example: The most popular lichen

genus used in traditional medicine is *Usnea sp.*; *Xanthoparmelia scabrosa*, is a component of several aphrodisiac preparations. *Peltigera leucophlebia* (ruffled freckled pelt) was used to treat candidiasis/thrush disease. *Cetraria islandica* has been utilized for treating mild oral and pharyngeal mucosal irritation, dyspepsia, and appetite loss. *Cetraria islandica* is used to treat cancer; while *Lobaria pulmonaria* (lungwort lichen) used to treat tuberculosis and asthma in European traditional medicine. In Indian Medicine System, *Parmelia chinense* powder is used to treat wounds, liquid for headaches and as diuretic drug; *Parmelia sancti-angeli* is used to cure a condition similar to ringworm. In Nepal, *Parmelia nepalense* is used to heal toothaches and sore throats. *Usnea longissima* exhibits natural antitumor & antioxidant properties (Zhao et al., 2021; Elkhateeb et al., 2022).

Perfumes, incense and essential oils extracted from lichen used in cosmetic industry

Lichens contain various aromatic substances in their thallus hence, employed as a component in the production of various cosmetic products, toothpaste, salves, perfumes, scented items like hawan samagri, dhoop, etc. In Japan, lichens are also utilized in paints. Additionally, extracts from lichen along with various botanicals are used as popular ingredients in personal care products like baby creams, face and body lotions, etc. According to some studies, an estimate concludes that, lichen-derived chemicals have been used in numerous (>90) colognes, deodorants, and, perfumes (Sheu et al., 2006). Usnic acid is known as most used lichen-derived product. In both Europe and USA, lichen-derived products are widely used. An acid extract from lichen, *Evernia prunastri* is used as an aromatic component in scents and fragrances. Additionally, lichens like *Pseudorina*, *Evernia* and *Ramalina* contain fragrant & volatile essential oils.

Dyes extracted from lichen used in textile industry

Lichen plays a significant role in textile industry as they have been used for extraction of natural dyes used to dye materials which accounts for great commercial and economic value. Numerous lichen species synthesize secondary substances, pigments, poisons or toxins that keep herbivores away but these substances have been economically used as dyes and also help in identification of lichens. They are frequently used to make dyes as they can produce various colors, including yellow, brown, green, orange, purple, red, and reddish-brown. Dye extraction technique involves boiling of lichens in water, combined with water/pine sap alone or first burned to ash before, which are utilized for dyeing fabrics, furnitures, etc. *Parmelia saxatilis*, *Lobaria pulmonaria*, *Parmotrema*, and *Umbilicaria* are a few examples (Devkota et al., 2017).

Researchers have reported that there are over 2,000-year-old accounts of lichens being used by ancient people to create red and purple dyes and utilizing them for coloring their wool and silk. Lichen family Roccellaceae has a significant historical and economical value; its various species are used for extraction of dye which is used to dye silk, wool, wood, leather, feathers, and, marble (Cardon, 2007). *Roccella* and *Lecanora/Ochlerechia sp.* are known to have orchil (red dye) and litmus (purple-violet dye). *Roccella tinctoria* (dyer's weed lichen) is used

for extraction of pH indicator dye known as litmus by boiling, which detects substances as acid or base (Rather et al., 2016). A lichen species, *Parmotrema crinitum* is also used to extract dye. The synthetic equivalents have now largely replaced red dye (orcein) and other dyes obtained by various lichen species. Lichen species, *Ochrolechia tartarea*, *Lasallia pustulata*, *Xanthoria parietina* (common orange lichen) and *Parmelia saxatilis* (crottle grey lichen) found in the Scottish Highlands and are used for extraction of dyes and traditionally dyeing wool for making Harris Tweed and other fabrics (Cardon, 2007).

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CHAPTER

24

ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN ALLEVIATING HEAVY METAL STRESS FROM AGRICULTURAL SOIL

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A**bstract**

Heavy metal stress inhibits growth of plants and efficiency; environmental change and agricultural malpractices like insufficient fertiliser and pesticide use have caused these effects and damaged the ecosystem; there is an urgent need for environmentally friendly management techniques like the use of arbuscular mycorrhizal fungi for boosting crop productivity. Arbuscular mycorrhizal fungus associations are well known for promoting plant growth on severely disturbed sites, particularly those that are contaminated with heavy metals. AMF associations are essential, functional components of plant roots. According to reports, they play a significant role in metal tolerance and accumulation and are seen on the roots of plants growing in heavy metal contaminated soils. The majority of research on heavy metal toxicity to soil microbes has focused on effects where microbial function loss can be seen; however, the effects on biodiversity within microbial populations and communities have received little attention. This review emphasises how HM-contaminated soils and AMF interact.

Keywords: *Mycorrhizal Fungi, Heavy Metal, Contaminated Soil, Plant Growth, Mineral Nutrition.*

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Introduction

Arbuscular mycorrhizal fungi (AMF) mediate a series of complex communication events between plants and fungi to promote host plants to thrive under stress conditions increasing the rate of photosynthesis, water uptake and other gas exchange-related traits (Birhane et al., 2012). Many authors have reported that AM fungi are responsible for resistance to various types of stresses like drought, salinity, herbivores, temperature, metals, and diseases caused. (Rodriguez et al., 2008; Ahanger et al., 2014; Salam et al., 2017). The interdependent relationship between AMF and plants is so important that it is estimated that 90% of plant species on Earth form this type of relationship. AMF association is mainly found in flowering plants, but also associated Gymnosperms, bryophytes, and ferns (Zhu et al., 2010; Ahanger et al., 2014). AMF symbiosis forms vesicles, arbuscules and hyphae inside roots and also forms spores and hyphae in the rhizospheric soil of plants. AM fungal hyphae formed network in the rhizospheric soil. The plant roots significantly improve the ability of roots to enter a large surface area of the soil, thus enhancing plant growth (Bowles et al., 2016). AMF improves plant nutrition by increasing the availability and translocation of various nutrients (Rouphael et al., 2015). AMF association is helpful in improving soil quality by changing soil texture and structure, and finally affects plant health (Zou et al., 2016; Thirkell et al., 2017). AM fungal hyphae can accelerate the process of decomposition of organic matter present in soil (Paterson et al., 2016). In addition, mycorrhizal fungi can affect the fixation of atmospheric carbon dioxide by hostplants, increasing the "sinking of effect" and the movement of photo assimilates from aerial part of plant to roots. Considering the importance of AMF and the research progress related to its application in agriculture. This chapter is mainly focus on the role of AMF is helpful in alleviating heavy metal stress from agricultural soil and act as biofertilizer. AMF can promote plant growth in heavy metal contaminated soil. Heavy metal (HM) pollution that is a global environmental problem that has attracted much attention in recent years.

HM pollution is a potential threat to ecosystems and human health, and has been recognized as one of the most serious threats to the sustainability of agricultural ecosystems (Wang et al., 2018; Kamran et al., 2019; Wang et al., 2019). Human activities discharge hazardous wastes into soil and water, greatly accelerating the process of environmental pollution.

HM is not biodegradable, and their excessive concentration in the soil will enter the food chain, such as "cadmium rice" and "toxic vegetables", which are important issues related to human health and safety (Huang et al., 2018; Gong and Tian, 2019). On heavy metals, the lead content should not be more than 0.2 mg per kg, arsenic not more than 0.05 mg per kg, cadmium not more than 0.1 mg per kg, and tin not more than 5.0 mg per kg.

HM can accumulate in grains and become part of the food chain. Once they are introduced into the human diet, they will cause many human health problems (Fan et al., 2018; Zhang et al., 2019;).Once HM enter the environment, they will stay there for many years. The Ministry of Environmental Protection (MEP) and the Ministry of Land and Energy (MLR) recently announced the current status of soil pollution in China. Agricultural plantations growing in unfavourable environments face various types of abiotic stress from heavy metals. All these factors will not only reduce the growth of plants, but also make the environment more

vulnerable to threats to the survival of plant populations. In plants, metal stress interferes with plant metabolism and slows plant growth by reducing the absorption of essential macro- and micronutrients (Wu et al., 2014; Zhao et al., 2015; Turan, 2021). In addition, the toxicity of HM affects plant cell mechanisms, such as inhibiting root growth, changing regulatory mechanisms, and accumulating reactive oxygen species, hindering the absorption of essential nutrients and leading to lipid peroxidation in membranes, which can lead to plant cell death in severe cases (Saleem et al., 2021; Seneviratne et al., 2019; Dixit and Hering, 2015). Previous reports indicate that vascular plants have adopted various strategies to mitigate the negative effects of toxic metals through sequestration, leakage, isolation or detoxification (Hu et al., 2013). Some plants that do not have the ability to detoxify HMs, these toxic metals are transferred to the aerial parts of the plant. The high concentration of HMs in the edible parts of plants can pose a serious threat to the food chain and ultimately to human health. Therefore, there is an urgent need to eliminate or at least reduce the negative impact of heavy metals in contaminated agricultural soils to ensure environmental safety and human health (Yang et al., 2019; Sun et al., 2019).

Bioremediation and phytoremediation are the most important and appropriate strategies to remove HM from ecosystems (Lebeau et al., 2008). Plant extraction and stabilization are the most studied phytoremediation mechanisms. In the plant extraction process, the plants accumulate HM in the aerial parts of the plant by eliminating HM from the soil (Pajević et al., 2016; Miransari, 2011), while the second method immobilizes the HMs in the plant roots.

Heavy Metal Contaminated Soil and Microbial Population

In the agricultural soil different types of microbes are present, which are responsible for nitrogen fixation, assimilation and degradation of organic matter present in soil, to release mineral nutrients (Baath et al., 1997; Brookes, 1995). If the heavy metals remain in the soil due to continuous and uncontrolled additions, then they alter these important biochemical processes and change the ecological balance. The toxic effects of HM on microorganisms are manifested in many aspects, such as the reduction of litter decomposition and nitrogen fixation, the reduction of the efficiency of the nutrient cycle (Baath et al., 1997) and the deterioration of the synthesis and enzymatic activity in soil, sediments and water. Due to their relationship to soil functions, soil microbial populations and activities have been proposed as useful indicators of soil improvement and degradation. Furthermore, the activity of soil enzymes is considered a sensitive and early indicator of natural and man-made disturbances (Giller et al., 1998). Those studies highlight the importance of metal-microbe interactions and microbial processes from sorption and desorption of metals on the cell surfaces to reduction/oxidation phenomena in determining the fate of heavy metals in the environment. In addition to soil microbial activity and functions can be determined by molecular fingerprinting methods can also provide qualitative and quantitative measurements of microbial diversity and community composition in uncontaminated and contaminated soils (White et al., 1998), due to their heritage of microbial genes present in the studied state system (Head et al., 1998; Van Elsas et al., 1998). The microbial community present in soil is considered to be a sensitive biological indicator of metal pollution mainly on bioavailability and biogeochemical processes (Hinojosa et al., 2005). It has been shown that certain

concentrations of HMs will have long-term toxic effects on the ecosystem and have obvious negative effects on biologically mediated soil processes (Lee et al., 2002). In general, the accumulation of HMs in the soil will reduce the soil microbial biomass (Brookes and McGrath, 1984; Chander et al., 1995) and the activities of several enzymes, resulting in a decrease in functional diversity. Changes in soil ecosystem and microbial community structure (Frostegard et al., 1993). However, metal exposure can also lead to the development of metal tolerant microbial populations (Ellis et al., 2003).

Tolerance and Adaptation of AMF against Heavy Metals

Resistance is the ability of microbes to resist the generally effective effects of contaminants on them, while tolerance refers to the ability of microbes to adapt to the persistence for contaminants. The literature proposes a series of "classical" ecological principles that explain the process of increasing tolerance or resistance in the community (Boivin et al., 2002). According to Leyval et al., (2001) tolerance and resistance to the toxic effects of heavy metals depend on the mechanisms involved. In summary, as described in epidemiological studies (Foster and Hall, 1990; Tosun et al., 2005), tolerance to metals can be defined as a phenomenon by which microorganisms increase the toxicity of exposure to heavy metal resistance to pressure. The metal tolerance of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi have been analysed by different methods, including: AM spore number, root colonization, and abundance of ECM fruiting bodies (Weissenhorn et al., 1993, 1994; Del Val et al., 1999). But these methods do not provide information on the conditions, limits, and thresholds that ensure the survival and growth of AMF, nor do they provide information on the genetic basis of polymetallic resistance and tolerance. Furthermore, AMF coexists with other microbes present in the rhizospheric soil plants, that can tolerate and accumulate the toxic heavy metals. It can confuse with the actual interaction between AMF and the metals present in the environment. Now days, to assess the tolerance of microbes of soils contaminated with metals, experts have adopted the concept of community pollution tolerance method (PICT) (Nikli Ěska et al., 2006). They showed that long-term exposure to Zn imposes stress on soil microbes, they develop tolerance. They summarized that the long-term accumulation of zinc in the soil is responsible for the microbial community to adapt for this metal. In fact, it is often found that the microbial community recovers after being initially inhibited by a large number of metals (Khodaverdiloo et al., 2011). First of all, due to the immobilization reaction that occurs in the rhizosphere, the availability of metals gradually declines. Mycorrhizal fungi have also been shown to be related to metal plants in highly contaminated soil. However, it should be mentioned that AMF inoculation can be totally repressed under specific severe metal circumstances (Weissenhorn et al., 1994). According to Delval et al. (1999) the number of spores decreased as the amount of heavy metal increased, while in soils with moderate sludge pollution, species richness and diversity increased, but declined in soils where sludge pollution from heavy metals was the highest. According to several observations and remarks, AMF from metal-contaminated locations has adapted successfully and evolved a tolerance for metal toxicity (Weissenhorn et al., 1993, 1994; Leyval et al., 2001; Toler et al., 2005; Sudova et al., 2007). Mycorrhizal fungi have also been shown to be related to metal plants in highly contaminated soil. However, It should be

mentioned that AMF inoculation can be totally repressed under specific severe metal circumstances (Weissenhorn et al., 1994). According to Delval et al. (1999), when the quantity of HMs increase, the number of spores dropped, while in soils with moderate sludge pollution, species richness and diversity increased, but decreased in soils with the highest heavy metal pollution rate of sludge. Due to the toxicity of heavy metal, concentration of heavy metals may reduce the number and viability of HM (Dixon, 1988), or it may have no effect on the colonization of mycorrhizae (Leyval et al., 1997). AM fungi long-term colonisation behaviour in soil contaminated with metals was examined by Biro et al. (2005) under regulated conditions. The soil used has been collected after 12 years of the metal adaptation process. Among them are 13 types of four gradients (0, 30, 90, and 270 mg/kg of dry soil), trace element salts including Al, As, Ba, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sr, and Zn were present. Barley (*Hordeum vulgare* Linn.) was used as the test plant. They found a strong dose-dependence on the overall arbuscular abundance. It has been discovered that the AMF sporulation is the process that is most vulnerable to long-term metal stress (loid). They claimed that the amount of AM fungi Spores was greatly reduced by Al, As, Ba, Cd, Cr, Cu, Pb, Se, Sr, and Zn. At present title is known about the possible mechanism of AM fungi and metal interaction, the cellular and molecular mechanisms of AM fungi tolerance to heavy metal contaminated soil (Leyval et al., 2001; Martin et al., 2007). The mobilisation of intracellular deposits or the tolerance and absorption of HM in extracellular media are mediated by metal transporters and plant-encoded transporters, respectively (Göhre et al., 2006; Hildebrandt et al., 2007). According to Göhre et al., 2006 hypothesis metal may be released at the front fibre interface and subsequently taken up by plant-encoded transporters.

AMF- Induced Increase of Nutrient in the Host Plants

Furthermore fungal hyphae also hydrolyses organic phosphorus. After absorbing the hyphae of the fungi the inorganic phosphorus is transported to the structure of the fungus in the root and immediately transferred to the arbuscular area in the arbuscular cells. The intracellular hyphae of AMF penetrate the epidermis of the root and the cortex of the host cell and exchange nutrients with the host forming a special structure called arbuscular (Kobae, 2019; Bonfante et al., 2012). However, in most plant species the mycorrhizal pathway of zinc absorption is challenging due to the close interaction between phosphorus and zinc nutrition (Ryan et al; 2003; Watts-Williams et al; 2015). Mycorrhizal plant content will rise as a result of a lack of P, Zn, K, and other micronutrients. However, due to the high supply of P the ability of AM to absorb Zn and other nutrients may be reduced due to the low rate of mycorrhiza colonization (WattsWilliamset al; 2014). Data from more than 200 studies indicate that AM has a positive effect on iron nutrition, although this has a significant effect on plant roots (Lehmann et al., 2015). A recent survey (Cui et al; 2019) confirmed that AMF can reduce the toxicity of Cd in soybeans. AMF promotes the distribution of mineral elements in soybean shoots and roots. AMF not only increases P but also Mg and compared to non-mycorrhizal plants AMF-inoculated soybean plants have higher magnesium uptake indicating that they have more protection against cadmium stress because magnesium is primarily involved in abiotic stress tolerance (Ferrol et al., 2016). AMF (*Glomus caledonium*) reduces arsenic in the soil and increases phosphorus uptake leading to increased biomass of the maize plant compared to non-mycorrhizal plants (Bai et al; 2008). Calonne Salmon (2018)

studied the effects of *Rhizophagus truncatula* in *Medicago truncatula* on phosphorus uptake and promotion of plant biomass in a circulating semi-hydroponic culture system with polycyclic aromatic hydrocarbons and plants inoculated with AMF have higher (Orthophosphate) Pi uptake is higher than that of non-mycorrhizal plants. This higher Pi uptake leads to higher plant growth and provides tolerance to the plant. Finally, the effectiveness of this interaction varies according to the plant and the AMF species (Janos; 2007), the fungal isolates the characteristics of the soil and the type of toxic metal and the degree of toxicity of the metal.

Impact of AM Fungi in Phytoremediation of Heavy Metal Contaminated Soils

The nature of some plants accumulates a lot of heavy metals. Although hyperaccumulator plants are widely used for plant extraction their biomass is generally low which does not lead to phytoremediation of HM-contaminated soil. In other words phytoremediation tends to take a long time mainly due to the low bioavailability of heavy metals in the soil or the low biomass of hyper-accumulating plants (Khodaverdiloo et al., 2011). The affinity of the soil for the adsorption of a particular metal, which in turn depends on the properties of the soil, provides a significant impact on the mobility and bioavailability of HMs as well as their potential phytotoxicity (Khodaverdiloo et al., 2011). The low bioavailability of cationic heavy metals is more important in young calcareous soils of low development in arid semiarid areas. These soils have a high affinity for irreversibly adsorbed HMs most likely precipitated by metallic carbonates (Khodaverdiloo et al., 2011). Numerous studies have demonstrated that AMF can lessen host plants' uptake of metals. Hence shielding them from the toxicity of heavy metals (Leyval et al; 1997; Zaidi et al., 2017).

Soil disturbance generally reduces host plant spore numbers and subsequent root colonization (Waaland et al; 1987). However, AMF species that have adapted to the local soil conditions can promote plant development more effectively than non-native species. Indigenous AMF ecotypes are the result of long-term adaptation to soils with exceptional features. (Rahmanian et al., 2011). Therefore, a promising biotechnological approach to successfully restore damaged ecosystems is the inoculation of plants with local AMF that can be applied under pressure (Mathur et al., 2007). According to Lasat (2002), the plant and the metal may have different effects on how well metallic roots are absorbed. Higher root length density and possibly more hyphae allow plants to explore larger volumes of soil thereby increasing the availability of cations (metals) that are not available to non-mycorrhizal plants (Mohammad et al; 1995).

The introduction of microbes resistant to heavy metal can significantly reduce plant biomass, as demonstrated by (Rahmanian et al; 2011). They attributed the reduction to the fact that plants have easier access to the relatively fixed Cd present in the soil and more absorption of metal pollutants caused by microorganisms (Karimi et al., 2011). It was a study that found that co-inoculating AM fungi and bacteria that promote plant development (PGPR) led to greater absorption and accumulation of Cd and Zn by corn (*Zea mays*) compared to sterile

conditions. However, at a given level of soil contamination plants accumulate higher levels of Cd and Zn in PGPR-inoculated soil than in AM fungi inoculated soil. They concluded that PGPR is more effective than AM fungi for the co-mediation of Cd and Zn in plant microbial systems. Inoculation of arbuscular mycorrhizal fungi (AMF) with remediating plants has been found to accelerate the phytoremediation process by enhancing plant biomass and their metal accumulation potential while improving the soil physicochemical and biological characteristics.

AMF may promote plant metal extraction when metal concentrations are low in soils and also help plants to accumulate a major chunk of toxic metals in plant roots to stop their transfer to aerial components when soils contain a lot of metals. Singh et al. (2019) investigated the effects of inoculating four AM fungi species with *Zea mays* on the removal of Cr, Cd, Ni, and Pb from the tannery sludge. These species included *Rhizophagus fasciculatus*, *R. intraradices*, *Funneliformis mosseae*, and *Glomus aggregatum*. All four AMF species, they found, increased metal buildup in the roots but lowered it in the shoots. In comparison to the non-inoculated control plants, the metal translocation factor was much lower. These findings provide crucial proof of AM fungal potential to improve metal Phyto stabilization. The effects of two AM fungi, *F. mosseae* and *R. intraradices*, on plant growth-related parameters, *Pb* accumulation, photosynthesis, and antioxidant enzymatic activity in *Robinia pseudoacacia* were also assessed by Yang et al. (2020).

The fact that inoculated plants had enhanced biomass, photosynthetic pigment, gas exchange capability, and different enzymatic activity implies that both AM fungi species were able to shield plants from cellular damage by removing ROS under Pb stress. These two AMF species have the capacity to promote metal Phyto stabilization, as evidenced by the lowered Pb concentration in the leaves of *R. pseudoacacia* that has been infected with AM fungi.

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CHAPTER

25

A COMPREHENSIVE STUDY OF BIOLOGICAL PROPERTIES OF FUNGAL ENDOPHYTES: A REVIEW

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A**bstract**

A wide variety of microorganisms lives inside plants and are colonized there. Many of these bacteria do not directly affect their hosts manner that's why they known as endophytes. Endophytic fungi are abundant in secondary metabolites, which are biologically active substances in higher plants. The fungal derivatives are essential to human life, and their components are the main source of medicines for viral, microbial, and cancerous illnesses. The natural compounds isolated from the endophytes acts as growth inhibitor for plant pathogenic organism. Resources from endophytes are used in the pharmaceutical and agricultural industries as well as in phytoremediation.

Keywords: *Abiotic Stress, Drug Resistance, Phytoremediation, Symbiosis, Siderophores, Xenobiotics.*

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Introduction

De Bary (1886) invented the word "endophytes," which translates from the Greek as "plants within" or "endophytes." Symbiotic to pathogenic relationships between endophytes and plants exist. By preventing harmful organisms from invading, endophytes are beneficial to the host plant. It has been split into two main categories, the clavicipitaceous and non-clavicipitaceous, based on variances in its evolution, taxonomy, plant host, and ecological activities. Only a few species of grass are susceptible to clavicipitaceous infection, in contrast to nonclavicipitaceous, which is present in the asymptomatic tissues of other higher plants. (Rodriguez et al. 2008).

Endophytes have just recently been thoroughly investigated for their potential as novel sources of strong new medications. Penicillin, which comes from the fungus *Penicillium notatum*, and *Bacitracin*, which comes from the common bacterium *Bacillus subtilis*, are only two examples of the valuable and powerful therapies for both fungi and bacteria, have given to contemporary medicine or pharmaceuticals.

Additionally, a Pacific Yew tree endophyte produces the powerful chemotherapy drug Taxol. Given that so many bacteria are evolving resistance to some of the present medications, it is very crucial to find new treatments. Endophytic fungi are a group of fungi that colonize the inner and living tissues of plants without immediately causing harm. Recent research has shown how common these fungi are; Endophytic funguses, which live inside of plants and even lichen, are thought to exist in 1 million different species. Endophytic fungi are recognized to have an impact on the structure, diversity, and community of plants and make up a sizeable and quantitative portion of the fungal biodiversity.

How do endophytes work?

There are several variants to Petrini's definition, which states that "all organisms inhabiting plant organs that can colonize internal plant tissues without causing apparent harm to the host" are included in the list of definitions that are most frequently used. De Bary coined the specialized word "endophyte," which was used for a while to refer to "any organisms occurring within plant tissues." or "A group that colonizes living, internal plant tissues without having an immediate negative impact" or "Endophytes are any fungi isolated from internal symptomless plant tissues" or "Fungi and bacteria that enter the tissues of living plants and cause undetectable infections that only affect plant tissues, with no disease-related symptoms."

Endophytes studies' historical development

Freeman wrote one of the earliest publications describing an endophytic fungus in 1904, citing four additional papers on endophytes that were published in 1898. This study examined a fungus found in Persian darnel, an annual grass that many wheat growers now view as a dangerous plant. Similar asymptomatic endophytes were discovered in a wide variety of grasses between 1930 and 1990 as a result of various findings made on related investigations. Reports on endophytes are also available in between Reports on endophytes are also available from 1990 to 2000 on endophytes from Europe, including endophytes from palms, grasses, and woody plants. Similar contributions to knowledge of endophytes have been published by several authors from 2000-2012.

Occurrence and Biodiversity of Endophytes

Darwin was the first to propose the connection between fungal diversity and impacts on ecosystem processes. Numerous theoretical models and experimental studies contributed significantly to diversity, including improvements in primary productivity, nutrient retention, nutrient flow, water accessibility, and disease invasion resistance. The variety of benefits that endophytes offer to their hosts is taken into account when determining their diversity, in addition to the hosts' morphology and uniqueness. Endophytes have now been isolated from all types of plants, including lichens, big trees, palms, and sea grasses. The quantity of endophyte strains and species varies widely and is strongly influenced by the scope of the investigation. Few scientists reported that, (i) the numbers of endophyte depend on how much care, time and Petri-dishes are used in a study; a meticulous researcher will laboriously isolate Thousands of strains and consequently more species (ii) temperate plants yield many communities of endophytes as compared tropical plants; and (iii) different tissues may yield diverse endophyte communities. However, Rungjindamai et al. observed that some endophytes may also be classed into Basidiomycetes. The majority of isolated endophytes have been classified as Ascomycetes. However, there were significant differences in their rates of plant colonization and isolation of endophytic fungi. More endophytic fungi are present in some medicinal plants than others.

Endophytic fungi and host plant interactions

There are many different types of connections between fungal endophytes and their host plants, ranging from symbiotic to hostile or somewhat harmful. Endophytes may produce potential substances used in agriculture, industry and modern medicine such as novel antibiotics, antimycotics, immunosuppressant and anticancer compounds. Additionally, research on endophytic fungi and their interactions with hosts will help us understand the ecology and evolution of both endophytes and their hosts, including the development of plant symbioses with endophytes and the ecological factors that affect the direction and intensity of this interaction. About 6500 endophytic fungi were isolated from trees and herbaceous plants by Schulz et al., who then screened them for physiologically active substances. They discovered a connection between biotope and biological activity.

Methods for Isolation of Endophytes

The microbes are found in the tissues' intercellular gaps as well as maybe inside the plant cells. More recently, after plant samples in the form of leaves, stems, or portions of roots have been collected, the other endophytes are encouraged to leave the host and to grow on Petri plates containing agar medium, and later in nutrient-rich liquid media. In this context, structural tools like X-ray and electron diffraction are not very useful. Small plant samples, such as leaves, stems, or roots, are typically collected from the field and transported to a lab in plastic bags. The admission of non-endophytic bacteria into the sample of dead plants should be carefully controlled. By soaking in 70% ethanol, plant surfaces are sterilized in the lab to get rid of any microbial epiphytes. Following a week of incubation at room temperature, the hyphal ends of the fungal growth can be observed oozing from the plant sample. This is done within the laminar-flow hood using a sterilized knife. The microbial colonies are repeatedly re-plated until a pure culture is formed. The minute cutting of these

growths is then transplanted onto new water agar plates or onto more nutrient-rich potato dextrose plates.

Endophyte Identification

Based on the morphology of the hyphae or colony in the fungal culture, the traits of the spores, and the reproductive structures, endophytic fungal strains can be identified morphologically. If these characteristics were distinct, the identities of selected main groups were then confirmed using molecular techniques in accordance with the steps used by Promptuttha et al. The fungi strains were individually inoculated on potato dextrose agar, potato carrot agar, and water agar in Petri dishes to induce sporulation. The slides were later mounted in lactophenol and sealed with nail varnish or parafilm after all fungal features were measured in water mounts. At least twice each in every experiment and observation. According to their cultural traits, the cultures that were unable to sporulate were classified as sterile mycelia and separated into various species. It is a typical issue associated with endophytes that some fungal isolates could not be recognized down to the species or genus level.

However, polymerase chain reaction (PCR) and subsequent DNA sequencing are the most recent approaches to identifying endophytic fungus. Utilizing primers that amplify DNA encoding ribosomal RNA, the PCR can be carried out on cultivated endophytes. The best method for identifying fungal endophytes is through the amplification of Internal Transcribed Spacer (ITS) sections, which are repetitive DNA structures that code for ribosomal RNA. The AFTOL [Assembly of the Fungal Tree of Life] project and Genbank both provide very extensive databases of sequences for these constantly evolving regions. The 18S RNA is another sequence that can occasionally be helpful in classifying different fungi. In comparison to ITS sequence data, there is less variability in this sequence between species, particularly among families. Since many of the fungi isolated from plants are expected to be novel, it is Pandi et al.: needful to have primers covering as many taxonomic groups as possible. Identifying the species can be done by sequencing the amplified DNA and comparing it to the database; if the sequence is known.

Anticancer Activity

Taxol is a diterpenoid secondary metabolite with strong functionalization that is frequently used as an anticancer medication (Wani et al. 1971). It was initially separated from the western yew's bark, *Taxus brevifolia*. During the cell division, taxol prevents the depolymerization of tubulin. Production of taxol from different genus of endophytes by fermentation is thoroughly cheaper method (Page et al. 1999). Numerous genera of endophytic fungus, including *Alternaria*, *Fusarium*, *Monochaetia*, *Pestalotia*, *Pestalotiopsis*, *Pithomyces*, and *Taxomyces*, have been identified to contain the anticancer agent taxol (Strobel et al. 1996). More than 15 fungi genera were produced paclitaxel and its analogues (Zhao et al. 2010). According to Pandi et al. (2011), a breast cancer cell line was sensitive to the taxol that was isolated from *Lasiodiplodiatheobromae*. The main mycotoxin medication generated by the fungus genus *Alternaria* is called alternariol 9-methyl ether.

It has the ability to cause DNA strand breakage, micronuclei, and gene alterations in a variety of cultured mammalian cells, as well as mitochondrial death in human colon cancer cells (Meng et al. 2012). Giridharan et al. (2012) discovered that colon cancer cells undergo

apoptosis when exposed to sclerotiorin, an isolated compound from *Cephalotheca faveolata*, which has anti-proliferative properties. *Fusarium species* and *Aspergillus fumigatus* endophytic extracts displayed inhibitory action against HeLa cervix cancer cell lines (Ruma et al. 2013). The endophytic fungi, *A. terreus* showed cytotoxic effect against HepG2 cancer cell line (Suja et al. 2014). *Phoma* sp. developed polyketide compounds with strong anti-mouse leukemia cell activity (Santiago et al. 2014). *Alternaria alternata* ethyl acetate extract shown good cytotoxicity as well as anticancer efficacy against human breast cancer cell lines. (Arivudainambi et al. 2014). Chen et al (2015) reported that cytochalasins from endophytic fungi, *Phomamultistrata* exhibited moderate anticancer activity against SMMC-7721 (hepatocellular carcinoma cell line), SK-BR-3 (breast cancer cell line), PANC-1 (pancreatic cancer cell line), HL-60 (human myeloid leukemia cell line) and A-549 (lung cancer cell line).

Antimicrobial Activity

Increased drug-resistant germs and fungi are causing health issues around the world. Because many pathogenic germs have been exposed to the same class of medicines incorrectly or repeatedly, they have become resistant to them. Intensive searches for many newer and more effective antibiotics that can deal with lethal problems is underway now a days. A dynamic area of research is the separation of new secondary metabolites from endophytes (Huang et al. 2008). Fungal endophytes such as *Cladosporium* sp., *Cylindrocarpon* sp., *Saussureain volucrata*, *Fusarium solani*, *Cordyceps memorabilis*, *P. longicolla* and *Dothideomycete* sp., *Phaeosphaeria avenaria*, *Leptosphaeria* sp., *Fusarium* sp., *P.chrysanthemicola*, are found to have antimicrobial activity against human pathogenic bacteria and fungi such as *Candida tropicalis*, *C. albicans*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*, *Shigella flexneri*, *S. epidermidis*, *Cryptococcus neoformans*, *Micrococcus luteus*, *Enterococcus shirae*, *Aspergillus fumigatus* and *Salmonella typhi* (Lv et al. 2010; Tayung et al. 2011; Rhoden et al. 2012).

The *Phomopsis* sp. showed antibacterial efficacy against *C. albicans*, *B. subtilis*, *M. luteus*, *K. pneumoniae*, and *E. coli*. (Jayanthi et al. 2011). Silva et al. (2011) reported that secondary metabolites of *A. niger*, *Curvularia pallescens*, *Guignardia bidwelii*, *Paecilomyces variotii* and *Mycelia sterilia* showed antibacterial activity against *S. aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *M. luteus*, *E. coli* and *Pseudomonas aeruginosa*. *Gibberella* sp., exhibited the antimicrobial activity against *S. aureus* and *C. neoformans* (Liang et al. 2012). According to Bungihian et al. (2013), the extracts from *Colletotrichum gloeosporioides* and *Chaetomium globosum* have antibacterial effects on *Mycobacterium TB*, *Gordonia terrae*, *S. aureus* and *E. coli*. *Streptococcus pyogenes* and *Enterococcus faecalis* were also susceptible to the antibacterial effects of the extracts derived from *C. gloeosporioides* (Nath et al. 2014). *S. aureus* and *Bacillus subtilis* were resistant to the extract from *Diaporthe arengae* (Patil et al. 2014). The earlier research clearly shows that Endophytic fungi derived compounds are being used in the development of antimicrobial drug.

Insecticidal Activity

Plants that have endophytic fungus can defend themselves against infections and pests (Arnold et al. 2003). By creating secondary metabolites such as alkaloids that are harmful to insects and animals, foliar endophytes can minimize herbivory. (Scharidl 2001). The

endophytic fungus *P. oblonga* were originally used by Webber (1981) to defend elm trees from the *Physocnemum brevilineum* insect. The endophytic fungi (*Acremonium coenophialum*) shows insecticidal activity against aphids (*Rhopalosiphum padi*, *Schizaphis graminum*) and milkweed bug (*Oncopeltus fasciatus*) (Johnson et al. 1985). *Cladosporium herbarum*, *A. alternata*, *Rhodotorula rubra*, *Epicoccum nigrum*, *Cryptococcus* sp., *Penicillium* sp., *Fusarium graminearum* were act as protectants to plants from herbivores (Larran et al. 2002; dos santos et al. 2003). The diverse entomopathogenic fungi genera *Acremonium beauveria*, *Cladosporium clonostachys*, and *Paecilomyces*, among which *B. bassiana* and *Clonostachys rosea* were pathogenic to coffee berry borer (Vega et al. 2008), were isolated from the coffee plants. *B. bassiana* was found to control the borer insects in coffee seedlings (Posada and Vega 2006). Baskar et al. (2012) stated that *B. bassiana* isolated from Pulegy exhibited larvicidal and growth inhibitory activities against *Spodoptera litura* gossypii Glover (Hemiptera: Aphididae) is significantly insecticidal to the endophytic fungi *Claviceps purpurea* (Shi et al. 2013). Senthilkumar et al. (2014), Phomopsis species-specific phytochemicals utilized as insecticides include dodecanoic acid, ethylester, phthalic acid, and octyl 2- pentyl ester. The *Cladosporium oxysporum* showed insecticidal activity against *A. Fabae* (Bensaci et al. 2015). When topically administered to *S. litura* larvae, extracts from *Emericella nidulans*, *A. oryzae*, *A. tamarii*, and *A. versicolor* were reported to have insecticidal action in all four cases.

Plant Growth Stimulants

Endophytic metabolites offers many varieties of fitness to host plants boosted by boosting plant tolerance to biotic and abiotic challenges. As a result, endophytes can actively or passively promote the plant growth through a variety of methods. Numerous endophytes can solubilize phosphate, improve phosphorus (P) absorption, fix nitrogen, produce siderophores, and produce plant hormones like ethylene, gibberellins, and indole acetic acid (IAA), auxin, abscising, which are important for plant growth regulations (Malinowski and Belesky 2000; Boddey et al. 2003; Loiret et al. 2004, Goodman et al. 1986; Barraquio et al. 1997; Sandhiya et al. 2005, Firáková et al. 2007, Malinowski et al. 1999; Zou et al. 1999;). Gibberellic acid (GA) is a potent phytohormone that regulates plant growth. Fungal endophyte, *Cladosporium sphaerospermum* from the plant, *Glycine max* (L) Merr. produce Gibberellic Acid 3, Gibberellic Acid 4 and Gibberellic Acid 7. It induces plant growth in rice and soybean (Hamayun et al. 2009). In contrast to *Distylium chinense* seeds, a pestalotin analogue isolated from the *Pestalotiopsis* microspore significantly increased germination rate (85.56%) and demonstrated gibberellins activity (Li et al. 2015). The *Fusarium tricinctum* and *Alternaria alternata* derivatives of indole acetic acid enhanced the plant growth (Khan et al. 2015).

Crop Protection

Endophytic fungi are also capable of enhancing resistance to diseases, and many mechanisms are responsible for this resistance. The processes of endophyte-induced resistance are linked to the host's nutritional condition and aim to improve plant fitness by increasing their ability to withstand as abiotic stress (Aguilar and Barea 1996). Endophytic fungi *Colletotrichum* sp., *Cryptosporiopsis* cf. *Quercina* are found effective against phytopathogens such as *Rhizoctonia cerealis*, *Phytophthora capsici*, *Pyricularia oryzae*, *Gaeumannomyces graminis* (Lu et al. 2000, Li et al. 2000;).

Phytoremediation

The goal of phytoremediation is to reduce the damage to land and water brought on by too much toxic organic pesticide, environmental deterioration, improved industrial sewage, dangerous fumes, and biodiversity loss. Endophytes are frequently utilized in biological control to eradicate pests, diseases, and environmental toxins (Guo et al. 2008). Endophytes are used in phytoremediation, which is the removal of heavy metals and xenobiotic from the environment using plants as a medium (Ma et al. 2011). However, the dominance of endophytes as in the form of phytoremediation depends on the tolerating and accumulating capacity of microbes of plants in high concentrations of pollutant, because microorganisms are capable to bioaccumulate heavy metals and many other pollutants from the environment, or because they can promote plant development and pollutant uptake from soil by plants through mobilization or immobilization of pollutants (Ma et al. 2011). Plants, *Festuca arundinaceaschreb* and *F. Pratensis* Huds are infected with endophytic fungi *Neotyphodium coenophialum* and *N. uncinatum* and uninfected plants are grown on petroleum contaminated soil. When uninfected plants are compared with endophyte infected plants, we can observe that the root and shoot biomass is more in endophyte infected plants (Soleimani et al. 2010a). There may be a direct or indirect function in phytoremediation, with the former occurring indirectly by promoting the growth of plants with the capacity for phytoremediation and accelerating the process, and the latter occurring directly by degrading and accumulating pollutants on its own. The *Neotyphodium* endophytes enhance the Cadmium tolerance activity in couple of plant species of *Festuca arundinacea* and *Festuca pratensis* when compared to non-endophyte infected plants (Soleimani et al. 2010b). *Penicillium funiculosum*, a fungus that mediates stress, proved efficient against copper stress and promoted plant development. It can also be employed in bioremediation of pollutants in farmed areas (Khan and Lee 2013).

Conclusion

Endophytic fungi are used in many different industries. Endophytes have the ability to create a wide variety of bioactive substances. Endophytic fungus can create secondary metabolites that can function as biocontrol agents. A possible source for many pharmacological compounds may be endophytic fungi that have been identified from medicinal plants. It is expensive and time-consuming to isolate a component from plants and produce a product on a large scale. However, endophytic fungi derived from medicinal plants are able to create useful chemicals and are simple to cultivate in big quantities via fermentation. Future medicinal, agricultural, and other businesses would have access to inexpensive endophytic fungus products. Because of this, Role will undoubtedly isolate more new chemicals as a result of study on endophytic fungus.

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A

bstract

This study related to indoor air quality (IAQ) and its profound implications for human health and well-being. With people spending the majority of their leisure time within the confines of their homes, the significance of IAQ cannot be underestimated. This study specifically focuses on the impact of biological pollutants, notably fungi, on indoor air quality and its consequences. Biological pollutants, particularly fungi, are found to be major contributors to the deterioration of IAQ. They can induce a range of allergic reactions, including asthma, rhinitis, conjunctivitis, urticaria, and atopic dermatitis, depending on the immune response elicited. These reactions highlight the need for a deeper understanding of the role of fungi in indoor environments. The associated symptoms encompass a wide array of neurotoxic effects, acute and chronic health disorders, including cancer. Mycotoxins pose a significant health risk, particularly through inhalation exposure, adding urgency to the need for proper IAQ management. It advocates for proactive measures, including improved ventilation, humidity control, and mold inspection, to mitigate the health risks associated with indoor air pollution. Education and awareness about the potential hazards of IAQ are critical for public health, aiming to create and maintain healthier indoor environments that enhance the quality of life and reduce health risks for individuals.

Keywords: *Asthma, Rhinitis, Conjunctivitis, Urticaria, Atopic Dermatitis, Neurotoxic, Mycotoxins.*

Introduction

The significance of indoor air quality (IAQ) cannot be understated, given that people spend approximately 90% of their leisure time within the confines of their homes. Surprisingly, the awareness of indoor air pollution has often been eclipsed by concerns about outdoor air pollution. It was not until the period between 1978 and 2016 that a mere fourteen international conferences were convened to address issues surrounding indoor air quality (Smith et al., 2018). As early as 1984, the World Health Organization (WHO) published a report indicating that up to 30% of buildings worldwide could be subjected to complaints regarding their indoor air quality (WHO, 1984). Alarming as this may be, the problem has not abated in recent years; if anything, it has exacerbated, as evidenced by the latest WHO report that provides guidelines on indoor air quality, with a focus on moisture and mold (WHO, 2020). It is estimated that indoor humidity issues afflict anywhere from 10% to 50% of indoor spaces in highly developed countries across Europe and beyond (Mendell et al., 2011). Furthermore, the increasing incidence of respiratory problems associated with residing in houses plagued by moisture-related issues and the presence of microorganisms in various building materials is a cause for concern (Flappan et al., 2015).

Allergic Reactions of Fungi

The presence of fungi in residential settings is often linked to allergic reactions (Gergen et al., 2005). The ability of fungi to trigger inflammatory responses largely depends on their growth environment and, specifically, the type of substrate they inhabit. Allergic reactions to fungi can manifest as various conditions, including bronchial asthma, rhinitis, conjunctivitis, urticaria, or atopic dermatitis (Bush and Prochnau, 2004). Fungi, depending on their species, can induce all four types of hypersensitivity reactions (Agarwal et al., 2013).

The most common allergic reaction induced by fungi is hypersensitivity of type I, an immediate, IgE-dependent response (Bush and Prochnau, 2004). Clinically, this type of immune response presents as asthma, rhinitis, sinusitis, urticaria, or bronchial obstruction. In this scenario, fungal allergens stimulate the production of IgE antibodies, which bind to mast cells and basophils. Symptoms typically appear within 10-30 minutes after exposure to the allergen (Bush and Prochnau, 2004).

Type II reactions are less common and are typically triggered by fungal antigens, such as mannan, primarily found in the cell walls of *Aspergillus* and *Candida* species (Agarwal et al., 2013). These reactions involve cytotoxic responses mainly driven by IgG and IgM antibodies or cells like cytotoxic lymphocytes, natural killer (NK) cells, or activated macrophages targeting antigens present on blood cells or cell membranes.

Hypersensitivity of type III reactions to fungal antigens are observed in cases of bronchopulmonary aspergillosis & allergic alveolitis (Agarwal et al., 2013). These reactions are typically associated with immune complexes composed of fungal antigens and antibodies. Such complexes are formed either due to the presence of small amounts of fungi in the body (in the case of ABPA) or repeated exposure to small conidia, like those produced by *Aspergillus fumigatus*. Clinical symptoms of type III hypersensitivity reactions occur approximately 4-6 hours after allergen exposure.

Lastly, type IV hypersensitivity reactions develop 24-48 hours after exposure to antigens and involve Th1 (CD4+) sensitized lymphocytes (Agarwal et al., 2013). The antigen triggering this response can be fungal cell structures, haptens produced by certain fungi, or, as recently discovered, may be associated with Archaea species. Clinically, type IV hypersensitivity reactions may manifest as contact dermatitis, urticaria, or granulomatous cases characterized by distinctive inflammatory infiltrates.

Fungal spores, naturally occurring in both indoor and outdoor air in small quantities, can trigger various allergic reactions in susceptible individuals (Agarwal et al., 2013). The prevalence of molds tends to peak during the summer months, gradually decreasing from September to November. Notably, high concentrations of spores from genera like *Alternaria* and *Cladosporium* are associated with exacerbations of bronchial asthma in late summer (Kurup and Shen, 2005).

However, the concentration of spores or conidia from specific fungi in indoor air may not necessarily follow a seasonal pattern (Kurup and Shen, 2005). For instance, *Serpula lacrymans* releases copious basidiospores daily, posing a risk not only due to potential allergic reactions but also because of the emission of various volatile organic compounds (VOCs) during its active growth and fruiting body decay (Wålinder et al., 1996). Furthermore, this fungus can be detrimental to building materials due to its strong cellulosic and lignolytic properties.

Interestingly, what is commonly referred to as "allergy to dust" or seasonal hay fever can often be attributed to fungal spores, which constitute a significant portion of household dust (Bush and Prochnau, 2004). House dust serves as an important reservoir for molds, reflecting the airborne mycobiota of indoor spaces (Nikulin et al., 2007). Under suitable humidity conditions, dust provides the necessary nutrients for fungal growth, given its substantial organic content and often optimal pH.

Individuals typically inhale about 10,000 liters of air per day, with even clean outdoor air containing up to two dozen fungal spores, bacterial cells, and various small organic particles per liter (Adams et al., 2013). Consequently, the respiratory system is consistently exposed to a multitude of allergens. Allergic reactions induced by molds can sometimes mimic symptoms associated with influenza. Importantly, whether molds are classified as pathogenic or non-pathogenic, both categories pose health risks. Typically, pathogenic fungal species that cause mycoses are not allergenic.

Common complaints linked to mold allergen exposure include runny nose, conjunctivitis, cough, upper respiratory tract congestion, chest pain, and urticaria (Adams et al., 2013).

Allergenic fungi can also exacerbate symptoms in individuals with conditions like atopic dermatitis, allergic alveoli, or bronchial pneumonia (ABPA) (Agarwal et al., 2013).

Volatile Compounds Production by Fungi

Fungi are well-known for their capacity to release a variety of volatile organic compounds (VOCs) as part of their primary or secondary metabolic processes (Hung et al., 2015). This phenomenon is closely linked to a condition known as sick building syndrome (SBS), particularly when molds thrive in enclosed spaces with poor ventilation and high relative humidity (Korpi et al., 1997). Fungi tend to exhibit heightened metabolic activity under increased relative humidity conditions.

Symptoms associated with VOCs emitted by fungi often include discomfort, headaches, eye irritation, pharyngitis, dry cough, dizziness, blurred vision, difficulty concentrating, heightened sensitivity to Odors, fatigue, apathy, and an increased susceptibility to colds (Burge et al., 1987). These predominantly neurotoxic symptoms tend to intensify with higher concentrations of bioaerosols in indoor air. Interestingly, these symptoms typically dissipate rapidly after leaving the affected building or room.

Such detrimental conditions can be confined to a single room, extend to an entire apartment, or even encompass an entire building, particularly if it was constructed with materials ill-suited to the prevailing regional climate (Hung et al., 2015). The growth of fungi in these environments is invariably accompanied by the release of carbon dioxide and various VOCs. These fungal-secreted compounds encompass a range of chemical groups, including aromatic and chlorinated hydrocarbons, alcohols, ketones, aldehydes, and esters (Ahearn et al., 2010). Commonly detected alcohols include 1-octanol, 2-octanol, methylbutanol, and ethylhexanol. Additionally, terpenes and ketones, such as 2-pentanone, 2-hexanone, 2-heptanone, 3-octanone, α -pinene, β -pinene, camphene, limonene, and methylfuranes, are often produced. Aromatic hydrocarbons such as cyclohexane and benzene are also found among the compounds produced by fungi (Hung et al., 2015).

The specific types of MVOCs produced not only depend on the fungal species but also on the nature of the substrate on which they grow (Ahearn et al., 2010). Furthermore, variations exist among strains within the same species. Fungi commonly isolated from indoor air, including those from the genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium*, have been shown to generate a wide array of volatile compounds (Hung et al., 2015). Some of these compounds include formaldehyde, acrolein, nonanal, 2-pentanone, 2-hexanone, 2-heptanone, limonene, methylfuranes, camphene, and α -pinene (Korpi et al., 1997). Additionally, formaldehyde and β -glucans produced by many fungal species have demonstrated strong irritation effects on mucosal membranes (Hung et al., 2015). It is worth noting that not only fungi but also bacteria, actinobacteria, and certain building materials, furniture, carpets, and other sources can contribute to the presence of MVOCs in indoor environments (Ahearn et al., 2010).

Mycotoxin Production After Secondary Metabolism

Highlighting the detrimental impact of fungi within the immediate human environment necessitates acknowledgment of their toxic effects on the human body (Bennett and Klich, 2003). In terms of human and animal health, a well-recognized concern revolves around mycotoxins.

Mycotoxins are toxic byproducts of molds' secondary metabolism (Bennett and Klich, 2003). It is believed that fungi produce these compounds as a form of competition with other microorganisms, including other fungal species, for ecological niches. Over 400 distinct mycotoxins have been identified so far (Magan and Aldred, 2007). These substances can induce both acute and chronic disorders in humans. To trigger acute intoxication, which can be fatal, a milligram dose per kilogram of body weight is required. Mycotoxin poisoning often occurs through the consumption of food contaminated with these compounds (Bennett and Klich, 2003). However, it's worth noting that mycotoxins can also accumulate within fungal structures and diffuse into the surrounding environment. Inhalation of spores, conidia, or mycelium fragments can lead to primary mycotoxicosis. For instance, *Stachybotrys chartarum* produces mycotoxins concentrated in conidia, phialides, and conidiophores, with some of these toxic compounds also being released into the environment (Nielsen et al., 2002). Other species like *Aspergillus flavus* and *Aspergillus parasiticus* accumulate mycotoxins in conidia and sclerotia (Bennett and Klich, 2003).

The notably increased incidence of cancer observed in individuals inhabiting mold-infested spaces is often attributed to prolonged exposure to mycotoxins (Etzel, 2003). Studies conducted in Poland have indicated that approximately a quarter of dwellings exhibit dampness, putting around 24% of residents at risk from mycotoxins and allergens produced by fungi (Norbäck et al., 2016).

It is suggested that tumor-related changes, particularly liver or kidney cancer, may result from prolonged exposure to low doses of mycotoxins (Bennett and Klich, 2003). Among the most significant mycotoxins commonly encountered in the environment are aflatoxins, ochratoxin A, zearalenone, trichothecenes, and fumonisins (Magan and Aldred, 2007). Aflatoxins are particularly hazardous due to their carcinogenic properties associated with the benzofuran ring. Several species of the *Aspergillus* genus, including *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, *A. clavatus*, and *A. chevalierii*, are often isolated from materials containing cellulose or collagen (Nielsen et al., 2002). The production and intensity of mycotoxin production depend on fungal strains, genotypes, developmental stages, relative humidity, temperature, and the chemical composition of the substrate in which the fungus grows (Magan and Aldred, 2007). The source of nitrogen plays a crucial role in toxic compound production. Mycotoxins are produced most intensively at higher temperatures and elevated relative humidity, especially in poorly ventilated dwellings. For instance, the optimal temperature for aflatoxin formation is around 33°C. Moreover, certain conditions like inorganic nitrogen levels, phosphates, oxygen depletion, and elevated carbon dioxide can inhibit mycotoxin production (Magan and Aldred, 2007).

It is particularly concerning that many mycotoxins exhibit an affinity for specific organs and tissues (Bennett and Klich, 2003). Ochratoxin A, for example, has an affinity for the kidneys and liver, posing a significant risk due to the presence of *Aspergillus ochraceus* in the environment. Trichothecenes tend to affect the gastrointestinal system, while zearalenone negatively impacts the reproductive system (Etzel, 2003).

In addition to the adverse effects of mycotoxins, fungi can also act as pathogens, causing superficial, subcutaneous, or systemic infections (Nucci and Perfect, 2008). Certain species from the *Aspergillus* genus, frequently found in indoor air, can act as opportunistic pathogens, leading to conditions like lung aspergillosis, keratitis, or otitis in predisposed individuals.

As previously mentioned, house dust can serve as a reservoir for fungal spores and propagules, including those of dermatophytes, especially in homes with humidity issues (Viswanath et al., 2019). The presence of these propagules is more likely in residences with different domestic animals that shed corneous epidermis or dander, promoting the development of dermatophyte infections caused by zoophilic dermatophytes such as *Microsporum canis* and *Trichophyton mentagrophytes* (Elewski, 1998).

In summary, the presence of molds in dwellings is consistently associated with health issues, which vary depending on individual sensitivity (Etzel, 2003). The prevalence of fungi in homes, as discussed in this article, becomes a significant concern when considering that roughly every fourth individual in Poland is exposed to fungal spores daily (Norbäck et al., 2016). Fungal spores and bacteria are omnipresent in buildings and can readily adapt to their available niches, provided there is sufficient relative humidity and a minimum level of water in the substrate (Hung et al., 2015). Importantly, some fungi can develop even at significantly lower relative humidity levels and substrate humidity (Korpi et al., 1997).

Result

The research findings shed light on the critical importance of indoor air quality (IAQ) and its direct correlation with human health and well-being. This study primarily focused on the role of biological pollutants, particularly fungi, in deteriorating IAQ and the consequent health implications.

Biological Pollutants and Allergic Reactions

The presence of fungi in indoor environments was found to be a significant contributor to allergic reactions among occupants. Fungi can induce a range of allergic responses, including bronchial asthma, rhinitis, conjunctivitis, urticaria, and atopic dermatitis. These allergic reactions are often triggered by immune responses that vary based on the specific fungal species encountered. The study highlighted that fungi's ability to induce these allergic reactions is largely dependent on the type of immune response elicited.

One of the most common types of immune response observed was type I hypersensitivity, characterized by immediate IgE-dependent reactions. Symptoms of this response typically manifest within 10-30 minutes after exposure to the fungal allergen. Additionally, type II,

type III, and type IV hypersensitivity reactions were noted in specific cases, indicating the diverse ways fungi can impact human health through allergic responses.

Volatile Organic Compounds (VOCs) and Sick Building Syndrome (SBS)

Fungi were found to be significant contributors to the phenomenon known as sick building syndrome (SBS), primarily through the release of volatile organic compounds (VOCs). SBS encompasses a range of non-specific symptoms that affect building occupants and tend to improve when they leave the affected building. The study revealed that the symptoms associated with VOC exposure include discomfort, headaches, eye irritation, pharyngitis, dry cough, dizziness, blurred vision, difficulty concentrating, heightened sensitivity to odors, fatigue, apathy, and increased susceptibility to colds. These neurotoxic symptoms were shown to intensify with higher concentrations of bioaerosols in indoor air.

Importantly, the study highlighted that the production of VOCs by fungi is influenced by environmental factors, such as humidity levels and ventilation. Fungi exhibited heightened metabolic activity and increased VOC production under conditions of elevated relative humidity, making humidity control a crucial aspect of IAQ management.

Mycotoxins and Health Risks

The most alarming finding of this research was the production of mycotoxins by molds, which can have severe health consequences for humans. Mycotoxins are toxic byproducts of molds' secondary metabolism and can induce both acute and chronic disorders. The study identified several mycotoxins of concern, including aflatoxins, ochratoxin A, zearalenone, trichothecenes, and fumonisins.

Of particular concern were the carcinogenic properties associated with aflatoxins, which have been linked to cancer in individuals inhabiting mold-infested spaces. The research indicated that prolonged exposure to low doses of mycotoxins can result in tumor-related changes, particularly in the liver or kidneys.

The study also noted that mycotoxins can accumulate within fungal structures and diffuse into the surrounding environment, posing a risk of inhalation exposure. This emphasizes the need for stringent measures to control mold growth and the presence of mycotoxins in indoor spaces.

Discussion

The results of this research underscore the critical importance of addressing biological pollutants, particularly fungi, in indoor environments to improve IAQ and safeguard human health. Several key points warrant further discussion:

1. **IAQ Management:** The findings emphasize the necessity of proactive IAQ management strategies. Effective ventilation, humidity control, and regular inspection for mold growth are essential to mitigate the health risks associated with indoor air pollution. Public health authorities and individuals alike should prioritize these measures to create healthier indoor environments.

2. **Allergenic Responses:** Understanding the various immune responses triggered by fungi is crucial. This knowledge can aid in identifying susceptible individuals and implementing measures to reduce their exposure to fungal allergens. Additionally, healthcare professionals should consider fungal exposure when diagnosing and treating patients with allergic reactions.

3. **VOC Emissions:** Managing VOC emissions from fungi is paramount to addressing SBS. Proper ventilation and humidity control can help reduce the concentration of VOCs in indoor air, alleviating the associated symptoms and improving overall occupant comfort and well-being.

4. **Mycotoxins and Health Risks:** The research underscores the need for rigorous measures to prevent and remediate mold infestations. Mycotoxins pose serious health risks, and their potential carcinogenic properties necessitate proactive steps to minimize exposure. This includes thorough mold inspection, removal, and ensuring optimal indoor environmental conditions.

This study provides a comprehensive understanding of the impact of biological pollutants, particularly fungi, on indoor air quality and human health. It highlights the urgent need for improved IAQ management practices, increased awareness of the health risks associated with indoor air pollution, and the implementation of measures to create and maintain healthier indoor environments. By addressing these issues, we can significantly enhance the quality of life and reduce the health risks for individuals spending a substantial portion of their lives indoors.

Conclusion

Indoor air quality (IAQ) is a matter of utmost importance, considering that people spend the majority of their leisure time inside their homes. This research has delved into the often-overlooked realm of IAQ, specifically focusing on the impact of biological pollutants, such as fungi, on the indoor environment. By understanding the consequences of biological pollutants, we can take meaningful steps toward improving indoor air quality, thereby enhancing our overall quality of life.

One of the key findings of this study is the significant role fungi play in deteriorating IAQ. Fungi can lead to a range of allergic reactions, including bronchial asthma, rhinitis, conjunctivitis, urticaria, and atopic dermatitis. These allergic responses can vary depending on the type of immune reaction triggered by specific fungal species, which may involve IgE-dependent, cytotoxic, immune complex, or Th1 sensitized lymphocyte responses. Importantly, indoor air is not immune to fungal spores, and common molds like *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Trichoderma* can pose health risks, particularly to individuals with preexisting conditions.

Furthermore, the production of volatile organic compounds (VOCs) by fungi, especially in enclosed spaces with poor ventilation and high humidity, contributes to a phenomenon known as sick building syndrome (SBS). The symptoms associated with VOC exposure can range from discomfort to neurotoxic effects, such as headaches, eye irritation, pharyngitis, and

difficulty concentrating. Addressing these issues is crucial for maintaining healthy indoor environments.

Perhaps most concerning is the production of mycotoxins by molds, which can have acute and chronic health effects on humans. Mycotoxins can lead to severe intoxication and are often linked to cancer and other chronic diseases. Aflatoxins, ochratoxin A, zearalenone, trichothecenes, and fumonisins are among the most significant mycotoxins, with certain fungi like *Aspergillus* species being known producers. These mycotoxins can accumulate in fungal structures and diffuse into the indoor environment, posing a risk of inhalation exposure.

In addition to mycotoxins, fungi can also act as pathogens, causing various infections, especially in individuals with compromised immune systems. *Aspergillus* species, frequently found in indoor air, can lead to conditions like lung aspergillosis, keratitis, or otitis.

The implications of this research are clear: IAQ is a critical factor in our overall well-being, and the presence of fungi in indoor environments demands serious attention. Steps must be taken to mitigate the risks associated with biological pollutants, including proper ventilation, humidity control, and regular inspection for mold growth. Education and awareness about the potential health hazards of indoor air pollution, particularly biological pollutants, are crucial for both individuals and public health authorities.

In conclusion, this research underscores the urgency of addressing indoor air quality and the impact of biological pollutants, such as fungi, on our health and well-being. It is imperative that we take proactive measures to create and maintain healthier indoor environments, ensuring the safety and comfort of those who spend a significant portion of their lives indoors. By doing so, we can improve the quality of life and reduce the health risks associated with indoor air pollution.

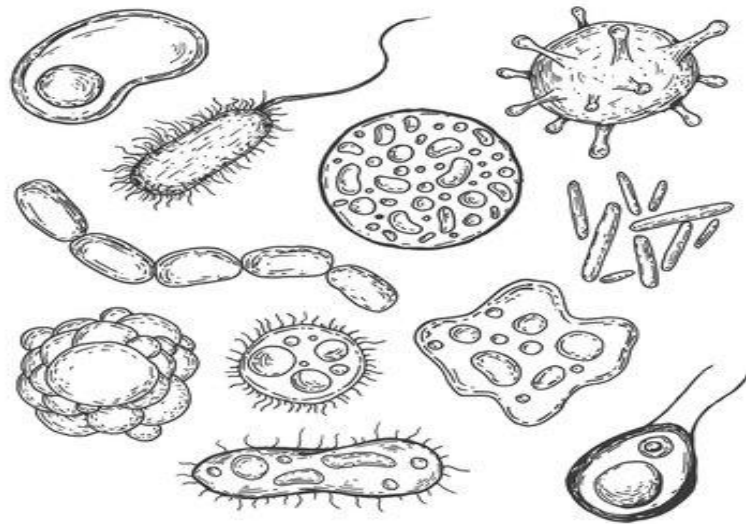
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Section-III

MICROBIOLOGY



MICROBIAL RESEARCH

CHAPTER

27

ECOLOGY AND BIOTECHNOLOGICAL APPLICATIONS OF MARINE CYANOBACTERIA: A REVIEW

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A**bstract**

Cyanobacteria have played a substantial role in the evolution of life by creating an oxygenic atmosphere through the process of photosynthesis. They have specific adaptive strategies to survive in extreme environments. They are ubiquitous in nature and can be spotted in both marine and freshwater ecosystems and are especially rich in oligotrophic waters of the tropical and subtropical marine environment. Cyanobacteria in the mangrove ecosystem provide fixed nitrogen, carbon, bioremediation and secreting plant growth-promoting substances etc, to the vegetation. Diversity of cyanobacteria in this biome has been traditionally underestimated. Cyanobacteria still play a significant role in modern coral reef ecosystems by forming a major portion of epiphytic, epilithic, and endolithic communities as well as of microbial mats. Cyanobacteria have gained a lot of attention in recent years because of their potential applications in the field of biotechnology. A wide range of secondary metabolites exhibiting pharmacodynamic properties such as antibacterial, antiviral, antifungal, anti-inflammatory and anticancer have been exhibited. In addition to these applications, cyanobacteria are also used in aquaculture, treatment of wastewater, food, fertilizers, vitamins, toxins, enzymes and pharmaceuticals. We present an overview of the literature describing the presence of cyanobacteria in marine and mangrove ecosystem and uses of these in various industries which provide an outlook on the challenges and future prospects of the field of cyanobacterial biotechnology. Future research should focus on isolating new cyanobacterial strains from

mangrove vegetation producing high value products and genetically modifying existing strains to ensure maximum production of the desired products.

Keywords: *Biotechnology, Cyanobacteria; Mangroves; Pharmacodynamic.*

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Introduction

Cyanobacteria are the largest group of photosynthetic prokaryotes, capable of fixing atmospheric carbon and nitrogen (Stal, 2000). Cyanobacteria exists in a variety of habitats. The cyanobacteria comprise a large component of marine plankton with global distribution. Cyanobacteria are abundant in most marine habitats due to their preference for alkaline conditions and an ability to tolerate high salt concentrations. Cyanobacterial species show resistance towards osmotic shock, extremes of temperature and reducing conditions. The most important discovery of the last two decades has been the great contribution of the cyanobacteria in the oceans (Waterbury and Rippka, 1989; Waterbury, 2006; Anburaj, 2011) and to the pelagic food web of the ocean. According to Desikachary (1959) about 20% of all known cyanobacteria occur in saline conditions and a majority of them are truly Marine. Most marine forms grow along the shore as benthic vegetation in the zone between the high and low tide marks. They have also been reported to occur in mangrove biotopes (Kathiresan and Bingham, 2001; Kathiresan and Qasim, 2005; Anburaj *et al.*, 2011). The unicellular cyanobacteria belonging the genus *Synechococcus* (Anburaj 2011) are an important component of phytoplankton in many marine and freshwater planktonic ecosystems. But the unicellular cyanobacteria especially in mangrove biotope are little focused (Sakthivel, 2004; Anburaj 2011; Anburaj *et al.*, 2011).

Cyanobacteria in marine biotope

Marine cyanobacteria are found in all the world's oceans but are most abundant in warmer temperate and tropical regions. The taxonomic diversity of marine cyanobacteria does not differ markedly from that of cyanobacteria found in terrestrial and freshwater habitats. The cyanobacteria are currently placed under five groups: Chroococcales, Pleurocapsales,

Oscillatoriales, Nostocales and Stigonematales (Anburaj 2011). Representatives from each of the five major taxonomic have been isolated from marine sources. However, particular marine habitats (intertidal and subtidal zones, coral reefs, salt marshes, and the open ocean) often harbor a restricted and characteristic diversity of forms which may differ spatially with geographical locations and temporarily with season (Anburaj, 2005; Anburaj, 2011). The intertidal and subtidal zones contain a diverse assemblage of cyanobacteria that are typically found attached to solid substrates such as rocks, mollusk shells, pieces of wood and eukaryotic macroalgae. Pleurocapsalean forms are characteristic of intertidal and subtidal habitats, where they frequently grow as epiliths or endoliths on calcareous substrates and as epiphytes on macroalgae. Compared to intertidal region, salt marshes support low diversity of cyanobacteria but in several cases, cyanobacteria are conspicuous in salt marshes as a result of the formation of dense algal mats. Oscillatorian forms usually make up the principal component of cyanobacteria in the algal mats, but a wide variety of unicellular and heterocystous forms are also usually present.

The open ocean harbors low numbers of planktonic forms of cyanobacteria, as compared to fresh water habitats (Whitton, 1973), where over 100 species of planktonic cyanobacteria have been described and 20 are capable of forming dense water blooms. The best known of water bloom is *Trichodesmium*, an Oscillatorian cyanobacterium that often forms dense blooms occupying vast areas in subtropical regions. This group of cyanobacteria is of interest to biological oceanographers because of its reputed ability to fix nitrogen. However, *Trichodesmium* has not been successfully cultured till date for experimental studies and applications.

Lyngbya majuscula (Oscillatoriaceae), also known as *Microcoleus lyngbyaceus* (Burja *et al*, 2002) is a filamentous, non-heterocystous marine cyanobacterium inhabiting tropical and sub-tropical estuarine and coastal waters (Anburaj, 2011). It is composed of thin, unbranched filaments, often olive- grey in colour. *L. majusculais* is often found forming benthic mats loosely attached to substrates such as seagrass, macroalgae or rock outcrops. Additionally, mats can rise to the surface through the accumulation of gas bubbles (Dennison *et al.*, 1999). This marine cyanobacterial species forms seasonal blooms in the Deception Bay Pumicestone passage areas of Moreton Bay, Queensland, for nearly a decade (Dennison & Abal, 1999) and the blooms have increased in severity and extent since early 1990. A similar seasonal change has been recorded in other cyanobacterial species in the salt marshes by Chapman (1960). Webber (1967) has identified 30 species of cyanobacteria from a salt marsh near Massachusetts, USA. Chapman (1977) has extensively studied the cyanobacterial communities in salt marshes from Europe, Iceland, America, India and New Zealand. In India, ecology of the marine cyanobacteria has been studied extensively by Iyyengar and Desikachary (1944); Devassy *et al.* (1978) mainly with *Trichodesmium* species.

Cyanobacteria in mangrove biotope

Cyanobacteria constitute one of the important resources of mangrove ecosystems along the tropical coasts (Kathiresan, 2000; Kathiresan and Bingham, 2001; Sakthivel, 2004; Kathiresan and Qasim, 2005; Kathiresan, 2011; Anburaj 2011; Kathiresan *et al.*, 2011;

Kathiresan et al., 2013; Kathiresan et al., 2016; Kathiresan et al., 2018). They colonize any submerged surface of sediments, roots, aerial roots, branches and tree trunks of mangroves (Kathiresan et al., 2000; Palaniselvam and Kathiresan, 2002; Nabeel *et al.*, 2009; Anburaj 2011; Kathiresan et al., 2011; Kathiresan et al., 2014; Kathiresan et al., 2016). The report of Millard and Broekhuysen (1970) is the early reference that has listed the cyanobacteria associated with mangrove along the southeast African coast. In India, the entire mangroves region of the Sundarbans is reported to be rich in benthic algal forms (Santra and Pal, 1988). Their populations in other mangrove areas of India have also been documented (Palaniselvam and Kathiresan, 2002; Sakthivel, 2004; Anburaj, 2011).

Cyanobacteria are reportedly present in the mangrove ecosystems of many countries (Anburaj 2011). Mangroves are occupied by diverse cyanobacterial communities, which reside on leaf, root litter, live roots, and often form extensive mats on the surrounding sediments; many of these communities are capable of fixing atmospheric nitrogen (Hoffmann, 1999). The genera *Oscillatoria*, *Lyngbya*, *Phormidium* and *Microcoleus* are widespread in these habitats, as heterocystous genera, like *Scytonema*, in some areas (Hoffmann, 1999). The taxonomic survey of the cyanobacteria in a red mangrove forest of the Brazil estuaries has revealed a total of 15 taxa in 8 families: *Synechococcaceae* (2 taxa), *Chroococcaceae* (1 taxa), *Hyellaceae* (1 taxa), *Xenococcaceae* (1 taxa), *Oscillatoriaceae* (2 taxa), *Scytonemataceae* (2 taxa), *Phormidiaceae* (5 taxa), and *Pseudannabaenaceae* (2 taxa) (Nogueira and Ferreira Correia, 2001). A total of ten genera of cyanobacteria has been recorded in mangroves; *Anabena*, *Rivularia*, *Aphanocapsa*, *Merismopedia*, *Lyngbya*, *Microcoleus*, *Oscillatoria*, *Phormidium*, *Schizothrix*, and *Spirulina* (Kyaruzi *et al.*, 2003).

The cyanobacteria colonize various parts of the tree mostly the aerial roots. The pneumatophores give microhabitat to specific cyanobacterial populations. Interestingly the cyanobacteria on the aerial roots exhibit sharp vertical zonation (Kathiresan and Bingham, 2001). Non-heterocystous, particularly filamentous cyanobacteria resembling *Microcoleus* sp., colonize the central zone, and by coccoidal cyanobacteria resembling *Aphanothecopsis* sp., mixed with unidentified filamentous cyanobacteria colonize the upper part (Venkataraman, 1981). *Lyngbya* sp., *Polysiphonia* sp., and *Oscillatoria* sp., are common epiphytic cyanobacteria of submerged root system of *Rhizophora* sp., (Krishnamurthy and Jayaseelan, 1983). The pneumatophores of mangroves are colonized by five cyanobacterial species; *Calothrix*, *Anabaena*, *Lyngbya*, *Hydrocoleum*, and *Schizothrix* as reported from Sundarbans (Santra *et al.*, 1988).

Cyanobacteria are an important component of the microbial flora of mangroves. Six species dominantly occur in the root-soil of mangroves and they are *Spirulina subsalsa*, *Phormidium tenue*, *Synechocystis salina*, *Oscillatoria willei* and *O. cortiana*. Their counts vary between 3.1×10^3 and 4.1×10^4 colony forming units per gram of soil with the maximum occurrence during summer (May) and the minimum in post monsoon (December) along the east coast of India (Nabeel *et al.*, 2009). The planktonic cyanobacteria are reportedly belonging to the species of *Trichodesmium*, *Synechococcus*, *Aphanotheca*, *Gloeocapsa*, *Gloeotheca*, *Merismopedia*, *Oscillatoria*, *Johannes baptista*, *Microcystis* and *Stigonema* in Sundarbans (Santra *et al.*, 1988; Saravanakumar *et al.*, 2016). Three species of planktonic cyanobacteria

Anabaena sp., *Oscillatoria* sp., and *Trichodesmium* sp., have been recorded in Pichavaram mangroves (Anburaj 2011). Population densities of planktonic cyanobacterial species decrease in monsoon and increases in pre-monsoon periods (Banerjee and Santra, 2001). The change in population density of cyanobacteria is due to the variations in environmental factors such as temperature, salinity, light, etc. (Banerjee and Santra, 2001; Anburaj 2011).

A study on mangrove-associated cyanobacteria has recorded the presence of 17 cyanobacterial species in the Muthupet estuary (Selvakumar and Sundararaman, 2001). This study reveals the unique associates of cyanobacteria on mangrove trees. Cyanobacterial species *Aphanocapsa koordersi*, *Johannes baptistia pellucid*, *Oscillatoria vizagapatensis*, and *O. tenuis* colonize on *Avicennia marina*. Cyanobacterial species, *Porphyosiphon natarsii*, *Phormidium* sp., *Oscillatoria calcuttensis*, and *Schizothrix telephorides* are found only on *Aegiceras corniculatum*. Cyanobacterial species *Aphanocapsa bullosa* and *A. littoralis* are specific to *Excoecaria agallocha* and the cyanobacterial species *Oscillatoria claricentrosa* occurs only on *Suaeda maritima* (Selvakumar and Sundararaman, 2001). The species specificity may be attributed to the root exudates and also to the environmental conditions. The root exudates may play a key role in forming a community structure by selectively stimulating and enriching certain groups of bacteria (Burgmann et al., 2005).

Ecological role of cyanobacteria in mangroves

Mangroves are among the most productive ecosystems in the world (Kathiresan and Bingham, 2001). They support rich macro and microbial floral components. The interactions of mangrove trees with soil and associated microbes are complex, as the trees and other biota must cope with a harsh intertidal environment (Anburaj 2009; Anburaj et al., 2012). The sedimentary bacteria play a significant role in energy flows and cycles of tropical mangrove forests. The bacteria are very high in densities and productivity in mangrove sediments of tropical Australia (Alongi et al., 2001; Anburaj et al., 2020), which is 2 to 10 times higher than that has been reported for other marine sediments. Mangrove soils are a source of microbially mediated gases, such as CO₂, CH₄, N₂, and N₂O (Anburaj 2011; Anburaj et al., 2017; Anburaj et al., 2020b). It is therefore, important to have better knowledge of rates and pathways of microbial processes in these tropical habitats to better estimate the role of mangroves in global carbon and nitrogen cycling.

Nitrogen is an essential biogenic component, responsible for productivity of any ecosystem. The nitrogen gas enters into the biosphere by the process (Tyagi, 1973), in which only a few species of microorganisms such as cyanobacteria, azotobacters, azospirillum, rhizobium is involved. Many of the cyanobacterial species have the power of nitrogen fixation by the presence of very special enlarged cells called heterocysts. According to Tyagi (1973), Apart from microbial nitrogen fixation, which is its prime function, the heterocyst is also credited with many other biological functions such as reproduction (Tyagi, 1973).

Biotechnological potentials of cyanobacteria

Cyanobacteria constitute a potential resource in varied applications such as mariculture, food, feed, fuel, fertilizer, medicine, industry and in combating pollution. They are important for

the production of glycerol, polysaccharides, lipids and fatty acids, which are needed for chemical industries. An exciting area of cyanobacterial research is biosynthesis of nanoparticles. Many microorganisms produce inorganic materials either intra- or extracellularly. For example, photoautotrophic cyanobacterium *Plectonema boryanum* forms extracellular silver nanoparticles (Langkeet *et al.*, 2007) and *Shewanella* sp., produces intracellular gold nanoparticles by microbial reduction of aurium chloride ions (Konishi *et al.*, 2006; Anburaj *et al.*, 2020a; Anburaj *et al.*, 2022). Similarly, unicellular cyanobacteria, *Synechococcus elongatus* and *Gloeocapsa* sp., are known to synthesize silver nanoparticles (Asmathunisha *et al.*, 2012; Anburaj, 2011; Asmathunisha *et al.*, 2018; Anburaj *et al.*, 2021).

Food and feed

A variety of fine chemicals such as pigments, vitamins and enzymes with varied applications can be obtained on a commercially viable scale from cyanobacteria. A number of cyanobacteria are rich in vitamins and many can excrete them into the surrounding environment (Borowitzka, 1988). The carotenoids and phycobiliproteins, characteristic of cyanobacteria have high commercial value. They are used as natural food colourants, as food additives to enhance the colour of the flesh of Salmonid fish and to improve the health and fertility of cattle (Thajuddin and Subramanian, 2005). *Phormidium valderianum* is an excellent source of phycocyanin, a blue natural colorant useful as a phycofluor in diagnostics (Anburaj, 2011).

Cyanobacteria secrete enzymes that can be exploited commercially in large-scale production of enzymes such as beta lactamase, protease and lipase (Prabhakaran, *et al.*, 1994). Several common and unique sequence-specific endonucleases are known from *Anabaena cylindrical* (Acy I), *Anabaena flos-aquae* (Afl I & Afl III), *Anabaena variabilis* (AvaI&AvaII), *Anabaena 54 variabilis* UW (AvrII), *Microcoleus* sp. UFEX 2220 (MstII), *Nostoc* sp. PCC 7524 (Nsp C I), which can be marketed at low cost since relative biomass production of cyanobacteria is much less expensive than bacteria (Elhai and Wolk, 1988). Cyanobacterial isolates with capacity to mineralize organic phosphorus have been reported with alkaline phosphatase activity. Enzymes such as chitinase, L-asparaginase, L-glutaminase, amylase, protease, lipase, cellulase, urease and superoxide dismutase have been reported from cyanobacteria (Elhai and Wolk, 1988).

Pharmaceuticals

Cyanobacteria in general and marine forms in particular are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications (Raghavan *et al.*, 2002; Annamalai *et al.*, 2007). Gustafson *et al.* (1989) have reported anti-HIV activity of marine cyanobacterial compounds from *Lyngbya lagerheimii* and *Phormidium tenue*. A massive programme of screening of extracts from the large culture collection of marine cyanobacteria in our research facility for antiviral, anti-bacterial, anti-fungal and immuno-modulatory activities has resulted in recovery of a compound from marine *Oscillatoria laete-virians* BDU 20801 that shows anti-candida activity (Deth, 1999).

Biofertilizer

The role of N₂-fixing cyanobacteria in maintenance of the fertility of rice fields has been well substantiated and documented all over the world. In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of field locations (Kathiresan and Bingham, 2001). A variety of cyanobacterial strains colonize rice fields wherein heterocystous species are capable of fixing atmospheric nitrogen. However, several non-heterocystous cyanobacteria are also able to fix atmospheric nitrogen under microaerophilic conditions. *In situ* estimations using acetylene reduction technique have shown an addition of 18–15 kg N ha⁻¹ yr⁻¹ due to the activity of diazotrophic cyanobacteria (Waterbury *et al.*, 1979). Beneficial effects of cyanobacterial inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Kaushik and Venkataraman, 1979).

Cyanobacterial toxins

Fresh water cyanobacteria blooms that have been implication on human livestock intoxications have been extensively studied (Carmichael, 1997). Bloom of marine cyanobacteria is also becoming an increasingly familiar occurrence within the tropical and sub-tropical regions of the world. Bloom forming taxa belonging to the order Chroococcales and Oscillatoriales produce marine toxic compounds. For example, *Synechococcus* species produce linolenic acid and phycocyanin compounds with antibiotic property, while *Synechocystis* species produce cytotoxic compounds such as nakitriol (Nagle *et al.*, 1995).

Cyanobacteria are rich source of toxic compounds as well antimicrobial and antiviral compounds (Nagle *et al.*, 1995). Jaspers *et al.* (1996) have alone reported a total of 208 compounds while Marinlit (2001) recorded 424 compounds, of which active compounds are mainly used for the enzyme's inhibition, herbicides, antimycotics, anti-malarial and immunosuppressive actions. Following the current decline in many natural product discovery programmes globally, a special focus is given on marine cyanobacteria-derived compounds for screening in new pharmaceutical property.

Concluding remark

Although cyanobacteria are known for their biological potential with anti tumor, anti-cancer, anti-HIV, anti-fungal, anti-bacterial, anti-oxidant, food additives, bio fertilizer, dye degradation, nanoparticle synthesis, a very few such compounds have been so far available. Marine cyanobacteria especially from mangrove biotope are one of the richest sources of known and novel bioactive compounds with wide range of applications. Hence, isolation and identification of new marine cyanobacterial species from marine environments such as mangrove environment will pave the possible way to identify novel compounds for their utilization in pharmaceutical, agricultural and food industries.

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CHAPTER

28

A STUDY ON CELLULOSE AND ITS DEGRADATION: A PROMISING BIOMATERIAL

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A**bstract**

Cellulose has been in use as fuel since history. In fact, it was man's first fuel being the major component of wood used for fires. Its conversion into synthetic fuels, feed and food could become a boon for the near future. In the era of global energy crisis's, this alternative source of energy could be proved as the most economic and reliable source for energy generation. In the current study, a detailed description of cellulose, its structural complication, biodegradation by various biological agencies majorly microbial conversion into valuable products and the possible end products are highlighted.

Keywords: *Cellulose, Energy Crisis, Biodegradation, Microbial Conversion.*

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Introduction

Cellulose is the most abundant bio-polymer on earth [Monserrate, 2001]. Cellulose is a linear polysaccharide of glucose residues with β -1, 4-glycosidic linkages. Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products [Ekperigin, 2007]. Found primarily in plant cell walls, cellulose is embedded in a hetero-matrix composed of xylan, other hemi-celluloses and lignin. Cellulose exhibits a high degree of polymerization: the individual glucan chains, or cellodextrins, can reach lengths of greater than 25,000 glucose residues [Desvaux, 2005].

Cellulose has no taste, is odourless, is hydrophilic with the contact angle of 20–30 degrees, [Bishop, 2007] is insoluble in water and most organic solvents, is chiral and is biodegradable. It was shown to melt at 467 °C in pulse tests [Dauenhauer, 2016]. It can be broken down chemically into its glucose units by treating it with concentrated mineral acids at high temperature [Wymer, 1994].

Cellulose produced by plants is composed of both highly amorphous regions containing large voids and other irregularities as well as tightly packed crystalline regions. Cellulose, because it is resistant to most forms of degradation, accumulates within the environment. It has been estimated that approximately half of the carbon fixed annually within terrestrial ecosystems is stored as cellulose. Cellulose synthesis is primarily associated with plants; however, some animals, bacteria and algal species can also produce the polymer [Boisset, 1999]. Cellulolytic species are found within the phyla Thermotogae, Proteobacteria, Actinobacteria, Spirochaetes, Firmicutes, Fibrobacteres and Bacteroids. Of these, approximately 80% of the isolated cellulolytic bacteria are found within phyla Firmicutes and Actinobacteria [Bergquist, 1999].

The experiment setup for simultaneous saccharification and fermentation of mixed bacterial culture with *Saccharomyces cerevisiae* resulted in production of ethanol. This result expressed the high cellulolytic potential of these selected bacterial isolates for decomposition of cellulose and its fermentation for production of ethanol. *Saccharomyces cerevisiae* utilizes the reducing sugar derived from cellulose degradation and converts it to ethanol [Kumar, 2009]. Current strategies to produce fuel ethanol from cellulose, referred to as “second-generation” biofuels, utilize simultaneous saccharification and Fermentation (SSF) or simultaneous saccharification and co-fermentation (SSCF) [Lynd, 2005] [Weimer, 2002].

Cellulase due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol [Vaithanomsat, 2009]. The present work concentrates on the isolation of cellulose-degrading bacteria from invertebrates such as termites, snails, caterpillars, and bookworms and assessment of their cellulolytic activity.

Methods of biomass conversion

Pyrolysis

Pyrolysis is the term used to describe the thermochemical conversion of biomass at high temperatures (400° to 1000° C) without the presence of air or a catalyst. When biomass is

broken down, it releases volatile substances that condense into bio-oil while solid, non-volatile particles are gathered to form bio-char [Yaman, 2014]. The two pyrolysis techniques are slow pyrolysis and fast pyrolysis, often known as flash pyrolysis. Slow pyrolysis has a temperature range of 400–600 °C, a residence time of hours to minutes, and a slow heating rate (0.1–1 °C) [Mohan, 2006]. At low temperatures, high heating rates, and brief residence times, bio-oil yield is at its highest. On the other hand, when temperatures are high with a long gas residence time and a low heating rate, fuel gas yield is at its highest. Usually fluidized bed reactor was used for carrying out Pyrolysis [Zhang, 2005]. Fast pyrolysis is considered to be remarkable technology for biofuel production where 70-80% conversion is achieved [Demirbas, 2001].

Transesterification

Transesterification is the reaction between an ester of one alcohol and three moles of an ester of another alcohol that results in the production of both an ester of the second alcohol and an alcohol from the reactant ester [Muniyappa, 1996]. A triglyceride molecule's free fatty acids are neutralised during chemical transesterification, which results in the creation of an alcohol ester and the removal of the byproduct glycerol. To make methyl ester, use methanol, while to make ethyl ester, use ethanol. Methanol is the main fuel used in the transesterification reaction. In most nations throughout the world, it is used to replace diesel [Ma, 1999]. It is not a recent procedure because scientists Duy & Patrich first used it in 1853 [Singh, 2010]. Transesterified vegetable oil was originally utilised to fuel heavy-duty vehicles in South Africa prior to World War II.

They are divided into two categories based on the feedstock used to produce biodiesel: first generation fuel and second-generation fuel. First generation fuel is biodiesel made from food oils such as sunflower, palm, rapeseed, and soyabean oil [Kansedo, 2009]. Second generation fuel is made from non-edible oils such as neem, jatropha, and castor. Unfortunately, edible oils were used in industries, which had a severe impact on the surplus food supply.

Hydrocracking

Hydrocracking is a technology that uses bifunctional (acid/metal) catalysts to convert high molecular weight compounds into low molecular weight compounds at high temperatures (200°C-400°C) and high hydrogen pressure (1-10MPa). Cracking and hydrogenation are the functions of bifunctional catalysts. For cracking, acid supports such as zeolites, silico aluminophosphates, and amorphous aluminosilicates are commonly used, and transition metals such as palladium (Pd), nickel (Ni), molybdenum (Mo), and platinum (Pt) are used to hydrogenate unsaturated molecules present in the feedstock and cracked products, thereby minimising chain polymerization and coke formation [Speight, 2004].

Catalytic Cracking

Catalytic cracking has various advantages, including cheap operational costs, compatibility with existing infrastructure, flexibility with varied sources of oil/fat, and product compatibility with engines and fuel standards [Demirbas, 2009]. Another advantage is that catalytic cracking can be utilised to produce gasoline, kerosene, and diesel-like fuels more

quickly. According to published reports, using catalysts for thermal cracking of vegetable oil leads in reduced temperature requirements, higher conversion rates, shorter residence times, and superior product quality [Ong, 2010]. Metal oxides, molecular sieves, activated alumina, and sodium carbonate are the most commonly employed catalysts. These catalysts have received a lot of interest recently because of their capacity to catalyse a variety of biological reactions.

Emulsification

The mixing of immiscible liquids with the help of surfactant is called emulsifications. The emulsification is a simple and economical method to upgrade the quality of bio-fuels. In emulsification processes, the energy content in the fuel is strengthened by reducing the viscosity of vegetable oils [Ma, 1999]. The addition of an emulsifying agent with the assistance of agitation is a critical stage in the emulsification process. By lowering the viscosity of the fuel, these emulsions improved its spray properties. There are four forms of emulsions: basic emulsion (either water in oil or oil in water), multiple emulsion (either oil in water in oil or water in oil in water), micro emulsion, and nano emulsion. Nano emulsions are the most stable of these emulsions and can be employed in compression ignition engines. The glycerine emulsions used in diesel-biodiesel blends lowered oxides of nitrogen emissions while also lowering engine operating temperatures [Sidhu, 2018].

Microbial Consortia for biofuel production

Kamra, (2005) investigated the rumen's microbial ecosystem, where diverse types of microorganisms operating on various substrates coexist with the host and work under various natural and feed-associated limitations, resulting in the bioconversion of lignocellulosic biomass. [Bakare, 2005] isolated Cellulase enzymes from *Pseudomonas fluorescens* to apparent homogeneity using several methods such as ammonium sulphate precipitation, DEAE Sephadex A-50 ion chromatography exchange, Sephadex G-100 Gel filtration chromatography, and PAGE. The enzymes were described by investigating their various qualities such as K_m values, optimal pH, optimum temperature, and metal ion action. [Mosier, 2005] demonstrated physical and chemical pretreatment strategies for removing structural and compositional obstacles in the cellulose hydrolysis process, resulting in an increase in enzyme hydrolysis rate.

Corcoran, (2008) produced cellulosic ethanol from sugar cane bagasse using dairy rumen microorganisms. The project concentrated on the hydrolysis stage, which converts cellulose to fermentable glucose. The optimal lab scale hydrolysis time period was discovered to be roughly 84 hours, during which time the glucose produced could be efficiently converted to ethanol, after which these sugars were changed to volatile fatty acids that could not be converted to ethanol. They conducted an energy study and created a large-scale conceptual design system based on the sustainability and efficiency of the lab scale system, predicting that it will be more efficient than present energy production techniques.

Kumar, (2009) mention the use of various types of recombinant microbes like *Saccharomyces cerevisiae* MT8-1, *Zymomonas mobilis* AX101, *Klebsiella oxytoca* P2 and *Escherichia coli*

KO11 for ethanol production after hydrolysis and found that thermophiles like *Thermoanaerobacterium saccharolyticum* JW/SL-YS485 were also efficient. They were further able to ferment monomers like glucose, xylose, mannose, galactose and arabinose as well as polymers like xylan and hemicelluloses and hypothesized that as the presence of glucose causes enzymatic inhibition of cellulase, thus it is better to carry out saccharification and fermentation simultaneously. In the same year [Mtui, 2009] highlighted various types of pretreatment methods and value-added products being obtained from the lignocellulosic wastes.

Khan, (2011) identified a bacterial strain that produces cellulase from cow faeces. [AL-Rawi, 2011] investigated Cellulase synthesis from rumen cellulolytic bacteria. [Verma, 2012] recovered cellulase from soil samples from the bacteria *Bacillus subtilis*. In the same year, [Das, 2012] identified and characterised various excellent rumen microorganisms for use in animal feed. Cellulase was isolated in its crude form, and its activity was determined using two methods: CMC activity and Filter Paper activity (FPA). [Gupta, 2012] isolated eight cellulose degrading bacteria in the same year and investigated their cellulolytic capability using various approaches such as Congo red agar media test, hydrolysis capacity, gravimetric analysis, DNS assay, FPA assay, and so on. Microorganisms that can produce cellulase enzymes (cellulolytic microorganisms) can degrade cellulose. Fungi and bacteria isolated from soil secrete several enzymes which degrade lignocellulosic biomass [Bruce, 2010].

These enzymes are commonly produced by some bacterial genera such as *Cellulomonas*, *Pseudomonas* [Nakamura, 1982] *Bacillus*, and *Micrococcus* [Immanuel, 2006] and fungi [Shin, 2000] that are widely used now in industrial applications. Cellulosic biomass hydrolysis requires successive action of three types of enzymes, which are cellobiohydrolase, endoglucanase carboxymethyl cellulase (CMCase), and β -glucosidases.

Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural agents of cellulose degradation [Lederberg, 1992]. The cellulose utilizing population includes aerobic and anaerobic mesophilic bacteria, filamentous fungi, thermophilic and alkaliphilic bacteria, actinomycetes and certain protozoa [Alexander, 1961]. However, fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular [Lynd, 2002].

Cellulose is commonly degraded by cellulase. The cellulase was first discovered in 1983 from the anaerobic, thermophilic spore-forming *Clostridium thermocellum* [Maki, 2011]. In addition to plants, non-photosynthetic organisms such as bacteria i.e., aerobic *Acetobacter xylinum*, marine invertebrates from the ascites family i.e., Tunicates, fungi, slime moulds and amoebae also produce cellulase [Tomme, 1995] [Lynd, 2002]

Fungi and bacteria with cellulolytic capability

Soft rot fungi

Aspergillus niger, *A. nidulans*, *A. oryzae*, *A. terreus*; *Fusarium solani*, *F. oxysporum*; *Humicola insolens*, *H. grisea*; *Melanocarpus albomyces*; *Penicillium brasilianum*, *P. occitanis*, *P. decumbans*, *P. janthinellum*; *Trichoderma reesei*, *T. harzianum*, *T.*

longibrachiatum, *T.atroviride*; *Chaetomium cellulyticum*, *C. thermophilum*; *Thermoascus aurantiacus*; *Mucorcircinelloides*; *Paelomyces inflatus*, *P. echinolatum*.

White rot fungi

Phanerochaete chrysosporium; *Sporotrichum thermophile*; *Trametes Versicolor*; *Agaricus arvensis*; *Pleurotus ostreatus*; *Phlebia gigantea*.

Brown rot fungi

Coniophora puteana; *Lanzites trabeum*; *Poria placenta*, *Tyromyces Palustris*; *Fomitopsis sp*

Aerobic bacteria

Acinetobacter junii, *A. amitratus*; *Acidothermus cellulolyticus*; *Anoxybacillus sp*; *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *B. Amyloliquefaciens*, *B. circulans*, *B. flexus*; *Bacteroides sp*; *Cellulomonas Biazotea*; *cellvibrio gilvus*; *Eubacterium cellulosolvens*, *Geobacillus sp*; *Microbispora bispora*; *Paenibacillus curdlanolyticus*; *Pseudomonas Cellulose*; *Salinivibrio sp*; *Rhodothermus marinus*.

Anaerobic bacteria

Acetovibrio cellulolyticus; *Butyrvibrio fibrisolvens*; *Clostridium Thermocellum*; *C. cellulolyticum*; *C. acetobutylum*; *C. papyrosolvens*; *Fibrobacter succinogenes*; *Ruminococcus albus*

Production of biofuel

Current technologies to produce biofuel from cellulosic materials involve gasification, pyrolysis/liquefaction and hydrolysis of biomass [Dautzenberg, 2011]. In order to produce biofuel from cellulose of biomass, the lignocellulosic biomass should be first disassembled to facilitate the isolation of cellulose from other constituents, i.e., lignin and hemicelluloses. Subsequently, cellulose macromolecules should be depolymerized, as depolymerization significantly improves the chemical and biological conversions of cellulose to biofuel. Then, the depolymerized cellulose, i.e., glucose, should be converted to biofuel via biological treatments, and finally the biofuel should be purified. Additionally, biomass should be deoxygenated as the presence of oxygen reduces the heat content of molecules and creates high polarity, which impairs its blending with existing fossil fuels [Cherubini, 2010]

Types of Biofuels

During the past few decades, several strategies have been developed for the conversion of biomass to liquid, solid, and gaseous fuels. Among all these categories of biofuels, liquid biofuels are easier in terms of storage and transport with a high energy density that attributes for its advantage and appears to be a promising alternative to fossil fuels [Yousuf, 2020]. Moreover, liquid biofuels can be used in existing boilers, engines, or turbines [Vamvuka, 2011].

Solid Biofuels- Bio-char

Gaseous Biofuels- Bio Gas, Bio Hydrogen

Liquid Biofuels- Bioethanol, Biomethanol, Biodiesel, Bio-Oil, Biobutanol, 2,5-Dimethylfuran

Four categories are used to group biofuels based on the type of feedstock used to produce them, their limitations as a renewable source, and their technological progress-

First generation biofuels are produced from edible feedstock, e.g., bioethanol from corn and sugar cane and biodiesel from oil seed crops (soybean, oil palm, rapeseed and sun-flower) using well understood, economically viable technologies and processes, such as fermentation, distillation and transesterification [Gumienna, 2014] [Astolfi, 2020]. These biofuels only provide minimum benefit over fossil fuels in terms of greenhouse gas emissions as they require a large amount of energy (from fossil fuels) to grow, collect and process.

Second generation biofuels are produced from agricultural by-products or cellulosic materials such as wood, leaves and grass and can be grown on marginal land [Jutakradsada, 2019] [Sivamani, 2020]. They are produced by converting cellulose into sugar units, which can then be converted to ultimately produce alcohol.

Third-generation biofuels are made from aquatic cultivated feedstock, i.e., algae [Saladini, 2016] [Kumar, 2018]. Algae have been shown to have great potential as biofuel feedstock, due to their capabilities of producing much higher yields with reduced resource inputs [Brennan, 2010] [John, 2011] [Daroch, 2013].

Fourth-generation biofuels are found from the bioengineered microorganisms, e.g., bioengineered algae, yeast, fungi and cyanobacteria [Jiang, 2014] [Shin, 2016].

Second, third and fourth generation biofuels are commonly referred to as ‘advanced biofuels’ and thought to hold many advantages over first generation fuels.

Bio Ethanol

Bioethanol is the most widely used liquid biofuel. The demand for bioethanol is expected to increase dramatically. Fuel ethanol can be used in a variety of ways. Ethanol is commonly used as an oxygenated fuel additive to reduce emissions of carbon monoxide, nitrous oxides and hydrocarbons. Ethanol has a higher-octane rating than petroleum fuels enabling combustion engines to run at higher compression ratios and thus give superior net performance [Wheals, 1999].

Bioethanol is produced by converting sugars directly from crops like sugarcane or sugar beets, indirectly through starch from corn, wheat, potatoes, or cassava, or through cellulose from biomass, into ethanol via fermentation followed by distillation [Wang, 2000].

The principle fuel used as a petrol substitute for road transport vehicles is bioethanol. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. Bioethanol is one of the cheapest biofuels which is produced by different types of lignocellulosic biomass. Bioethanol is produced from different resources of biomass by using different process, such

as pretreatment, hydrolysis and fermentation. Production of ethanol from agricultural and biodegradable wastes provides a variable solution to multiple environmental problems simultaneously creating sink for waste and renewable energy production as well. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and greenhouse gas emissions [Wang, 1999]. Mostly, fuel ethanol is produced from sugarcane, corn, wheat and sugar beets. The use of lignocellulosic energy crops, and particularly low-cost biomass residues, offers excellent perspectives for large-scale application of ethanol in transportation fuels [Ridder, 2000]. These materials will increase the ethanol production capacity and reduce of production cost to a competitive level. Bioethanol from these materials provides a highly cost-effective option for CO₂ emission reduction in the transportation sector [GM Europe, 2002]. Lignocellulosic materials, such as crop residues, grasses, sawdust, wood chips etc., are inexpensive, abundant and renewable source for ethanol production [Sun, 2002].

Bio Butanol

Biobutanol fuel is second generation alcoholic fuel with a higher energy density and lower volatility vs. ethanol. Butanol is produced by fermentation, from corn, grass, leaves and agricultural wastes. Butanol with a Reid Value of 0.33 psi. is safer to handle (less evaporative) when compared to gasoline at 4.5 and ethanol at 2.0. Butanol is an alcohol that does not have to be - blended with fossil fuels replaces gasoline one to one. Butanol can be shipped through existing pipelines. It is far less corrosive than ethanol. Butanol when consumed in an internal combustion engine yields no SOX, NOX or carbon monoxide; the CO₂ is 'Green'.

Acetone–butanol–ethanol (ABE) fermentation was carried out industrially throughout the United States during the first half of last century, but was discontinued in the early 1960s due to unfavorable economic conditions brought about by competition with the petrochemical industry [Ezeji, 2004]. Fermentation substrate is an important factor influencing the cost of butanol production [Qureshi, 2000]. Lignocellulose is the most abundant renewable resource on the planet, and has great potential as a substrate for fermentation. Hemicelluloses are the second most abundant polysaccharides in nature, and represent about 20 to 35% of lignocellulosic biomass [Koukiekolo, 2005]. Xylan or hemicellulose may contain Arabian, galactan, glucuronic, acetic, ferulic, and coumaric acids as well as xylose. The occurrence and quantity of these compounds depend on the sources of xylan [Olsson, 1996] [Koukiekolo, 2005]. Since substrate cost has the most influence on butanol price [Qureshi, 2000], we focused their research on the use of agricultural residues for fermentative production of butanol using *Clostridium beijerinckii* BA101, a genetically modified, hyper-butanol producing strain. In particular, they were focusing on the potential use of corn Stover, corn fiber, and fiber-rich distillers dried grain soluble (DDGS) as the substrates for growth and butanol production by *C. beijerinckii* BA101.

Bio-diesel

Biodiesel is an alternative fuel similar to conventional or 'fossil' diesel. Biodiesel can be produced from straight vegetable oil, animal oil/fats, tallow and waste cooking oil. The process used to convert these oils to Biodiesel is called transesterification. The largest

possible source of suitable oil comes from oil crops such as rapeseed, palm or soybean. Most biodiesel produced at present is produced from waste vegetable oil sourced from restaurants, chip shops, industrial food producers such as Birdseye etc.

Almost all biodiesel is produced using base catalyzed transesterification as it is the most economical process requiring only low temperatures and pressures and producing a 98% conversion yield. The Transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. During the etherification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel and crude glycerol. In most production methanol or ethanol is the alcohol used (methanol produces methyl esters, ethanol produces ethyl esters) and is base catalyzed by either potassium or sodium hydroxide. Potassium hydroxide has been found to be more suitable for the ethyl ester biodiesel production; either base can be used for the methyl ester. A common product of the transesterification process is Rape Methyl Ester (RME) produced from raw rapeseed oil reacted with methanol [Singh, 2013; **Biofuels: An alternative to conventional fuel and energy source**, <https://doi.org/10.1016/j.matpr.2021.08.227>]

Advantages and Disadvantages of Biofuel Production and Consumption

Biofuels are often sold in the global market as blends with fossil fuels, in order to be compatible with conventional vehicle engines and reduce the need for additives [Yusoff, 2020]. Brazil has successfully implemented public policies to increase biofuel consumption and in turn improve air quality. The introduction of flex-fuel vehicles, biofuel subsidies, and government mandates have all contributed to Brazil's current ranking as one of the cleanest energy countries in the world [Cardoso, 2019]. Indonesia has the potential to become the largest biofuel manufacturer in the world [Putrasari, 2016]. Despite first generation biofuels being successfully implemented globally, recent studies highlighted negative consequences of expanding their production further and argue for a greater effort on the production of advanced biofuels [Immerzeel, 2014] [Aro, 2016] [Lee, 2013].

Experts in ecology and global dynamics have long established that the removal of more than 1% of matter circulating in ecosystems leads to a violation of the stability and subsequent degradation of the biosphere. Humanity currently consumes almost 10% of the products as food, fodder and fuel from natural land ecosystem which is the main reason for the degradation of biosphere [Arutyunov, 2017].

The economic security of a country can be improved with a larger biofuel industry, especially through the creation of jobs in rural and under-developed areas [Banerjee, 2019] [Subramaniam, 2020]. In addition, not all countries have large crude oil reserves so a shift towards biofuel production would allow countries to reduce both their dependence on fossil fuels and, consequently, their import costs [Oliveira, 2017].

Biodiesel also has a better lubricity and is less toxic than conventional diesel, making it safer and easier to handle. In spite of these benefits, several problems stand in the way of using biofuels as a replacement to fossil fuels, for example, comparing with diesel, biodiesel has a

lower calorific value, higher NO_x emissions, higher copper strip corrosion and fuel pumping difficulty [Agarwal, 2006] [Demirbas, 2009]. However, the influence of the lower calorific value of biofuel on the performance of the engine is reduced by more efficient combustion due to the presence of oxygen in biodiesel and/or ethanol. Biofuels also have higher production costs in the current market due to requirement of more intensive processing procedures and, as with increased agriculture of any form, the production and end use of biofuels comes with concerns of deforestation, biodiversity loss and increased fertilizer and pesticide use [Webb, 2012].

The increased biofuel production can simultaneously improve food production by reducing GHG emissions which could be a current threat to food security. Reduced GHG emissions would lead to a lower global temperature, which could both increase crop yield and the quality of crops grown [Subramaniam, 2020]. A greater demand for biofuels could also encourage agricultural investment which would benefit both food and biofuel production, as well as bring opportunities to under-developed areas [Oliveira, 2017].

Advanced fuels share many advantages over first-generation biofuels, such as reduced land requirements and minimal fertilizer, pesticide, and fossil energy input [Hill, 2006]. Additional benefits have been recognized when using microalgae as a feedstock, such as faster biomass production, high oil content and a capability to grow throughout the year in both natural and artificial environments [Sharma, 2020].

A key benefit associated with the replacement of fossil fuels for biofuels is the reduced air pollution from motor vehicles [Arutyunov, 2017] [Larson, 2006]. Explosions are reported with the extraction of crude oil for fossil fuels and many aquatic reserves can be polluted during extraction, posing a damaging effect to sea-life [Zabbey, 2017], thus extraction of biofuels is far less damaging to the local environment.

Isolation and Screening of Cellulose degrading bacteria

The macerated gut of the collected organisms was inoculated in a basal salt media (NaNO₃ 2.5 g; KH₂PO₄ 2 g; MgSO₄ 0.2 g; NaCl 0.2 g; CaCl₂·6H₂O 0.1 g in a litre) containing filter paper (Whatman filter paper no. 1 of area 70.541 cm²) for the isolation of cellulolytic bacteria. These cultures were incubated for 7 days in a shaker incubator at 37°C at 100 rpm. Bacterial colonies capable of utilizing cellulose as sole source of carbon were isolated on cellulose agar media composed of KH₂PO₄ 0.5 g MgSO₄ 0.25 g cellulose 2.0 g agar 15 g gelatin 2 g and distilled water 1L and at pH 6.8–7.2.

Confirmation of cellulose-degrading ability of bacterial isolates was performed by streaking on the cellulose Congo-Red agar media with the following composition: KH₂PO₄ 0.5 g, MgSO₄ 0.25 g, cellulose 2 g, agar 15 g, Congo-Red 0.2 g, and gelatin 2 g; distilled water 1 L and at pH 6.8–7.2. The use of Congo-Red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discoloration of Congo-Red were taken as positive cellulose-degrading bacterial colonies [Lu, 2004], and only these were taken for further study. Cellulose-degrading potential of the positive isolates was also qualitatively estimated by calculating

hydrolysis capacity (HC), that is, the ratio of diameter of clearing zone and colony [Hendricks, 1995].

Leading countries based on biofuel production worldwide in 2021

Country	Production in petajoules
United States	1,435.8
Brazil	839.5
Indonesia	311.9
China	142.7
Germany	121.2
France	107
Thailand	89.8
Argentina	85.6
Netherlands	84.6
Spain	71.9

Commercial application

Paper products: Cellulose is the major constituent of paper, paperboard, and card stock. Electrical insulation paper: Cellulose is used in diverse forms as insulation in transformers, cables, and other electrical equipment. [Kohman, 1939].

Fibers: Cellulose is the main ingredient of textiles.

Consumables: Microcrystalline cellulose (E460i) and powdered cellulose (E460ii) are used as inactive fillers in drug tablets. [Weiner, 2000].

Building material: Hydroxyl bonding of cellulose in water produces a sprayable, moldable material as an alternative to the use of plastics and resins. The recyclable material can be made water- and fire-resistant. It provides sufficient strength for use as a building material. [Zeoform, 2013].

Pharmaceuticals: Cellulose derivatives, such as microcrystalline cellulose (MCC), have the advantages of retaining water, being a stabilizer and thickening agent, and in reinforcement of drug tablets. [Thoorens, 2014].

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CHAPTER

29

TOP FIVE PATHOGENIC BACTERIA IN PLANT PATHOLOGY

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A

bstract

The majority of plant bacteriologists believe that their particular microbe should be included on any list of the most important bacterial plant diseases. However, we are not aware of any such list. The purpose of this chapter was to poll all bacterial pathologists affiliated with the publication Molecular Plant Pathology and ask them to list the top ten bacterial pathogens in terms of their significance for science and the economy. The survey resulted in 458 votes from respondents around the world, which enabled the creation of a list of the top 10 bacterial plant pathogens. In order of appearance on the list, there are the following: (1) *Pseudomonas syringae* pathovars (2) *Ralstonia solanacearum* (3) *Agrobacterium tumefaciens* (4) *Xanthomonas oryzae* pv. *Oryzae* (5) *Xanthomonas campestris* pathovars *Candidatus Liberibacter asiaticus*, *Pseudomonas savastanoi*, and *Clavibacter michiganensis* (*michiganensis* and *sepedonicus*) are granted honourable mentions for just missing the Top 5. In order to spark discussion and debate within the community of plant bacteriology and establish a standard, this review article provides a brief section on each bacterium in the Top 5 list and its significance. It will be interesting to observe how attitudes develop and which bacterial diseases enter and exit the Top 5 in upcoming years.

Keywords: *Bacterium. Pathogenic Bacteria, Pathology, Plants.*

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Introduction

Recently, the journal Molecular Plant Pathology explored which viruses would make up a list of the top 10 plant viruses according to the contributors' assessments of their significance in terms of science or business. (Villamor *et al.*, 2019). This was followed by a similar review on fungi (Wingfield *et al.*, 2012). These studies were conducted because several papers, reviews, and grant applications assert that a specific plant virus or fungal disease is extremely important—and probably for good reason. A similar survey was conducted for plant pathogenic bacteria as a result of the interest that the surveys of plant viruses and fungal pathogens had generated. As before, bacterial pathologists affiliated with the journal Molecular Plant Pathology were contacted and asked to suggest three plant pathogenic bacteria that they would expect to see on a list of the most important bacterial pathogens in terms of both science and commerce. The chapter format and layout naturally resemble those of the Top 10 Virus and Top 10 Fungal pathogens. (Villamor *et al.*, 2019; Wingfield *et al.*, 2012). The poll received 458 responses from respondents worldwide, enabling the creation of a Top 10 list of bacterial plant pathogens for the journal Molecular Plant Pathology (Table-1).

Top 10 bacterial plant pathogens are shown in **Table-1**. The ranking of bacteria, as determined by plant bacteriologists affiliated with the journal Molecular Plant Pathology, is shown in the table.

Rank	Bacterial Pathogen	Author of bacterial description
1	<i>Pseudomonas syringae</i> pathovars	John Mansfield
2	<i>Ralstonia solanacearum</i>	Stéphane Genin
3	<i>Agrobacterium tumefaciens</i>	Shimpei Magori, Vitaly Citovsky
4	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Malinee Sriariyanum, Pamela Ronald
5	<i>Xanthomonas campestris</i> pathovars	Max Dow

Pseudomonas syringae is the bacteria, or group of pathovars, that is most strongly supporting itself on scientific and commercial grounds. Both individual pathovars and groups of pathovars received votes. It is obvious that *P. syringae* continues to produce commercially significant plant diseases and has had a significant impact on our scientific understanding of microbial pathogenicity.

Ralstonia solanacearum, which ranks quite highly in terms of economic significance globally, is in second position. This is especially true given that it has a very broad host range, with impacted crops ranging from potato to banana.

Agrobacterium tumefaciens, which is third, makes a solid showing based solely on its scientific significance. Despite the fact that this bacterium can seriously harm some crops, it received support for its significance in scientific discoveries and applications.

Xanthomonas species, which are clearly unique in their pathologies and host targets, are in positions four, five, and six and each has received a considerable amount of support.

Xanthomonads with relatively narrow crop targets, like *Xanthomonas oryzae* pv. *oryzae*, one of the most dangerous diseases of rice, and *Xanthomonas axonopodis* pv. *manihotis*, the cause of cassava bacterial blight (CBB), are ranked fourth and sixth, respectively. The fifth-placed pathovar of the *Xanthomonas campestris* genus, which causes illnesses in a variety of crops worldwide.

Erwinia amylovora, which causes the well-known fire blight disease of ornamentals, fruit trees, and bushes, is ranked eighth. This condition has a long scientific history and continues to have economic significance.

The eighth spot in the Top 10 is rightfully occupied by *Xylella fastidiosa* because it is linked to various significant diseases of trees and crops. It also makes the significant scientific claim that its genome was the first phytopathogen's to have been sequenced (apart from plant viruses). It was chosen to combine two *Dickeya* species, *Dickeya dadantii* and *D. solani*, for the ninth-place entry because *Dickeya* received a substantial number of votes, many of which were simply referred to as *Dickeya* spp. This is likely understandable given that these bacteria's taxonomy could be said to be in flux. Despite the fact that the name *Dickeya solani* has not yet received official recognition, it is obvious that *Dickeya* spp. cause economically significant illnesses, especially in potato.

The tenth entry, *Pectobacterium carotovorum* (which also includes *P. atrosepticum*), earned a spot in the Top 10 due to the economic losses associated with the soft rot diseases as well as its role in various scientific breakthroughs. This is in addition to certain ongoing translational advancements, such participation in the management of a few leukaemias. While identifying the opinions of contributors to Molecular Plant Pathology for the Top 10 Most Important Plant Pathogenic Bacteria was the goal of this chapter, the authors are well aware that importance and priority might differ locally across continents and disciplines. We also recognise that not all bacteria can rank among the top 10 due to logical number restrictions, despite the fact that such bacteria can still be viewed as being of paramount importance. Therefore, we thought it fair to provide bacteria that just missed making the Top 10 list honourable mentions, such as *Clavibacter michiganensis* (*michiganensis* and *sepedonicus*). (Nandi et al., 2018). *Pseudomonas savastanoi* (Bardaji et al., 2011); and *Candidatus liberibacter* (pv. *asiaticus*) (Li et al., 2009). all clearly important.

The Top 5 are covered in one-page summaries in this evaluation, along with supporting data and important reading recommendations. We anticipate that the evaluation will spark discussion and debate within the field of plant bacteriology and establish a standard. In the next years, it will be interesting to see how attitudes shift and which bacteria make it into and off of the Top 5 list.

1. *Pseudomonas syringae*

A team of pathovars earning an award seems a little unfair, similar to a relay team winning the 400-meter individual Olympic gold medal. of course, it may be argued that because *Pseudomonas syringae* is an incredibly adaptable species, the pathovar classification is unnecessary. The details that are now becoming available from genome sequencing are reviving this discussion. *P. syringae* excels on all counts when it comes to the criteria for this

prize, which included importance to basic research and influence on food production and/or the environment. *P. syringae*'s economic impact is growing due to the comeback of old diseases such as bacterial tomato speck (pv. tomato; Shenge *et al.*, 2008) and the emergence of new infections of importance worldwide, such as bleeding canker of horse-chestnut (pv. aesculi; Nowell *et al.*, 2016). The European Handbook of Plant Diseases (Lucas, 2009) describes 28 pathovars, each attacking a different host species. pv. aesculi can now be added to this list. Several pathovars, such as *pathovars savastanoi* and *morsprunorum*, harm trees over time, frequently by causing distortions and cankers. Annual crop infections are rarer, and outbreaks are frequently brought on by sowing contaminated seed. Many accounts emphasise *P. syringae*'s seed-borne nature, however it is a remarkable adaptable pathogen that may appear in some seemingly odd places, like snow melt waters (Morris *et al.*, 2010). Disease outbreaks are frequently devastating once new infections have been established, assuming good rainfall and temperature conditions, as demonstrated with bean halo blight caused by pv. phaseolicola (Ramos *et al.*, 2012).

Research into the molecular biology of virulence and plant defence against *P. syringae* has provided fresh perspectives on microbial pathogenicity, both with reference to illnesses that affect plants specifically as well as human health more broadly. For basic research on bacterial invasion and plant defence, the pathovars phaseolicola and tomato have proven to be excellent models (Preston, 2000; Xin, 2018).

Pseudomonas syringae is the market leader when it comes to the impact of high-throughput sequencing technologies on our understanding of pathogenicity. It's interesting to note that Collinge *et al.* (2022) forecast that "at least two dozen new *P. syringae* genomes will be released this year" was true was confirmed by the seminal study by Ravindran *et al.* (2015). The fact that pathovars colonising distinctly dissimilar plants are currently being closely grouped together may come as a surprise to some. For instance, the pathovars pv. savastanoi (olive) and pv. phaseolicola (bean) both belong to the same clade. Genomic analysis, initiated by Monteil *et al.* (2016) and Lindeberg *et al.* (2009), has perhaps the most potential for unravelling the determinants of host specificity. In addition to being the top group of plant pathogenic bacteria, *Pseudomonas syringae* pathovars also likely rank at the top of the list of all known pathogens, including fungi and oomycetes. The advancements made with *P. syringae* have greatly influenced research on the effector biology of filamentous pathogens (Cunnac *et al.*, 2011).

2. *Ralstonia solanacearum*

Probably the most harmful plant pathogenic bacterium in the world is *Ralstonia solanacearum*. The fact that the *R. solanacearum* species is made up of a sizable number of strains that differ in their geographic origin, host range, and pathogenic behaviour is one reason for this (Genin, 2010). This diverse group has been classified into four major phylotypes (phylogenetic groupings of strains) and is now recognised as a "species complex." The species as a total infects 200 plant species across more than 50 families, and it is the cause of potato brown rot, bacterial wilt of tomato, tobacco, eggplant, and several ornamentals, as well as Moko disease of banana.

The soil-borne pathogen *Ralstonia solanacearum* attacks plants by entering through fractures, cuts, or root tips at the locations where lateral roots originate. The bacterium then invades the xylem vessels and colonises the root cortex and travels via the plant's vascular system to reach the stem and aerial portions. **(Fig.1).** *Ralstonia solanacearum* can rapidly multiply in the xylem up to very high cell densities, leading to wilting symptoms and plant death. *R. solanacearum* is endemic in many poor nations in inter-tropical zones, where the disease's incidence is particularly severe for agriculture. The organism also causes significant losses in locations where it is under quarantine because of eradication regulations and limitations on continued cultivation on contaminated soil. *R. solanacearum* is a model system for the investigation of bacterial pathogenicity since it is a root and vascular pathogen. The pathosystems that have been created with model plants like *Arabidopsis* or the legume *Medicago truncatula* have facilitated genetic and molecular studies on both the plant and bacterial partners. The bacterium was one of the first plant pathogen genomes to be completely sequenced (Guidot *et al.*, 2007).

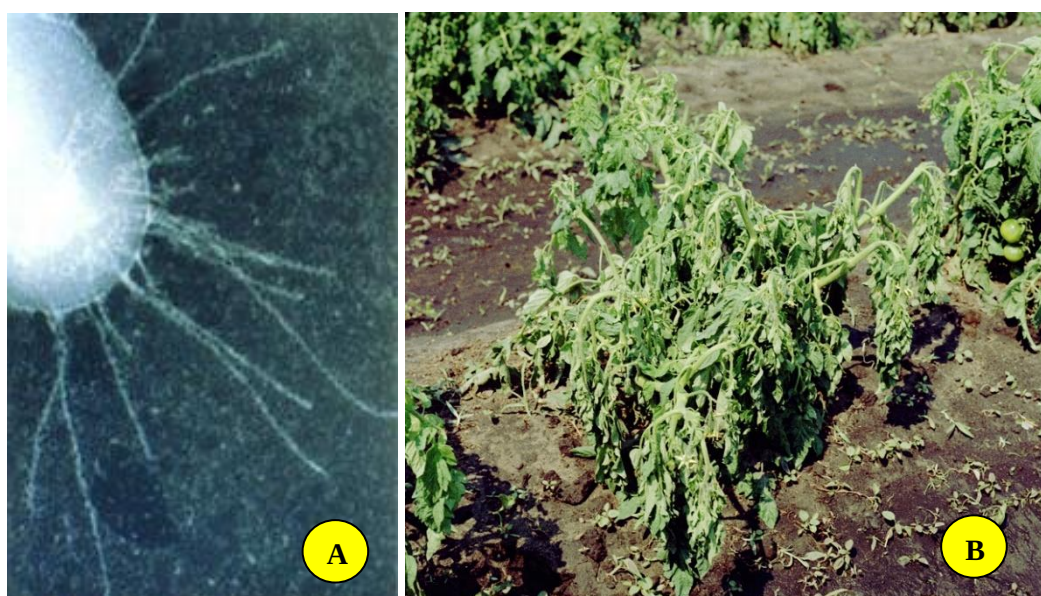


Fig.1: (A) *Ralstonia solanacearum* and (B) disease wilting symptoms on tomato.

Future studies in this area will focus on gaining a deeper understanding of the molecular mechanisms underpinning this adaptable group of strains' ability to coexist with such a wide variety of hosts. Another significant problem is figuring out how to harness our growing understanding of the complex processes *R. solanacearum* created to increase plant sensitivity to construct new and enduring defence systems to combat this terrible disease.

3. *Agrobacterium tumefaciens*

Crown gall tumour, one of the most devastating plant diseases affecting diverse crop species globally, was first linked to *Agrobacterium tumefaciens* by Tindall 2014. This soil-borne bacteria lowers crop production and growth vigour considerably by inducing neoplastic growths **(Fig.2)** at wound sites on host plants.



Fig.2. A crown gall by *Agrobacterium tumefaciens*

The 'Eureka' moment came in the late 1970s when Mary-Dell Chilton, Eugene Nester, and their associates demonstrated that the specific DNA segment (today referred to as the T-DNA) of the bacterial tumour-inducing (Ti) plasmid was present in the genome of infected plant cells (Hooykaas *et al.*, 1984). The importance of *Agrobacterium* as the first creature capable of trans-kingdom gene transfer was highlighted by this important discovery. Since then, a tonne of knowledge has been gained regarding the molecular mechanisms underlying *A. tumefaciens*-mediated genetic transformation, which has emerged as a highly complex process governed by a variety of bacterial and host factors (reviewed by Gelvin, 2010; Pitzschke and Hirt, 2010; Tzfira and Citovsky, 2002; Zupan *et al.*, 2000). In a nutshell, *A. tumefaciens* detects phenolic compounds released from plant wound tissues and triggers the development of a number of effectors known as virulence (Vir) proteins. The production of a single stranded copy of T-DNA (T-strand) and its transportation into the host cell via a type IV secretion system are mediated by some of these components. Several Vir proteins are also transported into plant cells together with the T-strand. The nuclear import of the T-strand and its subsequent integration into the host genome are made easier by these exported effectors working in conjunction with numerous host factors. The expression of genes involved in the manufacture of auxin and cytokinin from the integrated T-DNA results in aberrant cell proliferation in the infected tissues and the development of tumours, such as crown galls (**Fig.2**).

Plant pathologists and biologists are never able to stop being amazed by *Agrobacterium tumefaciens*. Even after a century of study, we are continually learning new mechanisms that underlie how *A. tumefaciens* interacts with its hosts and are just now starting to comprehend just how cunning this virus is. For instance, recent studies have revealed that *A. tumefaciens* can subvert the host defence machinery for the active promotion of infection (Pruss *et al.*, 2008). Therefore, *A. tumefaciens* will continue to be used as a potent tool for plant genetic engineering as well as a top-notch model organism to understand host-pathogen interactions in the near future.

4. *Xanthomonas oryzae*

Both tropical and temperate regions are affected by bacterial leaf blight (BLB), which is brought on by *Xanthomonas oryzae* pv. *oryzae* (Xoo). BLB can also be found in the USA, Australia, Africa, Latin America, and the Caribbean (Iñó-liu *et al.*, 1993; Mew *et al.*, 1993). Yield losses of 10%–50% from BLB have been reported (Ou, 1972). Outbreaks of BLB are most common during the monsoon season in South-East Asia and India (Mew *et al.*, 1993). Rice was introduced for cultivation into the USA (North Carolina) more than 200 years ago and has been cultivated in other parts of the USA for over 100 years. Although many rice diseases have either been introduced or developed on rice during the history of its cultivation in the USA, Xoo has not established in the USA. The climates of rice-producing areas in the USA and USA rice cultivation practices are not conducive to the long-term survival or spread of Xoo. For these reasons, Xoo is of low risk to US agriculture. (1969; Mizukami and Wakimoto). Utilising resistant rice varieties effectively controls BLB. However, this resistance is frequently overcome since Xoo possesses the ability to express effectors that decrease various host defence responses (Triplett *et al.*, 2011).

A rod-shaped, Gram-negative bacterium called *Xanthomonas oryzae* pv. *oryzae* infects plants. Extracellular polysaccharide (EPS) and xanthomonadin, a yellow soluble pigment, are both produced by it. According to Huang *et al.* (1989) EPS is crucial for preventing wind- and rain-borne dissemination as well as protecting bacteria from desiccation.

5. *Xanthomonas campestris*

Worldwide, *Xanthomonas campestris* pathovars are responsible for important agricultural diseases. *Xanthomonas campestris* pv. *campestris* (Xcc), the cause of the black rot of crucifers that affects all cultivated brassicas, and *X. campestris* pv. *vesicatoria* are two of the most notable of these infections. (Xcv), the cause of bacterial spot, has been reclassified as *X. euvesicatoria* of tomato, pepper, and *X. campestris* pv. *malvacearum* (Xcm, currently) *X. axonopodis* pv. *malvacearum*), a cotton disease that results in angular leaf spot. Although black rot is also economically significant in temperate places, such as Cornwall and other western portions of the UK, it is particularly severe in areas with a warm and humid climate. Xcc is significant for producing the EPS xanthan, a food additive, pharmaceutical, oil drilling, and additive industries. Studies of these microorganisms have had a significant impact on science, and not just in the field of molecular plant pathology. The concept that a gene-for-gene pattern controls interactions between bacterial pathogens and plants was originally supported by research on Xcm (Gabriel *et al.*, 1986). By isolating the genes avirulence on pepper cultivars having the Bs1, Bs2, or Bs3 (for bacterial spot) resistance genes, work on Xcv established the genetic basis of the activation of disease resistance in pepper (Boch and Bonas, 2010; Astua-Monge *et al.*, 2000). In *Xanthomonas* spp., AvrBs3 is the model member of the vast family of TAL type III effector proteins. Later, it was shown that this effector is transported to the plant cell's nucleus, where it regulates gene expression by interacting with plant promoters (Boch and Bonas, 2010). The 'code' guiding promoter recognition by the majority of effectors in this family has been established (Fig.3).



Fig.3: Black rot disease symptoms on cabbage caused by *Xanthomonas campestris* showing the characteristic blackening of the leaf veins

Xanthan biosynthesis genes were discovered as a result of research on the Xcc (Capage *et al.*, 1987; Vorhölter *et al.*, 2008), as well as the rpf gene cluster, which controls the production of extracellular enzymes and xanthan and is associated with virulence. The cell-cell communication mechanism controlled by DSF, which was later identified as a cis-unsaturated fatty acid, was discovered through studies of the activity of the Rpf gene products (Ryan and Dow, 2011).

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CHAPTER

30

THE ROLE OF *Aspergillus tubingensis* IN MYCOREMEDIATION OF POLYURETHANE

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A**bstract**

Environmental pollution is one of the pollutions that has a major impact on all living organisms and their ecosystem in one way or another. Environmental pollution is mainly due to the accumulation of plastics such as polyurethane, polyethylene terephthalate, polypropylene, and many more. These polymers are harmful to animals, birds, aquatic organisms, and human beings too. Almost one million birds die annually due to plastic pollution. This harmful plastic is of two types which are thermoplastic and thermosetting. The thermosetting polymers are resistant to heat and are hard to break down. This makes the polymer take years to degrade. But with the help of recent research, it was found that *Aspergillus tubingensis*, a species of fungi produces an enzyme called serine hydrolase which makes degradation easier and quicker compared to other degradation methods. This enzyme breaks down the bonds between the substrate thereby reducing the molecular weight of the polymer. This enables the polymer to degrade within days rather than decades. Using the FASTA alignment files of serine hydrolase of *Aspergillus tubingensis*, the phylogeny of species with similar protein sequences enabled us to identify enzymes of similar species with the same activity.

Keywords: *Aspergillus tubingensis*, *Aspergillus niger*, Polyurethane, Mycoremediation, Serine Hydrolase.

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Introduction

Plastic is one of the leading causes of environmental pollution all around the world. Plastic is made out of different materials like polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and polycarbonates (PC). Every year around 19-23 million tonnes of plastic is being dumped across the globe which accounts for the death of many animals. More than one lakh marine animals are estimated to be killed every year by the entanglement of plastic. Plastic is not only hazardous to animals and aquatic ecosystems but also to the soil. The toxic chemicals present in the plastic can leach into the soil and thereby reduce the quality and fertility of the soil.

Polyurethane (Fig.1)

Among the plastic waste dumped, polyurethane is responsible for one-third of the environmental pollution. Plastic polymers and polyurethanes are usually made up of a combination of diisocyanates and polyols. Polyurethanes is a thermosetting polymer but some specific types exhibit thermoplastic properties too. As polyurethane is a thermosetting polymer, it is hard to degenerate. In a recent study conducted by the Kunming Institute of Botany, and the Chinese Academy of Sciences, the researchers found that the fungal species, *Aspergillus tubingensis* which is typically found in the soil has the ability to degrade the plastics. It can secrete an enzyme that breaks apart the bonds between individual molecules and disintegrates the polymer within weeks rather than decades.

Aspergillus tubingensis

Aspergillus tubingensis belongs to the family of trichocomaceae. The dark-pigmented fungus was discovered in the year 1934 by Raoul Mosseray. It is an asexual fungus which is closely similar to the taxa *Aspergillus niger*. The two taxa can be differentiated with the help of the indole test where *Aspergillus tubingensis* produces a positive result. *Aspergillus tubingensis* is morphologically similar to *Aspergillus* but differs by the presence of production of asparagine. *Aspergillus tubingensis* has the ability to grow in elevated temperatures that range from 30-37 degrees Celsius and is also highly resistant to ultraviolet light. *Aspergillus tubingensis* which grows in a 15-20°C temperature range is capable of producing mycotoxin and ochratoxin which are hazardous toxins present in them.

Polyurethane Structure

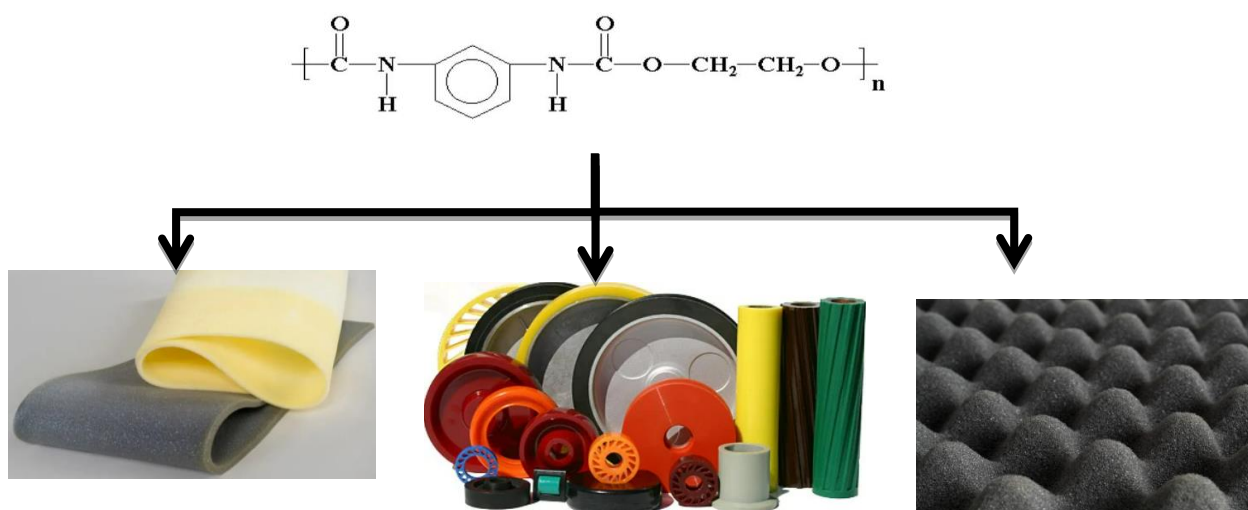


Fig.1: Structure of Polyurethane

Mycoremedial Activity of *Aspergillus tubingensis*

There are several mycoremedial activities done by the fungal species that help to eliminate the polymers that do not degrade for decades. The effective degradation of plastics is done by the involvement of enzymes like esterases, lipases, cutinase, carboxylesterases, and proteases. Some pre-oxidant ions and lignocellulolytic enzymes are also helpful in the degradation of polymers. The enzymes improve the hydrophilicity of polymers by creating functional groups either by oxidation or hydrolysis. Due to this activity, the molecular weight of polymers changes from high to low which helps in breaking down the polymer in a count of a few days rather than decades.

The serine hydrolase enzyme family which includes amidases, esterases, lipases, proteases, and peptidases is found in *Aspergillus tubingensis*. Serine hydrolase plays an important role in the hydrolytic cleavage of ester or thioester or amide bonds that degrade the substrate. This is achieved by the formation of a covalent acyl-enzyme intermediate when an activated conserved serine nucleophile attacks the corresponding electrophilic substrate.

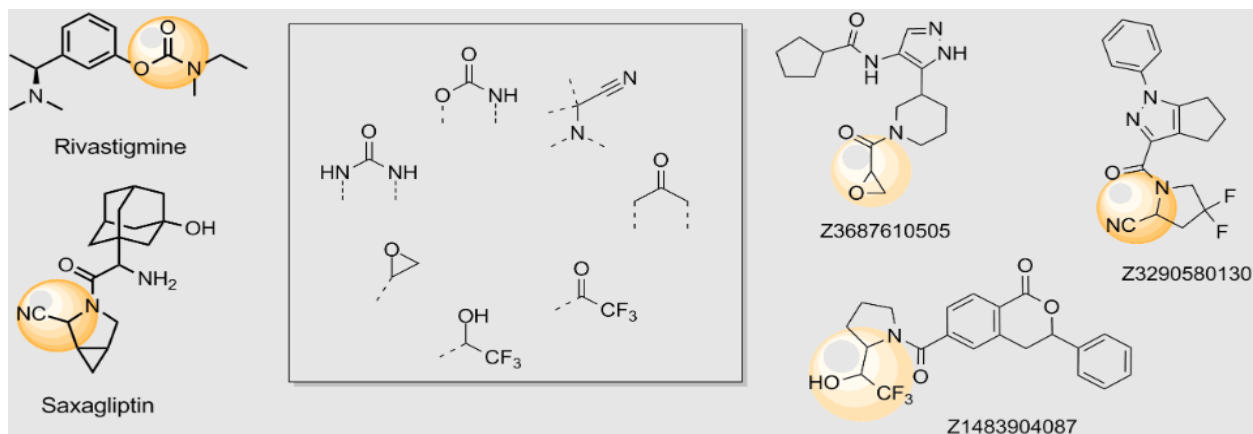


Fig.2: Structure of serine

Phylogeny Of Fungi

There are different types of fungal species which produce an enzyme that is capable of eliminating thermoplastic and thermosetting polymers.

The fungal species mostly belong to the genus *Aspergillus* such as *tubingensis*, *niger*, *awamori*, *fumigatus*, *phoenicis*, and *brasiliensis*. The family Aspergillaceae produces fungal laccases and peroxidases which are used for degrading polyethylene by acting on lignin on the other hand, esterases such as lipases and cutinases also help to degrade polyurethane and polyethylene terephthalate. These fungal ligninolytic enzymes are found to be very beneficial as they reduce the duration to eliminate the polymers on the surface of the earth and in return they also result in the production of oxygen.

Other than the family of Aspergillaceae, the fungal families like Pseudeurotiaceae and trichocomaceae which also eliminate plastics with the help of species like *Penicillium oxalicum* NS4, *Penicillium chrysogenum* NS10, *Pseudogymnoascus* sp. VKM F-3808, *Pseudogymnoascus* sp. VKM F-3557, etc... From the above-listed species, we are able to find the order Eurotiomycetes contains the largest number of plastic degraders among all the other microbes present in the world.

By the analysis of serine hydrolase of *Aspergillus tubingensis* using BLAST RID, the following data is collected.

The table given below shows the description of the serine hydrolase protein sequence of different species with similar alignment to that of *Aspergillus tubingensis* from BLAST using the database of NCBI.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
serine hydrolase [Aspergillus tubingensis]	Aspergillus tubingensis	912	912	100%	0.0	100.00%	438	XP_035351571.1
hypothetical protein ASPNIDRAFT_40267 [Aspergillus niger]	Aspergillus niger	908	908	100%	0.0	99.54%	438	GAQ46368.1
hypothetical protein AtubIFM57143_000909 [Aspergillus tubingensis]	Aspergillus tubingensis	889	889	97%	0.0	99.77%	428	GLA94050.1
Methyltransferase domain family protein [Aspergillus niger]	Aspergillus niger	887	887	100%	0.0	96.58%	611	TPR07655.1
hypothetical protein AtubIFM54640_010262 [Aspergillus tubingensis]	Aspergillus tubingensis	885	885	97%	0.0	99.53%	428	GLA67283.1
beta-lactamase/transportase-like protein [Aspergillus niger CBS 101883]	Aspergillus niger CBS 101883	885	885	100%	0.0	96.58%	439	XP_025460446.1
beta-lactamase/transportase-like protein [Aspergillus niger ATCC 13496]	Aspergillus niger ATCC 13496	884	884	100%	0.0	96.58%	438	RDH15139.1
hypothetical protein CBS147346_2337 [Aspergillus niger]	Aspergillus niger	884	884	100%	0.0	96.80%	438	KAI3008902.1
hypothetical protein CBS133816_1816 [Aspergillus niger]	Aspergillus niger	882	882	100%	0.0	96.35%	439	KAI2831939.1
hypothetical protein CBS11852_3206 [Aspergillus niger]	Aspergillus niger	881	881	100%	0.0	96.12%	439	KAI2899639.1
hypothetical protein CBS147343_4679 [Aspergillus niger]	Aspergillus niger	879	879	100%	0.0	95.89%	439	KAI3074225.1
hypothetical protein CBS147324_2062 [Aspergillus niger]	Aspergillus niger	877	877	100%	0.0	96.12%	439	KAI2977388.1
hypothetical protein CBS13152_8182 [Aspergillus niger]	Aspergillus niger	873	873	100%	0.0	95.89%	438	KAI2884119.1
hypothetical protein AnigIFM62618_000916 [Aspergillus niger]	Aspergillus niger	872	872	100%	0.0	95.66%	438	GLA14534.1

The below given graphical representation shows the multiple sequence alignment from NCBI BLAST results to view alignment files in alignment FASTA.



Blast Tree View

This tree was produced using BLAST pairwise alignments. [more...](#)

Reset Tree

BLAST RID: [DNN4XS2R01N](#) Query ID: [Id|Query_534362](#) Database: [nr](#)

Tree method: Neighbor Joining Max Seq Difference: 0.85 Distance: Grishin (protein) Sequence Label: Sequence Title (if avail)

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

Find:

Multiple organisms | 69 leaves

- hypothetical protein CBS13152_8182 [Aspergillus niger]
 - hypothetical protein CBS1350_4840 [Aspergillus niger]
 - 6-aminocaproate-dimer hydrolase [Aspergillus awamori]
 - hypothetical protein AnigIFM49718_008157 [Aspergillus niger]
 - hypothetical protein AnigIFM62618_000916 [Aspergillus niger]
 - hypothetical protein AnigIFM60653_001424 [Aspergillus niger]
- ascomycete fungi | 2 leaves
- ascomycete fungi | 4 leaves
- ascomycete fungi and unknown | 7 leaves
 - hypothetical protein CBS147346_2337 [Aspergillus niger]
 - hypothetical protein AnigIFM63326_006092 [Aspergillus niger]
 - hypothetical protein CBS147324_2062 [Aspergillus niger]
 - hypothetical protein CBS11852_3206 [Aspergillus niger]
 - hypothetical protein AnigIFM63604_010402 [Aspergillus niger]
 - beta-lactamase/transportase-like protein [Aspergillus niger]
 - hypothetical protein AnigIFM59636_008424 [Aspergillus niger]
 - unnamed protein product [Aspergillus niger]
 - hypothetical protein CBS133816_1816 [Aspergillus niger]
 - hypothetical protein CBS147343_4679 [Aspergillus niger]
 - beta-lactamase/transportase-like protein [Aspergillus niger ATCC 13496]
 - beta-lactamase/transportase-like protein [Aspergillus niger CBS 101883]
 - Methyltransferase domain family protein [Aspergillus niger]

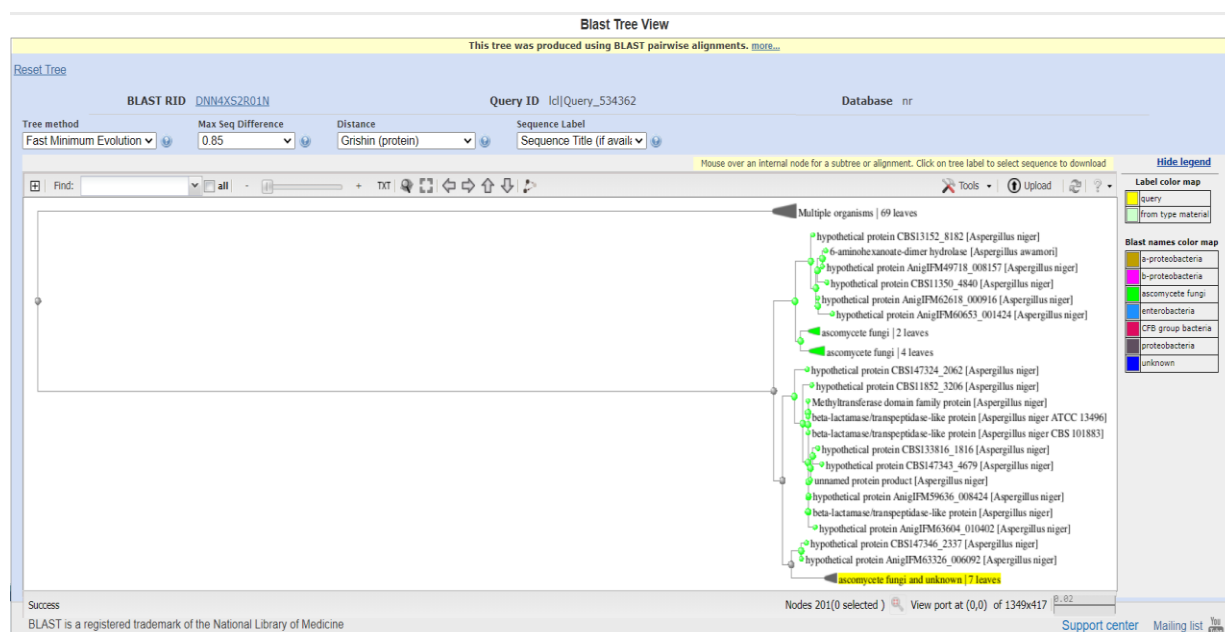
Success

BLAST is a registered trademark of the National Library of Medicine

Nodes 201(0 selected) View port at (0,0) of 1249x417

Support center Mailing list

The fast minimum evolution is one of the methods of blast tree view which is employed in the phylogenetic modeling. It searches for the phylogeny with shortest total sum of branch lengths with maximum parsimony. It is depicted below.



Conclusion

The family Aspergillaceae possesses the major species for the degradation of polymers that are both thermoplastic and thermosetting. This family of fungal species produces enzymes such as cutinase, proteases, lipases and lignocellulolytic enzymes. All these enzymes come under the serine hydrolase enzyme family. These enzymes reduce the molecular weight of the polymers significantly by breaking down the bonds between the substrate. Thereby reducing the duration of degradation of the polymers from decades to days.

An experimental analysis of the enzyme serine hydrolase of *Aspergillus tubingensis* was done using the FASTA alignment files along with the application of BLAST. The data was collected from National Center for Biotechnology Information, NCBI. It showed the phylogeny of species related to *Aspergillus tubingensis* in BLAST tree view. The protein sequence alignment also gave the description about the enzymes and species that have similar protein sequence to that of the enzyme serine hydrolase produced from *Aspergillus tubingensis*.

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CHAPTER

31

CYANOBACTERIA'S RISING INTERESTS IN NANOTECHNOLOGY AND GENETIC ENGINEERING TO BOOST PRODUCTION FOR FUTURE APPLICATIONS

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A**bstract**

Numerous sectors of study and development could be completely transformed by nanotechnology. The magic elixir that gives a component special property that are not there in its natural state is a procedure that uses many nanoparticles. It is improper to create microparticles using methods that contain toxic substances in the context of current research. Because of this, researchers are looking into safer ways to create Cyanobacteria, a type of microorganism with numerous new traits that may be used to synthesis nanoparticles. A description of the genetic engineering-assisted evolution of cyanobacterial strains follows. A discussion of recombination techniques that could be utilized to boost cyanobacteria's industrial potential is also included. This paper seeks to describe potential areas for future research as well as an outline of the work done to expand the market for cyanobacteria.

Keywords: *Cyanobacteria, Genetic Engineering, Nanoparticles.*

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Introduction

When combining many fields of science and technology, the word "nanotechnology" describes the processing, separation, consolidation, and deformation of materials by an atom or molecule. This has had a huge impact on every branch of science and technology, and it may even be the beginning of a new industrial revolution. Due to the combined effects of research and development (R&D), business, and commerce, notably in the medical, defense, and hygiene industries, the requirement for nanotechnology-derived nonmaterial will directly impact the global economy.

These particles come into three main categories: natural nanoparticles, incidental nanoparticles, and artificial nanoparticles. They are transients between bulk materials and molecular structures with a high ratio of surface area to mass. These can be further divided into natural carbon-containing nanoparticles, such as humic and fulvic acids, viruses, nanoglobules, and polyethyleneglycol, as well as anthropogenic carbon-containing nanoparticles, such as functionalized fullerenes, carbon nanotubes and DNA and peptide-based nanomaterials (Sangeetha et al., 2012), natural inorganic nanoparticles, such as sea salts, magnetite,

(Nowack and Bucheli, 2007; Buzea et al., 2007). These nanoparticles are extremely small in size and have a high surface-to-volume ratio. Numerous industries, including clinical diagnostics and treatments, electronics, cosmetics, textiles, food processing and packaging, wastewater treatment, environmental remediation, and agriculture, have found substantial use for noble metallic nanoparticles (Li et al., 2008; Mohanpuria et al., 2007; Shan et al., 2009; Bergeson 2010; Zhang and Fang, 2010; Zambrano- Zaragoza et al., 2011; Doria et al., 2012; Khot et al., 2012).

It focuses on producing NPs with little to no hazardous input and without endangering the environment or endangering human health. Biosystems like bacteria, viruses, algae, yeast, fungi, and plants have been extensively used in these environmentally friendly methods, making significant contributions to nanotechnology (Bhattacharya et al. 2005; Sharma et al., 2007; Bar et al., 2009; Song et al., 2009; Eroglu et al., 2013; Kharissova et al. 2013; Mittal et al. 2013).

The Future of Aquaculture: Genetically Modified Nanosized Cyanobacteria

To encourage safe and efficient fish production practises and to create ecologically acceptable means of preventing fish disease outbreaks, new tactics are required. Therefore, cyanobacteria are considered to be promising, environmentally friendly, and highly sustainable microbial cell factories for the production of value-added goods (Heidorn et al., 2011) when combined with the wide variety of genetic engineering technologies currently accessible. Researchers have demonstrated that recombinant proteins may be produced by cyanobacteria and loaded into EVs. This study lays the way for the use of cyanobacterial extracellular vesicles (EVs) as a cutting-edge biotechnological tool in fish, with potential applications in delivering proteins and enzymes to change fish diet or trigger specific

adaptive immune responses (Matinha-Cardoso et al., 2022). NKBG 15041c strain of *Synechococcus* sp. may be used as a long-term source of 13 fatty acids (13FA). The majority of the enzymes required to catalyze the formation of 13FA are housed in the cytoplasm, chloroplast, and endoplasmic reticulum in cyanobacteria, in contrast to eukaryotic microalgae, which have a more complicated intracellular structure (Heydarizadeh et al., 2013; Li et al., 2015; Liang et al., 2013). As antibiotic resistance genes (ARGs) proliferate in aquatic habitats, serious environmental safety issues are being experienced globally. It has been established that cyanobacterial extracellular DNA (eDNA) contributes more to and extends the survival of ARGs than intracellular DNA (iDNA). The two most prevalent genus types in cyanobacterial blooms, *Microcystis* and *Synechococcus*, have differing ARG abundances.

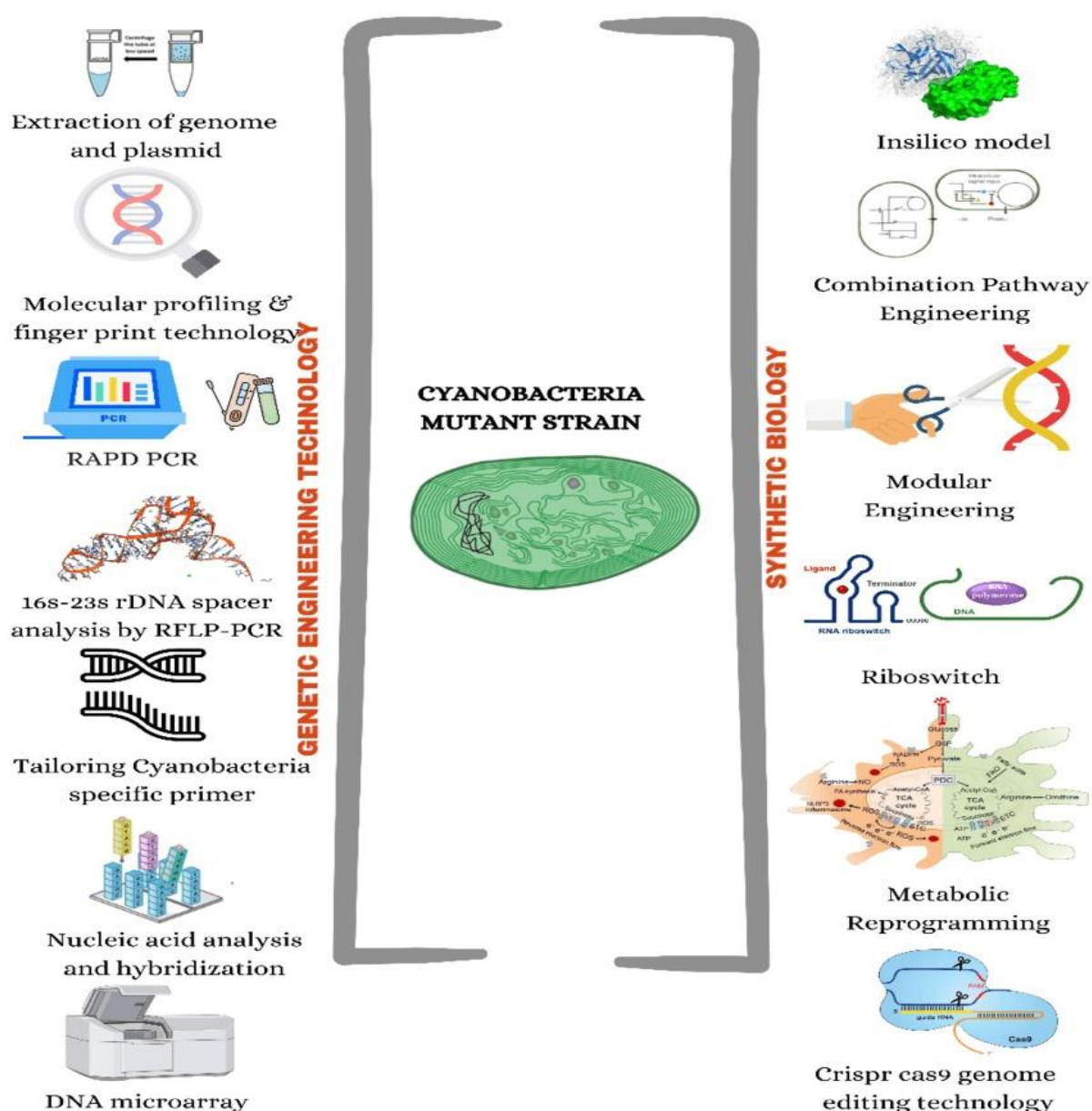


Figure-1: The use of cyanobacteria as a platform for genetic engineering and synthetic biology (Govindasamy et al., 2013)

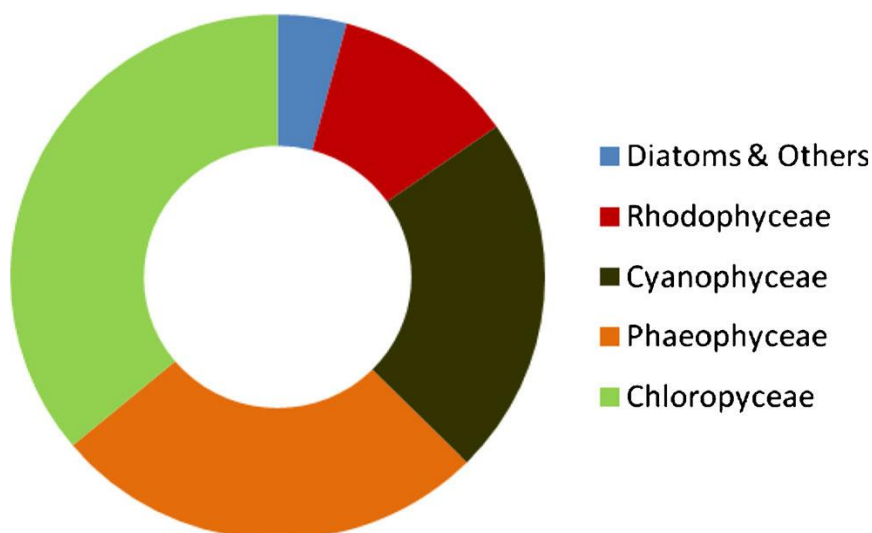


Figure-2: Different kinds of prospective algae have recently contributed to the manufacture of metallic nanoparticles. (Sharma et al., 2015)

High-Value Therapeutics Using Genetically Modified Cyanobacteria Nano-Formulation

Bioactive natural substances with significant commercial and therapeutic potential are produced by cyanobacteria. However, because of their poor productivity and the high cost of separating cyanobacteria species, only a small number of groups of them are produced in substantial quantities (Clarens et al., 2010; Norsker et al., 2011). Sulfated polysaccharides, which are highly valued in the nutraceutical, cosmetics, and pharmaceutical industries, can be produced from marine algae, particularly cyanobacterial species (Manivasagan and Oh, 2016). Due to their high biocompatibility, resilience, biodegradability, cost-effectiveness, non-toxic nature, and unique physiochemical properties, polysaccharide-based nanoparticles are also attracting a lot of interest from nanotechnologists as novel nanocarriers for cell imaging and therapeutic approaches (Lee et al., 2000). A number of studies have also examined the use of polysaccharide-based nanomaterials in medical applications like antibacterial activity, medication transport, gene therapy, and wound healing (Cardoso et al., 2016; Manivasagan et al., 2017). Nostoflan, an acidic polymer produced by *N. flagelliforme*, exhibits antiviral properties against viruses like influenza that have envelopes (Kanekiyo et al., 2005). The discovery of the natural oxadiazine nocuolin A (NoA) from several cyanobacterial genera, represents the subsets of the ostensible genes for synthesis of NoA in their genome, shows that these cyanobacterial genera have a distinctive biodiversity of natural products (Voracova et al., 2017).

Crop Improvement Using Gene Delivery Through Cyanobacteria-Mediated Nanoparticles

Human activities may cause soil to become less fertile, more salinous, or sterile, which could have a negative impact on agricultural productivity (Li et al., 2019). By fixing atmospheric nitrogen, producing an extracellular matrix, and producing the appropriate solutes,

engineered *Nostoc sp.* and *Anabaena sp.* are particularly well-suited to counteract the soil salinization condition (Li et al., 2019). Notable examples of organisms that fix nitrogen include *Anabaena*, *Aulosira*, *Nostoc*, *Calothrix*, *Plectonema*, etc. Supplements containing *Spirulina* may encourage sustainable agriculture, guaranteeing a supply of food to meet the needs of expanding populations and environmental objectives (Godlewska et al., 2019). By enhancing their capacity to bind water and nutrient content, cyanobacteria and microalgal biomass are employed to produce fertilizer that is used to revitalize impoverished soil (Zahra et al., 2020).

Biosynthesis Of Bio-Nanoparticles

By using 'green' methods, microbes may produce nanoparticles (NPs), which is an effective way to use microorganisms as handy nano-factories (Mishra et al., 2015). Recently, phyto-nanotechnology has opened up new pathways for the synthesis of nanoparticles and is a simple, quick, stable, and affordable process that is also environmentally beneficial. Biocompatibility, flexibility, and the medicinal usefulness of integrating nanoparticles using the all-purpose solvent, water, as a reducing medium are benefits of phyto-nanotechnology. Recently, using the leaf and root extracts from the therapeutic herbal plant *Panax ginseng*, gold and silver nanoparticles were successfully combined (Singh et al., 2015).

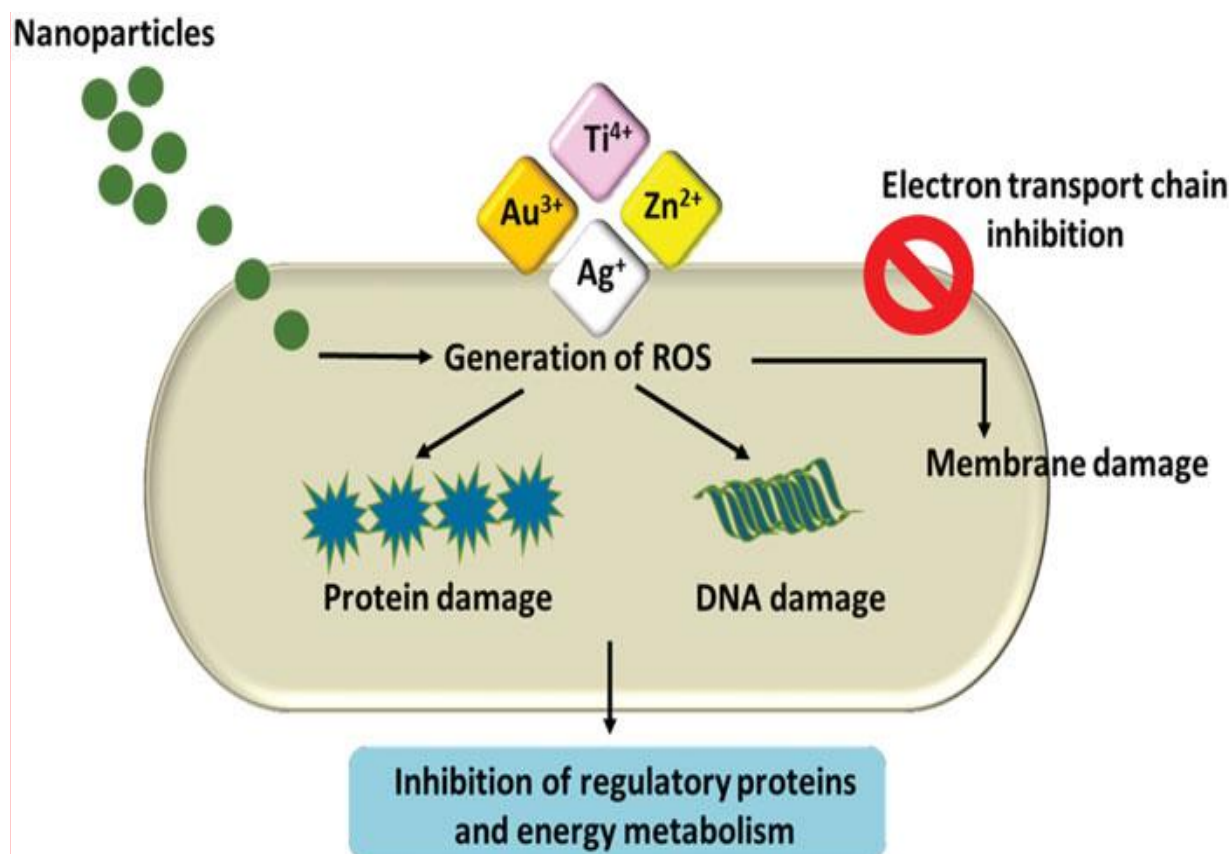


Figure-3: An overview of how nanoparticles affect microbes is as follows: they prevent the production of reactive oxygen species (ROS) and the electron transport chain, which damages DNA or proteins and ultimately leads to cell death (Khalid, 2019).

Mechanism Of Silver Nanoparticles' (Ag-Nps) Antibacterial Activity

According to a study by Morones et al., 2005, the bacterial cells (*Escherichia coli*) that were exposed to Ag-NPs died as a result of the nanoparticles' (NPs') adhesion to sulfur-containing proteins in the membranes, which increased the membrane's permeability. The existence of the peptidoglycan layer may play a role in the method by which Ag-NPs affect bacterial cells (without affecting mammalian cells), according to further research on the subject. The interaction of Ag-NPs exclusively affects bacterial cells since it is known as the particular membrane component of bacterial cells and is absent from mammalian cells (Kim et al., 2014). Researchers have also looked into the possibility that Ag-NP activity is more prominent in Gram-negative bacteria (Dixit et al., 2018). According to Kim et al.'s research, Ag-NPs' antibacterial action is caused by the generation of free radicals, which cause membrane damage. (Kim et al., 2014). Ag-NPs-polymer composites can also be used to increase anti-bacterial activity.

When coupled with Ag-NPs to form a composite, the cationic polysaccharide chitosan demonstrated more antibacterial action than Ag-NPs alone (Aymonier et al., 2002; Kumar and Munstedt, 2005; Melaive et al., 2005).

Mechanisms Of Silver Nanoparticles' Antifungal Action

According to reports, the attachment of Ag-NPs to cell membranes and their interaction with phosphorus-containing substances like DNA cause the cell to penetrate, which disrupts the replication and respiration activities in microbes. Ag-ions have a known strong cellular reaction with enzyme thiol groups that results in their deactivation (Rajeshkumar 2019). Experimental findings from a few research demonstrated that treatment with Ag-ions causes DNA to lose repeatability, which ends in cell death (Reidy et al., 2013). Similar to this, treatment with Ag-NPs causes the death of fungal cells by causing the creation of insoluble substances in the fungal cell wall, which starts the sulfhydryl groups' inactive mode and disrupts the lipids and enzymes in the cell wall.

Mechanism For Metal Nanoparticles' Antiviral Action

Metallic nanomaterials may diffuse into the cell and affect the cell by damaging the viral genome (DNA or RNA) in addition to directly interacting with viral cell surface glycoproteins (Lara et al., 2010). Metallic nanoparticles prevent reproduction by interacting with genetic elements in the cell, whereas an infection can spread by its own replication. The majority of Ag or Au-NP antiviral activity has been observed against enveloped viruses.

Silver Nanoparticles Are Biosynthesis

AgNPs have been linked to a wide range of potential applications, including antifungal, antimicrobial (Sharma et al. 2009b), wound healing (Silver et al. 2006; Tian et al. 2007), anticancer (Boca et al 2011) therapies, as well as catalytic activity (Mohanpuria et al., 2007). Their usefulness in the areas of medication administration, enzyme-released systems,

biosensor design, and ultrasound medical imaging has increased as a result of these features (Ngeontae et al. 2009; Vaidyanathan et al. 2009). These NPs have applications in sensing and imaging because of their sensitivity to metal surface absorption (Lee and El-Sayed 2006).

Utilising cyanobacteria for the biosynthesis of AgNPs

The filamentous cyanobacterium *Plectonema boryanum* UTEX 485 has been reported to produce spherical and octahedral AgNPs both intracellularly and extracellularly by incubating the algal extract at temperatures between 25 and 100 °C for up to 28 days (Lengke et al. 2007). The size of the extracellularly synthesised AgNPs ranged from 1 to 200 nm, while the intracellular AgNPs were less than 10 nm in size. From 25 to 100 °C, organic substances (protein) produced from dying cyanobacteria were thought to be the cause of extracellular bioreduction. *Oscillatoria willei* NTDM01 was found to reduce silver ions to spherical AgNPs with a size range of 100-200 nm on the cell surface, or extracellular.

Using *Arthrospira (Spirulina) platensis*, Tsibakhashvili et al. (2011) produced extracellular AgNPs and hypothesised that the number, size, and form of the NPs depend on the concentrations and length of time of silver ion exposure. *Arthrospira (Spirulina)*, *Aphanothece*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Gloeocapsa*, *Microcoleus*, *Synechococcus*, and *Aphanocapsa* extracts that were isolated from mangroves have also been investigated for the production of AgNPs. Several human pathogens, including *Escherichia coli*, *Bacillus subtilis*, *Corynebacterium*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, and *Vibrio cholerae*, were significantly inhibited by these biosynthesized AgNPs.

Biological Production of Gold Nanoparticles

AuNPs have the potential to be extremely useful in a variety of practical applications, including medicine, photonics, electronics, optics, biomedicine, and catalysis. The targeting of the nucleus by the AuNPs aids in intracellular therapeutic administration and diagnostics (Tkachenko et al. 2003). They have been applied in medicine for cancer therapy and computed tomography targeting (Kim et al. 2010). Using optically excited nanorods, heat can be generated that may inhibit the growth of malignancies (Hauck et al. 2008).

Utilising cyanobacteria for the biosynthesis of AuNPs

Lengke et al. (2006a) observed that the addition of gold(I) thiosulphate ($\text{Au}(\text{S}_2\text{O}_3)_2^-$) and gold (III) chloride (AuCl_4^-) solutions to a *P. boryanum* UTEX 475 extract resulted in the extracellular as well as intracellular synthesis of AuNPs ranging in size from 10 to 25 nm. Additionally, intracellular Au nanoparticle production smaller than 10 nm was seen. According to Govindaraju et al. (2008), the cyanobacterium was used in the biosynthesis of AuNPs, AgNPs, and a bimetallic nanoalloy (Au core-Ag shell NPs). The utilisation of *A. platensis* for the synthesis of AuNPs and AgNPs was also reported by Kalabegishvili et al. (2011), while (Uma Suganya et al., 2015) reported stable and uniform AuNPs of about 5 nm size that became ruby red after 48 hours employing *A. platensis*. These particles demonstrated antibacterial activity against *B. subtilis* and *S. aureus*, two gram-positive bacteria.

Various Algae are Used for the Biosynthesis of Further Nanoparticles

Various types of algae including cyanobacteria (MubarakAli et al. 2012; Singh et al. 2014), microalgae (Eroglu et al. 2013; Jena et al. 2014; Subramaniyam et al. 2015) and macroalgae have been found to reduce a variety of metals to form nanometals (Mahdavi et al. 2013; Abboud et al. 2014; Azizi et al. 2014). The microalga *Scenedesmus-24* was shown by Jena et al. (2014) to mediate Cd absorption and produce intracellular cadmium sulphide NPs that ranged in size from 150 to 175 nm. The aqueous extract of the brown alga, *S. muticum*, was used in the biosynthesis of cubic-shaped magnetic iron oxide NPs, Fe₃O₄ NPs, of 184 nm size by reduction of Fe³⁺ in FeCl₃ (0.1 M) solution for 90 min with steady stirring at room temperature (Mahdavi et al. 2013).

Recently, spherical monodispersed iron nanoparticles (FeNPs) of 20 to 50 nm diameters were reported to be produced from 0.1 M FeCl₂ solution in 48 hours of darkness by the green microalga *Chlorococcum* sp. (Subramaniyam et al. 2015). Azizi et al. (2014) reported using the brown alga *S. muticum* to biosynthesize hexagonal ZnONPs with a size range of 30-57 nm.

Conclusion

Algae are widely farmed in nanotechnology and are becoming significant organisms in green nanotechnology. This study tried to summarise the research on producing metallic nanoparticles by algae, furthering our understanding of this field of applied science known as phyco-nanotechnology. Algae have the potential for the large-scale synthesis of metallic nanoparticles with favourable effects on both the economy and the environment because they are extensively cultivated and found in a variety of environments. Chemical methods for manufacturing metallic NPs could be replaced with algae-mediated technologies.

By adjusting the pH and temperature of the reaction mixture, a variety of metallic NPs with various sizes, forms, and composites can be produced in a short period of time while also being more environmentally friendly and high-yielding. To avoid the negative impact of these nanoparticles on the flora and fauna, additional studies on bio-accumulators of nanoparticles and the toxicokinetics of nanoparticles must be assessed. Additionally, choosing affordable, ecologically safe nanotechnology with a varied spectrum of human life will be crucial for the successful and sustainable implementation of nanotechnology.

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CHAPTER

32

THE MAIN CULPRITS FOR BIOFOULING, DISCOLORATION, STAINING, AND BIODETERIORATION OF CULTURAL HERITAGE

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A**bstract**

Cultural heritage preservation is critical to preserving our common history and artistic expressions for future generations. Cultural artifacts and structures, on the other hand, are subject to biofouling, discoloration, staining, and biodeterioration, which are primarily caused by algae, fungi, and lichens. These seemingly insignificant bacteria exert significant impact in changing the visual and structural integrity of our cultural heritage. This chapter investigates the numerous effects of biofouling and biodeterioration on cultural heritage surfaces. To colonize and decompose organic and inorganic materials, algae, fungus, and lichens build biofilms and use various enzymatic and pigmentary mechanisms. Their presence has a wide range of repercussions, from surface discoloration to structural instability, offering considerable obstacles to conservation efforts. This chapter presents a broad perspective on the problems and potential in maintaining our cultural legacy by studying the underlying mechanisms and interactions between these microbes and cultural objects. The knowledge gained from this exploration enables us to devise effective measures for preserving the beauty and historical relevance of our shared cultural heritage.

Keywords: *Biofouling, Biodeterioration, Biofilms, Conservation, Cultural Heritage, Discoloration.*

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Introduction

Cultural heritage, comprising historical buildings, monuments, artworks, and artifacts, is an invaluable testament to human civilization's rich history and artistic expression. However, the preservation of these treasured legacies faces an array of challenges, including the detrimental effects of biofouling, discoloration, staining, and biodeterioration predominantly caused by algae, fungi, and lichens. These microorganisms, often unnoticed to the naked eye, wield significant power in altering the visual appearance and structural integrity of our cultural heritage.

Biofouling involves the colonization and growth of various microorganisms on the surfaces of cultural artifacts and structures. Algae, fungi, and lichens, among other biofouling agents, form biofilms that act as potent catalysts for biodeterioration processes. The metabolic activities of these microorganisms and the pigments they produce contribute to surface discoloration and staining, leading to gradual degradation over time.

Algae, being photosynthetic organisms, harness sunlight to proliferate on diverse substrates. Their pigments, such as chlorophylls, carotenoids, and phycobilins, can create a vibrant array of colors on the surface of cultural heritage. Fungi, on the other hand, employ various enzymes and pigments, including melanins and carotenoids, to break down organic materials and produce dark stains. Meanwhile, lichens integrate both algal and fungal components, resulting in a symbiotic relationship that can penetrate and erode the substrate.

This book chapter aims to comprehensively explore the manifestations and consequences of biofouling, discoloration, staining, and biodeterioration on cultural heritage predominantly caused by algae, fungi, and lichens. By understanding the underlying mechanisms and the interactions between these microorganisms and their hosts, we can develop effective strategies to safeguard our cultural legacy.

Through case studies of biofouling and biodeterioration on different types of cultural artifacts, we will gain valuable insights into the severity of the issue and the importance of early detection. Furthermore, we will explore preventive conservation measures, non-destructive cleaning techniques, and sustainable approaches that can be employed to mitigate the impact of biofouling on cultural heritage.

As we delve into the intricate world of these microorganisms and their intricate relationships with cultural materials, we recognize the significance of fostering a balance between preservation and the challenges of environmental factors. By addressing these issues head-on, we can ensure that future generations inherit a well-preserved and visually captivating cultural heritage that continues to inspire awe and admiration.

Biofouling of Cultural Heritage

Biofouling is the colonization and buildup of living organisms on submerged or exposed surfaces, which causes the substrate to deteriorate. Biofouling is a significant hazard to cultural heritage because it can affect the look, structural stability, and overall aesthetic value of historical buildings, monuments, and artifacts.

Mechanisms of Biofouling

Biofouling is a multi-stage process that involves interactions between live organisms and the substrate. Initial attachment, development and colonization, and the creation of a biofilm are the major phases in biofouling. Microorganisms connect to the surface via weak van der Waals forces and electrostatic interactions during the early attachment phase (Callow et al., 2011). Once attached, these microbes begin to grow and reproduce, eventually colonizing the substrate. As the biomass grows, a biofilm forms, creating an ideal environment for the growth of other species such as algae, fungus, and lichens (Flemming, et al., 2010).

A biofilm on cultural heritage surfaces produces a microclimate capable of retaining moisture, nutrients, and contaminants. This, in turn, accelerates the biodeterioration process by promoting the formation and proliferation of various biofouling agents (Cappitelli et al., 2008).

Algae as Biofouling Agents

Among the most common biofouling agents detected on cultural heritage surfaces are algae. They are a type of photosynthetic creature that ranges from microscopic unicellular species to gigantic multicellular seaweeds. Algal colonization on cultural material can cause discoloration, surface roughness, and the creation of acidic metabolites, all of which contribute to the substrate's destruction (Gaylarde et al., 2001).

Certain algal species, such as green algae (Chlorophyta) and diatoms, have been proven in studies to be particularly competent at colonizing stone and other porous substrates (Sanchez-Moral et al., 2003). They have the ability to infiltrate the substrate's microcracks and pores, accelerating deterioration by mechanical and chemical processes.

Fungi as Biofouling Agents

Fungi are another type of microbe that is frequently found in biofouling of cultural artifacts. They may colonize a variety of substrates such as wood, paper, textiles, and stone. Fungal growth on cultural artifacts can cause discoloration, staining, and surface alterations (Keiblinger et al., 2010).

One of the primary ways that fungi degrade cultural assets is by the synthesis of enzymes like cellulases and ligninases. These enzymes aid in the degradation of organic components present in the substrate, compromising the structural integrity and stability of the substance (Ilieş et al., 2022).

Lichens as Biofouling Agents

Lichens, which are composite organisms created by a symbiotic relationship between fungi and algae or cyanobacteria, are also substantial contributors to cultural heritage biofouling (Nimis et al., 2016). They can colonize a wide range of substrates, including stone, wood, and metal.

Lichens can create extracellular chemicals, including acids, that contribute to substrate deterioration. Furthermore, their delicate thallus structures can penetrate and physically

disturb the surface of cultural material, allowing other bacteria and contaminants to enter (Caneva et al., 2008).

Factors Influencing Biofouling Development on Cultural Heritage

Environmental conditions, substrate features, and the presence of other pollutants all influence the development and severity of biofouling on cultural heritage surfaces. Temperature, relative humidity, light exposure, and nutrient availability can all have a substantial impact on algae, fungus, and lichen growth and colonization (Krumbein et al., 2003).

Furthermore, the physical and chemical qualities of the substrate influence its susceptibility to biofouling. Porous materials, such as limestone and sandstone, provide more sites for microbial attachment, making them more susceptible to colonization than non-porous materials, such as metals (Warscheid et al., 2000).

Discoloration and Staining

On cultural heritage surfaces, discoloration and staining are common indications of biofouling and biodeterioration. Pigments produced by algae, fungi, and lichens add to these undesired visual changes, harming the aesthetic value and integrity of old structures, monuments, and relics.

Causes of Discoloration and Staining on Cultural Heritage

Discoloration and staining on cultural heritage surfaces are caused by a variety of biological and chemical processes beginning by algae, fungi, and lichens' colonization and metabolic activity. When these bacteria colonize a substrate, they create and release pigments that interact with the surface, causing color changes and stains (Ortega et al., 1995).

Furthermore, organic acids, enzymes, and other secondary metabolites produced by these bacteria might react with the substrate and contribute to the browning and staining process (Cappitelli et al., 2008). Furthermore, contaminants and particulate matter in the environment might be deposited by the biofilm, worsening the visual changes to cultural heritage (Warscheid et al., 2000).

Algal Pigments and Their Impact on Surfaces

Algae have pigments that allow them to photosynthesize and produce energy. Chlorophylls, carotenoids, and phycobilins are examples of these pigments (Wright et al., 2006). The pigments found in various algae groups affect the color of the biofilm, which can range from green to brown, red, or yellow.

Green algae, for example, are high in chlorophylls, which gives the biofilm its green color. Diatoms, a form of microalgae, can create pigments such as fucoxanthin, which gives the surface a brownish tint (Holzinger et al., 2006). Phycobiliproteins are produced by red and blue-green algae, resulting in reddish and bluish pigmentation, respectively (Lüning et al., 2003).

Fungal Pigments and Their Role in Staining

Fungi, by the synthesis of diverse pigments, also contribute to the deterioration and staining of cultural heritage surfaces. Fungal pigments include, among other things, melanins, carotenoids, flavins, and quinones (Sutherland et al., 2001). These pigments have the potential to interact with the substrate, resulting in surface discoloration and staining.

For example, black fungi (such as *Aspergillus niger*) create melanins, which darken the substrate. Similarly, certain fungal species produce yellow or orange pigments, resulting in striking surface color changes (Keiblinger et al., 2010). Fungal pigment stains can be extremely difficult to remove without causing damage to cultural heritage.

Lichen Pigments and Their Contribution to Discoloration

Lichens create a variety of pigments that contribute to the distinctive hue of their thalli. Chlorophylls, carotenoids, and anthraquinones are the most prevalent pigments found in lichens (Nimis et al., 2016). These pigments allow lichens to survive in a variety of environments and shield them from harmful radiation.

Lichen pigments can produce considerable surface degradation and stains in the setting of cultural heritage. For example, certain lichen species contain anthraquinones, which are strong colorants that can stain the substrate for a long time (Caneva et al., 2008).

Environmental Conditions Promoting Discoloration and Staining

Several environmental elements influence the development and intensity of discoloration and staining on cultural assets. Light exposure, temperature, humidity, and the presence of air pollutants are all factors that can speed up the creation and deposition of pigments on the surface (Bento et al., 2001).

High humidity encourages microbial growth and pigment formation, resulting in increased discoloration. Sunlight can also increase pigment synthesis in microorganisms, exacerbating visual changes on the cultural heritage surface (Piervittori, 2004).

Biodeterioration of Cultural Heritage

The biodeterioration of cultural heritage caused by algae, fungi, and lichens is a complex process that can lead to significant damage to various types of cultural artifacts and structures.

Understanding the Impact of Biofouling on Cultural Materials

Algae, fungus, and lichens colonize and grow on cultural heritage surfaces, triggering a cascade of biodeterioration processes. These microbes have the power to change the chemical, physical, and mechanical properties of the substrate, causing the culture material to deteriorate (Marques, 2013).

The existence of a biofilm and the consequent synthesis of acidic metabolites by microorganisms both contribute to substrate breakdown. Organic acids produced by microbial

metabolism can react with the surface, causing mineral dissolution, cation leaching, and material weakening (Gaylarde et al., 2001).

Effects of Algae on Different Types of Cultural Heritage

Algae can have an impact on a variety of cultural heritage items, including stone monuments, frescoes, and outdoor sculptures. Algae can penetrate porous stone surfaces and form biofilms that trap water and contaminants, speeding weathering processes (Cappitelli et al., 2008).

Algae can grow on the painted surface of frescoes, causing color changes and damage of the artwork. The paint's pigments and nutrients provide an ideal habitat for algae growth (Jurado et al., 2014).

Outdoor sculptures constructed of stone, metal, or other materials are also vulnerable to algal infestation. Algae can scratch the surface and form voids in the sculptures, causing structural damage (Ettenauer et al., 2012).

Fungal Biodeterioration: Deterioration Mechanisms and Consequences

Fungi are well-known for their propensity to biodegrade a wide range of cultural items, including wood, paper, textiles, and organic relics. The growth of fungi on wooden structures, such as old houses and shipwrecks, can cause degradation and structural instability (Gaylarde et al., 2011).

Fungal biodeterioration methods include the synthesis of enzymes like cellulases and ligninases, which degrade the cellulose and lignin found in wood and other organic materials (Sterflinger, 2010). The breakdown of these components weakens the material and exposes it to mechanical pressures and environmental contaminants.

Furthermore, fungi can produce colors and metabolic byproducts that stain and discolor organic surfaces, lowering the aesthetic value of cultural property (Pinzari et al., 2018).

Lichens' Role in the Biodeterioration of Cultural Artifacts

Lichens have the ability to have both physical and chemical effects on cultural items. Lichen thallus structures can infiltrate and erode the surface of stone monuments and buildings, resulting in physical damage (Nimis et al., 2016). Furthermore, lichen acids, particularly oxalic acid, can react with substrate mineral components, resulting in mineral leaching and erosion (Caneva et al., 2008).

Lichens can colonize the surface of outdoor sculptures and retain moisture, creating a microclimate conducive to biodeterioration. Lichen pigments can also create staining and deterioration on artwork (Liu et al., 2015).

Synergistic Effects of Algae, Fungi, and Lichens on Biodeterioration

Biodeterioration of cultural heritage is frequently caused by the simultaneous action of various biofouling agents, such as algae, fungi, and lichens. Interactions between these bacteria can hasten biodeterioration by forming a more complex and diverse biofilm on the surface.

Furthermore, metabolic by-products of one group of bacteria may promote the development and colonization of others, resulting in a synergistic impact that accelerates the degradation process (Gaylarde et al., 2012).

Case Studies

We shall look at case studies of biofouling, biodeterioration, and staining of cultural property produced mostly by algae, fungi, and lichens in this section. These case studies provide important insights on the effects of these microbes on various types of cultural artifacts and structures, as well as the efforts made to maintain and protect our cultural heritage.

Biofouling and Biodeterioration of Historic Buildings

Case Study: Green Algal Biofouling on Historical Facades

Green algal biofouling was detected on the exterior of various architectural sites in a case study conducted in a historical city with multiple stone buildings. Green algae, such as *Trebouxia* and *Trentepohlia*, caused unattractive greenish stains and discolouration on the stone surfaces (Krumbein et al., 2003).

Lesson Learned: The microclimate around old structures can foster algal growth. Preventive conservation methods, such as periodic cleaning and environmental monitoring, can help control algal colonization and decrease biodeterioration.

Algal Colonization of Monuments: Case Examples and Lessons Learned

Case Study: Algal Bloom on a Sculptural Monument

A bronze sculptural monument along the waterfront in a coastal city was plagued by recurring algae outbreaks. Green algae blooms not only stained the metal surface, but also provided an environment favourable to the attachment and growth of other biofouling agents, such as barnacles and molluscs (Ettenauer et al., 2014).



Fig.1, Wall showing Microbial biofouling, discoloration and staining (Senate Hall of University of Allahabad)

Lessons Learned: To minimize algae colonization and eventual biodeterioration, sculptures exposed to maritime settings must be maintained and cleaned on a regular basis. To protect the sculpture's integrity, environmentally friendly cleaning techniques should be considered.

Fungal Deterioration of Artworks: Notable Instances and Preservation Efforts

Case Study: Fungal Infestation in an Art Gallery

A fungus infection was discovered on certain artworks in an art gallery that had paintings, sketches, and fabrics. Fungi involved for biodeterioration included *Aspergillus* and *Penicillium* species, which created dark colours and destroyed the organic components of the artworks (Ortega-Morales et al., 2005).



Fig.2: Walls of Allahabad Fort showing microbial induced deterioration

Lessons Learned: To prevent fungal infections in art galleries, proper storage and environmental management are required. Regular monitoring and early action in the event of a fungal outbreak can aid in the preservation of precious cultural items.

Lichen-Induced Biodeterioration on Cultural Artifacts: Case Studies

Case Study: Lichen Damage on a Historical Monument

A medieval stone monument in a hilly location with many cultural monuments showed evidence of severe lichen-induced biodeterioration. Lichen infestation has resulted in physical surface damage, with thallus structures penetrating the stone's microcracks and triggering disintegration (Bento et al., 2001).



Fig.3: Walls of Akshay Vat of Allahabad Fort showing microbial induced deterioration

Lessons Learned: Once lichen-induced biodeterioration has progressed, it might be difficult to control. Regular monitoring and early intervention are required to prevent irreversible cultural heritage destruction.

Prevention and Control Strategies

Various preventative and management measures can be utilized to protect cultural heritage from the detrimental impacts of biofouling, discoloration, staining, and biodeterioration, which are mostly produced by algae, fungi, and lichens. These solutions try to reduce the effects of microbes while preserving the integrity of historical structures, monuments, and artifacts.

Monitoring and Early Detection of Biofouling

It is critical to conduct regular monitoring to detect early indicators of biofouling on cultural heritage surfaces. Implementing surveillance programs enables for the early detection of algae, fungal, and lichen colonization, allowing for timely action before severe damage occurs (Caneva et al., 2008).

Non-invasive monitoring techniques such as high-resolution photography and infrared thermography can be used to determine the amount of colonization without harming the cultural artifact (Manfredi et al., 2014).

Preventive Conservation Measures for Cultural Heritage

To produce an adverse environment for biofouling agents, preventive conservation measures must be implemented. Controlling environmental elements like temperature, humidity, and light exposure can help reduce the circumstances that algae, fungi, and lichens thrive in (Saiz-Jimenez, 1995).

To build barriers against biofouling, protective coatings and surface treatments can be applied to cultural heritage surfaces. To avoid long-term harm to the substrate, it is critical to choose compatible and reversible materials (Mohamed Rizk, 2021).

Non-destructive Cleaning Techniques for Discoloration Removal

When deterioration and staining occur on cultural heritage surfaces, it is critical to use effective yet non-destructive cleaning techniques. Brushing and microabrasion are mechanical cleaning methods that can remove surface layers of biofilm and stains without affecting the substrate (Gómez-Hera et al., 2005).

To ensure compatibility with the cultural artifact, chemical cleaning agents should be carefully selected and evaluated. To minimize environmental impact, biodegradable and low-toxicity cleaning solutions are used (Murthy et al., 2010).

Biocides and Their Application in Controlling Biofouling

In extreme cases of biofouling, the application of biocides may be required to inhibit the growth of algae, fungi, and lichen. Biocides can be used to prevent microbial colonization and biofilm formation on cultural heritage surfaces.

However, biocides should be used with caution because they can harm cultural artifacts and have negative environmental repercussions. To avoid unforeseen consequences, thorough risk evaluations and adherence to specified application techniques are required. (Snethlage et al., 2010).

Sustainable Approaches to Mitigate Biofouling without Harming Cultural Heritage

There has been an increase in interest in sustainable techniques to controlling biofouling on cultural monuments in recent years. Natural biocides generated from plant extracts and microorganisms are being investigated as environmentally benign alternatives to chemical biocides (Ranalli et al., 2020).

To prevent microbial attachment, bio-inspired coatings that replicate the self-cleaning capabilities of lotus leaves or other natural surfaces are being developed (Bosch-Roig et al., 2015).

Furthermore, studies into microbial communities that compete with biofouling agents or have bioprotective qualities are ongoing, with the goal of encouraging beneficial bacteria that can aid in the preservation of cultural heritage (Gadd, 2017).

Conclusion

Biofouling, discoloration, staining, and biodeterioration of cultural heritage, which is mostly caused by algae, fungi, and lichens, represent substantial difficulties to the preservation and safeguarding of our cultural heritage. We have looked at the various processes by which these bacteria invade and destroy cultural artifacts and structures, causing irreversible destruction and loss of historical significance. Algae, fungus, and lichens are opportunistic colonizers that use the biofilm milieu to establish themselves on cultural heritage surfaces. They produce

pigments, organic acids, and enzymes once formed, causing coloring, staining, and the disintegration of the substrate's structural components. These visual changes not only have an impact on the aesthetic appeal of cultural items, but they also jeopardize their long-term stability and structural integrity.

This chapter's case studies have emphasized the real-world effects of biofouling and biodeterioration on diverse types of cultural heritage. These examples, ranging from green algal biofouling on historical facades to fungal infestations in art galleries, highlight the importance of implementing preventive steps to protect our cultural legacy from microbial dangers. Monitoring and early detection of biofouling are critical for addressing these difficulties. Regular surveillance programs, along with non-invasive monitoring tools, enable the early detection of microbial colonization, allowing for fast action to prevent further deterioration.

Preventive conservation techniques are critical in making the environment unfavorable for biofouling agents. Controlling environmental elements like temperature, humidity, and light exposure can dramatically minimize the circumstances that algae, fungi, and lichens prefer. Non-destructive cleaning techniques should be used to remove biofilms and stains without affecting the substrate when discolouration and staining occur. Chemical cleaning solutions should be carefully selected to ensure compatibility with the cultural artifact, and environmentally friendly options should be sought.

While biocides may be required in extreme instances, they must be handled with prudence and in accordance with prescribed guidelines to avoid accidental cultural heritage damage. The pursuit of sustainable techniques to biofouling mitigation offers promising answers for cultural heritage protection. Eco-friendly biocides generated from natural sources, bio-inspired coatings, and the encouragement of beneficial microbes all present exciting opportunities to save our cultural heritage while maintaining environmental sustainability.

In conclusion, understanding biofouling, discoloration, staining, and biodeterioration on cultural heritage produced mostly by algae, fungi, and lichens is critical for the conservation and preservation of our historical treasures. We can ensure that future generations can appreciate and learn from the invaluable cultural heritage that defines our common human past via vigilance, preventive conservation, and sustainable practices.

As we progress our knowledge and methods in the preservation of cultural heritage, the collaboration of scholars, conservators, and caretakers becomes increasingly important in preserving these treasures for future generations.

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CHAPTER

33

ROLE OF MICROORGANISMS IN ECOSYSTEM RESTORATION

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A bstract

Microorganisms possess great metabolic diversity, and because of this distinguishing quality, they have a significant amount of biological relevance that has attracted research for more than a century. Modern research revealed many more novel characteristics of microorganisms that are at the threshold of massive implementation to address many of the issues we are facing today, such as food, energy, medicine, and environmental degradation. For ages, research by microbiologists, plant pathologists, ecologists, biochemists, and biotechnologists on microbial exploitation and overexploitation has been conducted with changing priorities across the globe. This book chapter will provide comprehensive knowledge about the microbes and their restoration processes involved in the repair and maintenance of the environment.

Keywords: *Restoration, Rehabilitation, Rejuvenation, Resilience.*

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Introduction

"Mother Earth" is the source of all life, both animate and inanimate. Soil, air, and water constitute some of the main habitats for microbes. Both the positive and negative effects of soil microflora on plant development and health have been documented. The microorganisms that cause damage to developing plants may not just be parasitic pathogens but could also be saprophytic microbes that have a negative impact on the growth of plants. Water bodies serve as carriers in the transportation of microorganisms in plants as well as in animals (Cho and Pachepksy, 2016). The faecal matter discharged into the water bodies contains faecal indicator organisms (FOIs), e.g., *E. coli* and *Enterococcus* sp., which indicate that the water channel got in touch with the land's faecal source, and these FOIs may not necessarily indicate that there are any infectious organisms in the water (Pachepsky, 2014).

The air microflora consists of spores of bacteria, viruses, fungi, protozoan cysts, spores of molds, algae, etc. *Micrococcus*, *Staphylococcus*, and *Aerococcus* are the three most prevalent bacteria in the air (Mancinelli and Shulls, 1978), and the most common fungi found in the air microflora are *Aspergillus*, *Alternaria*, *Penicillium*, *Rhizopus*, etc. Soil microorganisms include rhizobacteria, vesicular mycorrhizae (VAM fungi), and saprophytic rhizosphere fungi, which get involved in the plant machinery and act as plant growth-promoting factors. The interaction between soil microorganisms and higher plants may be dependent on each other (Duclaux, 1885). Plant-growth-promoting substances are being produced due to an increase in the microbial population. With the help of these microbes, there is a production of simple to complex organic matter, which gets mineralized and tends to increase crop production.

Rhizobacteria that have apparently co-evolved with the plants colonize the root system very aggressively. For example, *Rhizobium* sp., Actinomycetes, and *Frankia* opt for a symbiotic relationship, and free-living saprophytes increase the nutrient content in the soil. Later on, these minerals are uptaken by the root machinery of the plant with the help of vessels and tracheids, and these nutrients are supplemented to a different part of the plant, which helps the plant suppress the growth of harmful pathogens. Due to the excessive exploitation of microbes, the balance of the different cycles that are channelled in the environment got imbalanced to a great extent.

Result

The outcome of the study reveals that a major problem of the deteriorating environment is the rise in population to a great extent. Due to this, there is excessive demand for resources, which leads to the exploitation of microorganisms. There is a need for the conservation of microbial flora. For example, cyanobacteria or blue-green algae (BGA) possess metabolic systems that are similar to bacteria and an autotrophic method of nourishment like plants are found in free-living and symbiotic associations and act as nitrogen fixers in the environment, generating their own photosynthates from CO₂ and H₂O (Pabbi and Singh, 1998). Another contrasting characteristic is that they are used as biofertilizers. In tropical rice fields, they are widely used as biofertilizers and fix biological nitrogen present in the environment (Singh,

1961; Thajuddin & Subramanian, 2005). Some of the BGAs that are used in the rice field are *Anabaena*, *Tolypothrix*, and *Westiellopsis*, which are grown in open-air systems and in mixed culture (Venkataraman, 1972). Mycorrhizal fungi, *Glomus* sp., are used as a model case study as they have been used in ELISA tests (Varma, 1995a, Springer 2012), immunofluorescence, etc.

Discussion

The first thing that comes to mind is, what are the factors that cause the degradation of the environment? Several factors are responsible for the deterioration of our environment, which needs to be restored or rehabilitated. In India, a major factor is the "population blast" or "population explosion". The 16th Indian Census was going to be held in 2021, but due to the COVID-19 pandemic, it got postponed. According to the 2011 Census, it is known that every sixth person in the world is an Indian. Environmental deterioration is caused by the dynamic interplay of socioeconomic variables (Barman, Prabir, Ghosh, and Deb, 2022), institutional activity, and technological activity (Lin, Yang, Gross, and Whendee, Silver, 2021). Environmental changes might be caused by a variety of factors, including population expansion, urbanization, intensification of agriculture, increased energy consumption, and transportation. Poverty continues to be a major contributing factor to environmental problems. Economic factors include market failure, a lack of infrastructure, poor transportation services, and poor farming practices. Somewhere, humans are mainly responsible for the destruction of natural habitats due to their anthropogenic activities. If a chain of cycles regulated by microbes gets disturbed, the whole scenario at the end gets a harsh impact, which is seen as a result. Due to activities like agriculture, prospecting for oil and gas, economic growth, or water diversion, it could cease to provide the nourishment, water, shelter, and locations to rear young that species require in order to survive. All of these problems, whether they are anthropogenic or occur due to natural calamities, can be resolved with the help of these beneficial microbes. All ecosystems can regenerate and become resilient as a result of decreased environmental stress.

1. For instance, vegetated buffers are strips of vegetation that protect the water quality of riparian habitats from urban or industrial runoff.
2. Habitat rehabilitation is the process of making a location more suitable as a habitat for a certain species.
3. The process of remediation entails improving an already-existing ecosystem or creating a brand-new one to take the place of one that has been destroyed or damaged.
4. When a species or ecosystem is lost that is protected by law, mitigation is the necessary remedial activity.

Ecological cycles, a natural process where components are continually cycled in different forms across different biomes of the ecosystem, are the primary function of microorganisms in the repair of the environment. For example, the biogeochemical cycle, including the gaseous cycle (carbon, oxygen, nitrogen, and water cycle), sedimentary cycle (phosphorus,

sulfur, iron, calcium, and rock cycle), detritus cycle, and food chain, are the best examples of the recycling of nutrients with the help of microorganisms.

Table: The following table includes different types of cycles with their respective microbes.

S.No.	Cycles	Microbes associated with their respective cycle
1.	Gaseous cycle	
A.	Carbon cycle	Methanogenic bacteria (Cadena, Santiago, Cervantes, Luisa, Falcón, and García-Maldonado, 2019), <i>Thiobacillus ferrooxidans</i> , <i>Bacteroides succinogenes</i> , <i>Clostridium butyricum</i> , and <i>Syntrophomonas sp.</i> (Singhal, Pandey, Kumari, Chauhan, and Jha, 2021)
B.	Oxygen cycle	Eukaryotic algae and prokaryotic cyanobacteria (BGA) (Todar, 2012)
C.	Nitrogen cycle	Denitrifying fungi, nitrifying archaea, anammox bacteria, aerobic denitrifying bacteria, and heterotrophic nitrifying microorganisms (Hayatsu and Masanori Saito, 2008)
D.	Water cycle	Ice nucleating bacteria- <i>Pseudomonas syringae</i> (Louisiana State University, 2022).
2.	Sedimentary cycle	
A.	Phosphorus cycle	Fungi- <i>Asperigillus</i> , <i>Penicillium</i> , <i>Fusarium</i> , and Bacteria- <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Actinomycetes</i> , <i>Micrococcus</i> , and <i>Flavobacterium</i>
B.	Sulfur cycle	Sulfate-reducing bacteria- <i>Proteus</i> , <i>Campylobacter</i> , <i>Pseudomonas</i> , <i>Desulfuromonas</i> , and <i>Salmonella</i>
C.	Iron cycle	Family- Desulfobulbaceae, Desulfuromonadaceae, and Pelobacteraceae; Genera- <i>Desulfobacter</i> and <i>Geobacter</i> , <i>Clostridium</i> and <i>Bacillus</i> Family- Mariprofundaceae, Gallionellaceae; Genera- <i>Desulfococcus</i> and <i>Desulfobacterium</i> (Reyes and Michael, 2016)
D.	Calcium cycle	Cyanobacteria- <i>Anabaena</i>
E.	Rock cycle	Iron reducing bacteria- <i>Shewanella putrefaciens</i> , <i>Pseudomonas medosina</i> ; Acetogenic bacteria- <i>Eubacterium</i> ; Methanogens- <i>Methanobacterium</i> (Pedersen and Karsten, 1997); Sulfate reducing bacteria- <i>Desulfonema</i> (Teske and Yehuda Cohen, 1998)

Conclusion

Due to various anthropogenic changes by human beings like deforestation, excessive mining, land sliding due to excessive cutting of trees and forests, and destruction of soil microflora for making roads, houses, factories, etc., the discharge of harmful effluents in the water bodies without proper treatment and without the use of catalytic converters results in the

direct discharge of contaminants such as lead, ozone, sulfur dioxide, nitrogen dioxide, and carbon monoxide and some other gases into the environment. So, to tackle these problems, some strategies for the restoration of the environment should be adopted. Recycling is the key to the restoration process because, on earth, if we must survive with limited natural resources, they need to be converted and reused in other forms. Restoration can be done using the following methods:

1. Restoration of natural habitat
2. Rehabilitation of a functional ecosystem
3. Compensation of one ecosystem with another
4. Artificial ecosystem creation
5. Rehabilitation, recreation, reclamation, and recovery of degraded lands through afforestation
6. Human efforts towards reducing the discharge of toxic substances to neutralize acidity in water bodies
7. Recreation of wetlands
8. Translocation and reintroduction of captive-bred animals E.g., under "Project Cheetah," eight cheetahs were transported from Namibia to India (Kuno National Park, M.P.).

And some of the restoration projects in India:

1. "Project Tiger" in Jimm Corbett National Park
2. "Project Elephant" in the Dooars Region of West Bengal
3. "Project Dolphin" at Chilika Lake
4. Restoration projects in Karnataka for livelihood without deforestation
5. The revitalization of 13 significant rivers, including the Jhelum, Chenab, Ravi, Beas, Sutlej, Yamuna, Brahmaputra, Luni, Narmada, Godavari, Mahanadi, Krishna, and Cauvery, was the subject of recent detailed project reports (DPRs) from Bhupender Yadav, India's environment minister.
6. Land reclaimed in Mumbai
7. India has committed to increasing its forest and tree cover in order to create an additional 2.5-3 billion metric tonnes of CO₂ equivalent carbon sinks. As a result, current forest restoration initiatives like the "Green India Mission" and "National Afforestation Programme" exist. By 2030, these mandates restoring 26 million hectares of land and increasing the forest cover by 33%.

After studying all these possible aspects, we are able to understand the importance of microbes for the maintenance and upregulation of the environment and their beneficial role in human lives as well. The table gave very brief but illustrative knowledge about those microorganisms that are actually involved at several stages of the different cycles. And these microbes left a lasting effect on our livelihoods. Their involvement with soil, water, air, and detrital material made the restoration process easier, faster, and non-toxic. The use of microbes in restoration projects can indirectly save an endangered species from going extinct. Due to the active participation of microbes in the environment, the restoration process has also developed a major degree of resilience and resistance.

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