A DISSERTATION ON

Antibacterial screening of bioactive compounds of actinobacteria against multi-drug resistant and pathogenic bacteria

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY

IN PARTIAL FULFILLMENT

FOR THE

DEGREE OF MASTER OF SCIENCE

IN MICROBIOLOGY

BY

JAHNAVI YADAV

M.Sc. MICROBIOLOGY (IV SEMESTER)

Department of Biosciences

Integral University, Lucknow

UNDER THE SUPERVISION OF

Supervisor:

Dr. Uzma Afaq

Assistant Professor

Department of Biosciences

Integral University, Lucknow

Co-supervisor:

Dr. Arshi Siddiqui

Assistant Professor

Department of Biosciences

Integral University, Lucknow

INTEGRAL UNIVERSITY

Established Under U.P. Act no. 09 of 2004 by State Ligation

Approved by University Grants Commission

Phone No. : +91(0552)2890812,2890730,3296117,6451039

Kursi Road, Lucknow- 226026,Uttar Pradesh(India)

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Miss Jahnavi yadav** during the months March – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of the thesis has been written by the candidate herself. The thesis entitled **"Antibacterial screening of bioactive compounds of actinobacteria against-drug resistant and pathogenic bacteria"** therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Microbiology, Department of Biosciences, Integral University, Lucknow (U.P.).

Dr. Uzma Afaq

Assistant Professor

Department of Biosciences

Dr. Arshi siddiqui

Assistant Professor

Department of Biosciences

INTEGRAL UNIVERSITY

Established Under U.P. Act no. 09 of 2004 by State Ligation

Approved by University Grants Commission

Phone No. : +91(0552)2890812,2890730,3296117,6451039

Kursi Road, Lucknow- 226026,Uttar Pradesh(India)

TO WHOM IT MAY CONCERN

This is to certify that **Miss Jahnavi Yadav** of M.Sc. Microbiology (IVSemester), Integral University has completed four months dissertation work entitled **"Antibacterial screening of bioactive compounds of actinobacteria against-drug resistant and pathogenic bacteria"** successfully. She has completed this work from March–June, 2022 under the supervision of **Dr. Uzma Afaq** and co-supervision of **Dr. Arshi Siddiqui**. The dissertation was a compulsory part of her M.Sc. degree.

Dr. Snober S. Mir

Head

Department of Biosciences

Integral University, Lucknow

Acknowledgment

First of all I want to thanks to GOD for his love and blessing to me only that I am able to complete this project. I want to express my gratitude to all those who had accompanied and supported me

I want to express my sincere and humble gratitude to **Dr Snober S. Mir (Head of Bioscience Department, Integral university, Lucknow),** your immense knowledge and plentiful experience has always encouraged us during the dissertation. I am gratefulto you fir enlightenment us the first glance of research.

I would also like to thanks **Dr. Uzma Afaq** (Assistant professor Department of Bioscience, Integral University, Lucknow) for helping us with her invaluable expertise in formulating the dissertation methodology. Your insightful feedback pushed us to sharpen our thinking and brought our work to a high level.

Special thanks to **Syed Khalida Izhar** and all other laboratory staff members for their relentless help and advice. The generously devoted their valuable time for guidance and without their kind effort my work not be possible.

I also thank my group mates, I must write about my family for their unconditional love, support and encouragement. It is equally important to thanks my parents but this acknowledgement will never be complete if their name is not there.

Miss Jahnavi Yadav

List of Contents

Introduction

Actinomycetes are aerobic, spore forming gram-positive bacteria, belonging to the order actinomycetales characterized with substrate and aerial mycelium growth. They are the most abundant organisms that form thread-like filaments in the soil and are responsible for characteristically “earthy” smell of freshly turned healthy soil. They play major roles in the cycling of organic matter; inhibit the growth of several plant pathogens in the rhizosphere and decompose complex mixtures of polymer in dead plant, animal and fungal material results in production of many extracellular enzymes which are conductive to crop production. The major contribution in biological buffering of soils, biological control of soil environments by nitrogen fixation and degradation of high molecular weight compounds like hydrocarbons in the polluted soils are remarkable characteristics of actinomycetes. Besides this, they are known to improve the availability of nutrients, minerals, enhance the production of metabolites and promote plant growth regulators. Furthermore, actinobacteria do not contaminate the environment instead, they help sustainably in improving soil health by formation and stabilization of compost piles, formation of stable humus and combine with other soil microorganisms in breaking down the tough plant residues such as cellulose and animal residues to maintain the biotic equilibrium of soil by cooperating with nutria of soil by cooperating with nutrient cycling (Ranjani , Dhanasekaran et al 2016).

Actinomycetes are prokaryotic organisms that are classified as bacteria, but are unique enough to be discussed as an individual group. Actinomycete numbers are generally one to two orders of magnitude smaller than the total bacterial population. They are an important component of the bacterial community, especially under conditions of high pH, high temperature or water stress. Morphologically, actinomycetes resemble fungi because of their elongated cells that branch into filaments or hyphae. These hyphae can be distinguished from fungal hyphae on the basis of size with actinomycete hyphae much smaller than fungal hyphae. Characteristics and unique functions of actinomycetes, one distinguishing feature of this group of bacteria is that they are able to utilize a great variety of substrates found in soil, especially some of the less degradable insect and plant polymers such as chitin, cellulose and hemicellulose.

Although originally recognized as soil microorganisms, it is now being recognized that marine actinomycetes are also important. Specifically, marine actinomycetes have been shown to possess novel secondary metabolites that add a new dimenssion to microbial natural products (Jensen et al., 2005). Actinomycetes, one of the most diverse groups of filamentous bacteria, are

wellrecognized for their metabolic versatility. The bioactive potential of these bacteria facilitates their survival even in distress and unfavourable ecological conditions. This special issue is dedicated to the importance of multitude of primary and secondary metabolites produced by actinomycetes. The six articles published in this issue balance the biocatalytic and biocidal potential of actinomycetes.

The importance of large repertory of enzymes from actinomycetes and their potential in replacing chemical catalysts is discussed. Successful commercialization of these enzymes is an important step towards revolutionizing “green technology.” Reduction in the cost of enzyme production is demonstrated by production of endoglucanases from *Streptomyces* sp. on low-cost substrates. Such low-cost production initiatives can be extended to other enzymes and metabolites. Novel properties like thermal and ionic stabilities and a better turnover make these systems infallible and regenerative. The activity of enzymes from actinomycetes is not confined to substrate conversion alone butbroadened to biocontrol of quorum-sensing-dependent phytopathogens, as mediated by acyl-homoserine-lactone-degrading enzymes from endophytic actinomycetes.

Unexplored environments often appeal to researchers in the hope of accruing novel bacteria, a continuous quest which has actually led to discovery of unusually industrious microbes. Antimicrobial potential of actinobacteria isolated from the integumentof *Trachymyrmex* fungus-growing ants is on par with commercial antimicrobials, clearly manifesting a new explorable niche “actinobacterial symbionts of plants and animals.” The term “antimicrobials” often leads our thoughts to “medicine-related” but it’s “environment-related” applications are less contrived. *Streptomyceslunalinharesii* produces antimicrobial substances against sulfate-reducing bacteria commonly responsible for corrosion in the petroleum industry, with an ability to replace the existing biocides. Making the best out of the already good can be achieved for actinomycetes by strain improvement. Advanced microarray-driven reverse engineering strategies for the understanding and modulation of independently functioning regulatory pathways can allow these microfactories to overproduce important antibiotics.

In a nutshell, actinomycetes offer the most promising synthesizers of many industrially and commercially meaningful metabolites. Novel and unexplored habitats may offer bacterial assemblages not reached hitherto.

An integration of newer habitats, screening,and improvement technologies can offer promising

candidates for biotechnology and health-related applications (NeeluNawani et al 2013).

The discovery and development of actinomycete secondary metabolites (ASMs) have played pivotal roles in the fields of human medicine and its related biotechnology sectorsover the past several decades. The postgenomic era has enabled the development of a genome mining approach to isolate and characterize previously unsuspected ASM biosynthetic gene clusters (BGCs) hidden in the actinomycete genomes. Subsequently, BGC awakening techniques and biosynthetic mechanism studies have been pursued to maximize the biotechnological potential of actinomycetes. Moreover, chemical and synthetic biology approaches have allowed for higher production yields of novel and valuable ASMs, which could complement traditional culture-based approaches for chasing ASMs. This Special Issue focuses on recent advances in the study of actinomycetes, especially updated overviews of some significant ASMs and related technologies, including genome mining, BGC refactoring, and cell factory design. This Special Issue includes 11 original research papers and four review articles by renowned experts in the field, providing interested readers with specific examples of recent progressin the study of actinomycetes and ASMs (E.Kim et al 2021). Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. Among them, actinomycetales are an important group.(Berdy J et al 2005). The order actinomycetales is composed of approximately 80 genera, nearly all from terrestrial soils, where they live primarily as saprophytes, water and colonizing plants showing marked chemical and morphological diversity, but from a distinct evolutionary line (Goodfellow, Donnell et al 1989). A large number of actinomycetes have been isolated and screened from soil in the past several decades, accounting for 70%-80% of relevant secondary metabolites available commercially (Baltz RH et al 2008).

Actinomycetes are potential source of many bioactive compounds, which have diverse clinical effects and important applications in human medicine. It has been estimated thatapproximately one-third of the thousands of naturally occurring antibiotics have been obtained from actinomycetes (Takizawa, Colwell et al 1993).

The ability to produce microbial bioactive compounds makes actinobacteria one of the most explored microbes among prokaryotes. The secondary metabolites of actinobacteria are known for their role in various physiological, cellular, and biological processes. Actinomycetes are widely distributed in natural ecosystem habitats such as soil, rhizosphere soil, actinmycorrhizal plants, hypersaline soil, limestone, freshwater, marine, sponges, volcanic cave—hot spot, desert, air, insects gut, earthworm castings, goat feces, and endophytic actinomycetes. The most important

features of microbial bioactive compounds are that they have specific microbial producers: their diverse bioactivities and their unique chemical structures. Actinomycetes represent a source of biologically active secondary metabolites like antibiotics, biopesticide agents, plant growth hormones, antitumor compounds, antiviral agents, pharmacological compounds, pigments, enzymes, enzyme inhibitors, anti-inflammatory compounds, single-cell protein feed, and biosurfactant. The inquiry and discovery of novel microorganisms that produce new secondary metabolites can be required to stay critical in the race against new and rising diseases and antibiotic-resistant pathogens (Maniwasagan Kim et al 2013). Actinomycetes are broadly distributed in natural and man-made conditions and assume a vital role in organic matter degradation. They are additionally notable as a rich source of bioactive secondary metabolites (Berdy J et al 2012). Secondary metabolites are known as organic compounds which are not specifically associated with the normal growth of an organism, improvement, or propagation of it. Thediversity of Actinomycetes and their capacity to produce novel substances put this class in a noticeable position. They are in charge of the generation of about 50% of the exploredbioactive secondary metabolites, remarkably antibiotics, anticancer agents, anti- inflammatory agents, and enzymes (Mohan, Rajamanickam et al., 2018).

In light of the amazing reputation of actinomycetes, a lot of achievement has been centered on the fruitful isolation of new actinomycetes from various sources for medication screening programs in the past 50 years (Dewi , Agustiani et al., 2017). In the previous two decades, there has been a decrease in the revelation of new criticalcompounds from basic soil derived actinomycetes where they have produced huge numbers of previously described secondary metabolites (Mincer,Jensen et al., 2002). Consequently, this prompts increment in the finding of new actinomycete taxa from abnormal environments which thus prompts make new age of drug specialists (Bull Ward et al., 2002). Actinomycetes produce a wide array of biologically active compounds such as antibiotics, enzymes, and enzyme inhibitors (Salwana Sharmab et al., 2020).

As of late, the rate of discovery of new compounds from actinomycetes of terrestrial sources has been diminished, while the rate of re-isolation of known compounds has likewise been expanded. Consequently, it is significant that new actinomycetes taxa fromunderexploited or from unexplored habitats consider as very important sources of new bioactive compounds (Karuppiah, Mustaffa et al 2013).

REVIEW OF LITERATURE

The taxonomic and ecological positions of antibiotic producing actinomycetes are well recognized for their metabolic flexibility, commonly accompanied by the production of primary and secondary metabolites of economic significance. Various approaches including classical, chemo taxonomical, numerical taxonomic and molecular have been routinely employed for the identification of actinomycetes. The potential of actinomycetesin the discovery of novel compounds with activity against microorganisms has been realized, and hence opens exciting avenues in the field of actinomycetes. biotechnologyand biomedical research (Mukesh et al 2014). Actinomycetes are filamentous Grampositive bacteria, characterized by a complex life cycle belonging to the phylum Actinobacteria, which represents one of the largest taxonomic units among the 18 majorlineages currently recognized within the Domain Bacteria (Ventura et al. 2007). Actinobacteria are widely distributed in both terrestrial and aquatic ecosystems, mainly insoil, where they play an essential role in recycling refractory biomaterials by decomposingcomplex mixtures of polymers in dead plants, animals and fungal materials. They are alsoimportant in soil biodegradation and humus formation as they recycle the nutrients associated with recalcitrant polymers, such as chitin, keratin, and lignocelluloses, (Good fellow and Williams 1983, McCarthy and Williams 1992, Stach and Bull 2005) this produces several volatile substances like geosmin responsible of the characteristic “wet earth odor” (Wilkins 1996) and exhibit diverse physiological and metabolic properties, forexample the manufacture of extracellular enzymes (McCarthy and Williams 1992, Schrempf 2001). The bioactive secondary metabolites produced by microorganisms is reported to be around 23,000 of which 10,000 are produced by actinomycetes, thus representing 45% of all bioactive microbial metabolites discovered (Berdy 2005). Amongactinomycetes, approximately 7,600 compounds are produced by Streptomyces species.

Several of these secondary metabolites are potent antibiotics. As a result of which streptomycetes have become the primary antibiotic-producing organisms exploited by thepharmaceutical industry (Berdy 2005). Members of this group are producers of clinically useful antitumor drugs such as anthracyclines aclarubicin, daunomycin and doxorubicin peptides bleomycin and actinomycin D , aureolic acids mithramycin , enediynes neocarzinostatin , antimetabolites pentostatin, carzinophilin, mitomycins, etc (Newman and Cragg , Olano et al., 2009).The isolation of marine actinomycetes has been a great source of new compounds and their isolation all around the world from deepest sedimentsto the shallow costal sediments from the Mariana Trench, demonstrates that actinomycetes are ever-present in marine sediments, but at lower numbers than in soil

(Ghanem , Zheng , Fiedler , Maldonado et al. 2009). Marine actinomycetes have been found in symbiosis with different marine invertebrates, especially sponges. Marine actinomycetes have attracted great attention since they have developed unique metabolicand physiological capabilities that not only ensure survival in extreme habitats, but also offer the prospective to produce compounds with antitumor and other interesting pharmacological activities that would not be observed in terrestrial microorganisms , perhaps because of their close relationships with marine eukaryotic organisms includingmammals (Blunt et al. 2006, Mayer et al. 2007, Williams 2009, Blunt et al. 2009, Fenicalet al. 2002).Marine actinomycetes these studies are only beginning, several attempts to optimize their isolation and growth from several sources as well as the improvement of the fermentation process for the production of specific compounds and the developmentof tools to facilitate the genetic manipulation of the isolated biosynthesis gene clusters (Moore et al.2005). The composition of cell wall in actinomycetes varies greatly among different groups and is of considerable taxonomic significance. Four major cell wall typesare distinguished in these filamentous bacteria on the basis of the three features of peptidoglycan composition and structure. These features are (i) diaminopimelic acid isomer on tetrapeptide side chain position 3, (ii) sugar content of peptidoglycan, and (iii) the presence of glycine in interpeptide bridges. As is evident in, characteristic sugar patterns are present only in cell wall types II-IV of those actinomycetes with meso- diaminopimelic acid. which has each classification and identification, has its origin withinthe early supermolecule crossbreeding studies, however has achieved a new standing following the introduction of supermolecule sequencing techniques (O'Donnell et al. 1993). Importance of phyletic studies supported 16S rDNA sequences is increasing within the science of bacterium and actinomycetes. Sequences of 16S rDNA have provided actinomycetologists with a phyletic tree that enables the investigation of evolution of actinomycetes and conjointly provides the premise for identification. Analysis of the 16S rDNA begins by analytic DNA and amplifying the gene coding for 16S rRNA exploitation the enzyme chain reaction (Hapwood et al. 1985).

For the isolation of actinomycetes, various methods can be performed on the basis of different sources and media. Samples were collected from different ecological habitats. Further characterization can be performed to study the different strains of actinomycetes (Xu et al. 1999). Numerical taxonomy involves examining several strains for a large number of characters prior to assigning the test organism to a cluster based on shared options. The numerically definedtaxa are polythetic; therefore, no single property is either indispensable or adequate to entitle an organism

for membership of a group. Once classification has been achieved, cluster-specific or predictive characters is chosen for identification (Williams et al. 1983).Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae (Kampfer et al 1991). Over 500 species of Streptomyces bacteria have been described by Euzeby (Euzeby 2008). Streptomycetes have genomes with highGC-content and these are gram-positive (Madigan and Martinko 2005). Found predominantly in soil and decaying vegetation, mainly streptomycetes produce spores and are noted for their distinct “earthy” odor which results from production of a volatile metabolite, geosmin. Streptomycetes are characterized by a complex secondary metabolism. They make over two-thirds of the clinically useful antibiotics of natural origin(e.g. neomycin, chloramphenicol) (Kieser et al. 2000). At the start of the antibiotic era the fungal (penicillin, Griseofulvin) and bacterial (Gramicidin) species were in the forefront ofthe interest, but after the detection of streptomycin and afterward cholramphenicol, tetracyclines and macrolides the attention turned to the species of Streptomyces. In the fifties and sixties the majority (70%) of antibiotics was discovered from these species. Themost characteristic and a little bit surprising feature of the recent years just is this declining representation of the formerly exhaustively investigated Actinomycetes (Birnbaum et al. 1985). Actinomycetes have been revealed to be an excellent resource for L- asparaginase. A range of actinomycetes, mainly those isolated from soils such as Streptomyces griseus, S. karnatakensis, S. albidoflavus and Nocardia sp. have abilities to produce L-asparaginase enzyme (DeJong 1972, Narayana et al. 2007, Mostafa and Salama 1979). Microbial L-asparaginase has been generally used as a therapeutic agent in the cure of certain human cancers, mostly in acute lymphoblastic leukemia (Gallagheret al. 1989, Verma et al. 2007). Actinomycetes secrete amylases to the outside of the cells to carry out extracellular digestion.

Aamylase starch degrading amylolytic enzymes is of great significance in biotechnological applications such as food industry, fermentation and textile to paper industries (Pandey et al. 2000). Several actinomycetes and other actinobacteria are renowned as degraders of toxic materials and are used in bioremediation. They are significantly well adapted to survival in harsh environments. Some are able to grow at elevated temperatures (>50°C) and are essential to the composting method (Schmid et al. 1998). Actinomycetes are produced many antibiotics,that are best recognized and most valuable. These antibiotics include amphotericin, nystatin, chloramphenicol, gentamycin, erythromycin, vancomycin, tetracycline, novobiocin, neomycin, etc. (Schoenian et al. 2011). Urauchimycins be a Member of antimycin class, a set of well-identified

antifungals. Antimycins act by inhibiting the electron flow in the mitochondrial respiratory chain (Barrow et al. 1997). Antimycins havebeen identified in Streptomyces isolated from the integument of attine ants (Schoenian etal. 2011, Seipke et al. 2011, Seipke et al. 2012). The few current studies that focused onthe chemical characterization of bioactive compounds formed by Actinobacteria associated with attine ants support the potential isolation of novel molecules with biological activity (Oh et al. 2009, Barke et al. 2010, Carr et al. 2012, Haeder et al. 2009,Schoenian et al. 2011). Microbial diversity is a vast frontier and potential goldmine for thebiotechnology industry because it offers countless new genes and biochemical pathwaysto probe for enzymes, antibiotics and other useful molecules (Singh & Agrawal 2002). Actinobateria are of extraordinary significance in several areas of science and medicine, particularly in antibiotic production (Magarvey et al. 2004).Actinobateria are diverse group of Grampositive bacteria that usually grow by filament formation. They belong to the orderActinomycetales (Superkingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae). They have high G+C (>55%) content in their DNA. They arethe best common source of antibiotics, and provide approximately two-third of naturally occurring antibiotics, including many of medical importance (Okami & Hotta 1988). Need of new antimicrobial agents is greater than ever because of emergence of multidrug resistance in common pathogens, the rapid emergence of new infections and the use of multidrug resistant pathogens in bioterrorism (Spellberg et al. 2004). Resistance of bacteria to the effects of antibiotics has been a major problem in the treatment of diseases. Infectious diseases are still the second leading cause of death worldwide (WHO2002, Luzhetskyy et al. 2007). Though the recent quests for novel antibiotics have employed more recently established approach of target-based discovery using bacterial genomics, combinatorial chemistry, and high-throughput screening, these powerful toolshave not yet yielded any antibiotics approved for clinical use, and the prospects for their success are not encouraging (Baltz 2007).On the other hand ,programs aimed at the discovery of antibiotics from microbial sources have yielded an impressive number of compounds over the past 50 years, many of which have application in human medicine and agriculture (Busti et al. 2006). Hence, the traditional method of screening antibioticsfrom microorganisms is no longer considered glitzy science (Baltz 2007). Choice of naturalmaterials like soils in researches is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore hopefully, novel metabolites as a result of the geographical variation (Sen et al. 1993). Besides, theimportant approaches helpful in discovering new microbial species or unknown bioactivesubstances include isolation and characterization of microorganisms from

the most extreme habitations (Lee & Hwang 2002) and relatively unknown or unstudied areas (Moncheva et al. 2002). The role of Actinobacteria as producers of various clinically important antibiotics is well-established (Okachi, et al., 1977; Oskay, et al., 2004; Cook, et al., 2005). Numerous Actinobacteria have been isolated from various environments, from common environments such as farmland soils (Oskay, et al., 2004), compost litters(Grigova and Norris, 1990), to extreme environments such as marine sediments (Remyaand Vijaya, 2008) and soils from antartica (Moncheva, et al., 2000-2002). The vigorous exhaustion of Actinobacteria from various environments benefited the research community as this enabled both common and uncommon antibioticproducing Actinobacteria to be isolated, studied and manipulated for antibiotic production.Althoughthe antibiotic-producing Actinobacteria is still predominantly from the genus Streptomyces(Grigova and Norris, 1990), the antimicrobial properties of isolates from other lesser known genera such as Dactylosporangium, Micromonospora, Saccharomonospora, Saccharothrix, Promicromonospora, Kitasatosporia and Microbispora have recently been increasingly revealed (Okachi, et al., 1977; David and Kergomard, 1982;; Han, et al., 1999; Moncheva, et al., 2000-2002; Castiglione, et al., 2008; Gao and Huang, 2009). Bacteria have so far been the most promising resource for antibiotics in the past decadesand will undoubtedly remain an important resource of innovative bioactive natural products in the future. Approximately 45% of bioactive compounds obtained from microbes were produced by Actinobateria (Berdy, 2005). Actinobateria remain the most economically and biotechnologically useful microbes, producing 80% of the world's antibiotics, mostly from the genera Streptomyces and Micromonospora (Pandey et al., 2004).Many vitamins, antibiotics, enzymes and siderophores obtained by Actinobateria have pharmaceutical, veterinary, agricultural and clinical applications (Koehn and Carter,2005, Kekuda et al., 2010, Naine et al., 2011), in addition to antitumor and wound healingproperties (Janardhan et al., 2012, Jiao et al., 2013). ome actinobacteria are also known to form more intimate associations with plants and colonize their internal tissues. Within the order Actinomycetales there are examples of both endophytic and plant-pathogenic species. The best-characterized examples of the plant-pathogenic actinobacteria are thepotato scab-causing Streptomyces scabies, S. acidiscabies, and S. turgidiscabies. Pathogenicity has been associated with the presence of a conserved andtransmissible pathogenicity island (PAI) in their genomes. This PAI encodes for the biosynthesis of a phytotoxin, thaxtomin, and also contains plant virulence factor genes such as nec1. Actinorhizae (Frankia spp.) have been extensively researched as anendophytic association between a plant and an actinobacterium . However, examples ofactinobacteria other than Frankia

inhabiting the root tissues of healthy plants as endophytes are rare.Sardi et al. reported the presence of actinobacteria in root samples of crops and Italian native plants, with the majority of the isolates belonging to thegenus Streptomyces. de Araujo et al. found that actinobacteria could be isolated fromthe roots and leaves of maize (Zea mays L.), with the most commonly isolated genus being Microbispora, although Streptomyces and Streptosporangium spp. were also represented. Okazaki et al. (33) were also able to isolate Microbispora spp. at a much higher frequency from plant leaves than from the soil. Soil is a rigorously exploited ecological niche in order to discover useful biologically active natural products such as clinically important antibiotics (Thakur et al. 2007). The phylum Actinobacteria has been

identified as one of the major microbial populations in soil. They are widespread in soils and able to produce various useful secondary metabolites and compound with different properties (Wellington et al. 1994; Poornima and Ponmurugan 2006). Approximately 50%of actinobacteria are from the genus Streptomyces, and approximately 75% of commercially useful antibiotics are derived from this genus (Be´rdy 2005). Lately the increased of resistant pathogenic bacteria and fungus made the search for new antimicrobial drug continues to be of greatest importance in screening programs world wide. Until today, many bacteria-derived compoundshave been successfully develop intodrugs to treat various diseases (Aislabie et al. 2008). However, in recent years, the chances of discovering novel biologically active

molecules from various soil bacteria (including actinobacteria) has reduced, implying that a saturation effect might be occurring. The isolation of well known actinobacteria such as Streptomyces from differentenvironments was found to be producing similar compounds. This could be due to frequent genetic exchange between species (Bredholt et al. 2008) and has resulted in critical demand for new natural products and chemical compounds in pharmacology, which in turn has made the exploration of new habitats in unusual environments essentialto discover novel actinobacteria and metabolites (Barakate et al. 2002; Saadoun and Gharaibeh 2003; Lam 2007; Newman and Cragg 2007; Thakur et al. 2007). Poorly- explored areas of the world such as the Antarctic, Australia, China and Jordan suggest that a careful exploration of new habitats continue to be useful to find novel microorganisms and useful products (Okazaki and Naito 1986; Nolan and Cross 1988; Moncheva et al. 2002; Saadoun and Gharaibeh 2003). The Antarctic, one of the most poorly explored areas emerges as a substantially prospective region for the discovery of novel bacteria and bioactive metabolites (Moncheva et al. 2002; Van Trappen et al. 2002;Marinelli et al. 2004; Tindall 2004; Bull et al. 2005; Taton et al. 2006a; Taton et al. 2006b;Bull and Stach

2007). Barrientos Island is located at 62240 S, 59470 W, at the north entrance to English Strait between Greenwich Island and Robert Island. This island is occupied by various breeders like Gentoo penguins (Pygoscelis papua), Chinstrappenguins (Pygoscelis antarctica), southern giant petrels (Macronectes giganteus), kelp gulls (Larus dominicanus), and skuas (Catharacta spp.). The whole centre of the island is covered by extremely extensive moss carpet. Lichens Xanthoria spp., Caloplaca spp and other crustose lichen species are present. Furthermore the green alga Prasiola crispa is widespread. Robust screening models are important to increase the chances of discovering novel microbial metabolite. Therefore a total of four high-throughput screening models were used to detect the anti-microbial properties towards four different microorganisms (Hong et al. 2009). The 16S rRNA analysis was used for molecular identification of isolates, and the subsequent differentiation of actinobacteria isolates according to genera was accomplished by the ERIC-PCR and RAPD, as well as the composite analysis of both these fingerprinting methods. The aims of this study were-(1) To isolate actinobacteria from the soil of Barrientos Island using culturable method;(2) To identify the isolates using 16S rRNA analysis and to determine the bioactivity of secondary metabolites using high-throughput screening models; (3) To molecular profile actinobacteria from different genera by using the ERIC-PCR, RAPD and composite analysis of both methods. Actinobacteria are the most widely distributed group of microorganisms in nature. They are attractive, bodacious filamentous Gram positive bacteria having high GC content in their DNA. Actinobacteria are considered to be an intermediate group between bacteria and fungi. Majority of actinobacteria are free living, saprophyte found in soil, water and colonizing in plants. Actinobacteria are noteworthy as antibiotic producers, making three quarters of all known products; especially streptomycetes produced many antibiotics and other class of biologically active secondary metabolites, they cover around 80% of total antibiotic product, with other genera. It is anticipated that the isolation, characterization and the study on actinobacteria can be useful in the discovery of antibiotics from novel species of actinobacteria . Streptomycetes is the largest antibiotic producing genus in the microbial world. The number of antimicrobial compounds reported from streptomycetes has increased almost exponentially in the last two decades. About 4,000 antibiotic substances have been discovered from bacteria and fungi, many of them are produced by streptomycetes. Most of the streptomycetes produce a diverse array of antibiotics including aminoglycosides, anthracyclins, glycopeptides, polyether and tetracycline .Screening of microorganisms for the production of novel antibiotics has been intensively pursued for many years. Antibiotics have been used in many fields in- Correspondence to: Ramasamy Vijayakumar

Dept. of Microbiology, Bharathidasan University Constituent College Kurumbalur Perambalur Dt., India veterinary and pharmaceutical industry.

Actinobacteria have the capability to synthesize many different biologically active secondary metabolitessuch as antibiotics, herbicides, pesticides, anti-parasitic compounds and enzymes like cellulose and xylanase used in waste treatment. The abundance of terrestrial actinobacteria and their antibiotic productivity are known. The terrestrial actinobacteria would be an important source for the discovery of new antibiotics. Unfortunately, the rateof discovery of new compounds from existing genera obtained from terrestrial sources has decreased, while the rate of re-isolation of known compounds has decreased. Moreover, the rise in the number of drug-resistant pathogens and the limited success of strategies in proceedings new agents indicate an uncertain forecast for future antimicrobial therapy. Thus, it has been emphasized that new group of microbe from unexplored habits be pursed as sources of novel antibiotics and other small therapeutic agents . The perusal of the literature proved that there are not many reports of actinobacteria from textile effluent polluted soils.Keeping these points in view, the presentstudy has been undertaken to isolate and screen the antibiotic producing actinobacteria from dye polluted soils of Tirupur, Tamil Nadu. Further, the identified antagonistic actinobacteria were characterized based on morphological, biochemical, cultural and physiological characteristics.This study investigates the decolorization potential of actinobacteria from soil towards toxic triphenylmethane (TPM) dyes, i.e., malachite green (MG), methyl violet (MV), crystal violet (CV), and cotton blue (CB). The actinobacterial isolates were first isolated from fresh soil samples, plated onto actinobateria isolation agar(AIA), and both live and dead cells were prepared to evaluate their decolorization efficiency (DE). Isolates with positive decolorization activities were identified via partial sequencing of the 16S rRNA region. They were revealed as species of *Nocardiopsis* (*N. alba*), Streptomyces (*S. puniceus, S. bacillaris, S. albolongus, S. acidiscabies, S. albulus, S. pratensis, S. luridiscabiei, S. rubiginosus, S. albidochromogenes*), *Rhodococcus* (*R. sovatensis*), and *Kitasatospora* (*K. albolonga*).Currently, antibiotic resistance is occurring more and more severely and already has become a global challenge to public health however, new types of antibacterial drugs areso extremely limited that clinicians are forced to the situation as “Bad bugs, No drugs.” In early 2017, a request was made to the World Health Organization (WHO) by member states to develop a global priority pathogen list (PPL) of antibiotic-resistant bacteria to help in prioritising the research and development of new and effective antibiotic treatments. Actinobacteria, especially, the genus Streptomyces, are major producers of bioactive secondary

metabolites. After decades of screening, it has become increasingly difficult to discover new antibiotics from actinobacteria isolated from common soil environments. Nowadays, more and more researches are focused on special habitats and extreme environments , such as desert , marine , and mangrove , since microbes inspecial environments have to develop unique defense mechanism against the stress fromtheir habitats and can evolve adaptive biosynthetic pathways for synthesizing novel biological compounds .In fact, a large number of new bioactive compounds produced by actinobacterial strains residing in special environments have been discovered in recent years .Mangrove is unique intertidal ecosystem with the condition of high moisture, high salinity, low oxygen, and high organic matter content . Because the mangrove soil conditions are extremely different from common terrestrial conditions, microorganisms especially actinobacteria in mangrove soil have distinctive adaptation characteristics andhave the potential to produce novel bioactive metabolites Investigations in many countriesindicated that the mangrove actinobacteria have rich diversity and various biological activities. At the time of writing, at least 86 new actinobacterial species including 8 novelgenera have been isolated from mangrove. In addition, more than 84 new compounds produced by mangrove actinobacteria including some attractive structures such assalinosporamides, xiamycins, and novel indolocarbazoles have been reported. From north to south, mangroves in China mainly distribute along the southeast coast including Zhejiang province, Fujian province, Guangdong province, and Guangxi Zhuang Autonomous Region. Among them, Guangdong and Guangxi possess most of themangrove area. In order to explore the antibacterial resources and gain insight into the diversity of cultivable actinobacteria, mangrove soil samples from Futian, Guangdong, and Maoweihai, Guangxi, were collected and investigated. Due to the high prevalence of multidrug resistance among “ESKAPE” bacteria, defined by the Infectious Diseases Society of America as *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp., these pathogens in the global PPL of antibiotic- resistant bacteria were selected as the indicator bacteria in this study. In addition, a high-efficiency pDualrep2 reporter system was combined to accelerate the discovery of actinobacterial strains with clearly antibacterial mechanism from mangrove soil. Vessel formations occur through either vasculogenesis or angiogenesis, both in health and illness phenomena (Ribatti and Crivellato 2012). Angiogenesis (also known as neovascularization) is a physiological process through which new blood vessels are generated from pre-existing capillaries. Angiogenesis plays an important role in immunological and pathological processes such as cancer (Chung and Ferrara 2011; Zhao

and Adjei 2015), atherosclerosis (Pant et al. 2013), chronic inflammation (Boyle et al. 2014), proliferative diabetic retinopathy (Capitão and Soares 2016), age- related macular degeneration (Balaratnasingam et al. 2015), psoriasis (Marina et al. 2015) and acquired immune deficiency syndrome complications (Aoki andTosato 2003). Therapeutic inhibition of angiogenesis may be useful for the treatment of the mentioned diseases. The approaches to reach this goal had been inhibition of activation, proliferation and migration of endothelial cells. Moreover, angiogenesis is required for normal physiological processes, including the corpus luteum formation withinthe ovary (Woad and Robinson 2016) and endometrial regeneration during the menstrual cycle (Okada et al. 2016). Induction of angiogenesis can be also beneficial for thetreatment of some diseases, for example, wound healing (Shaw and Martin 2016). To date, various efforts have been executed to find out the natural compounds affecting on (Kim et al. 2015). Actinobacteria are classified among the most widely distributed bacterial taxa in various aquatic and terrestrial ecosystems. They are aerobic or anaerobic, Gram-positive bacteria and their genome size vary from 0·93 Mb in Tropheryma whipplei (Bentley et al. 2003) to12·7 Mb in Streptomyces rapamycinicus (Baranasic et al. 2013). Among total bioactive metabolites produced by micro-organisms, about 45% are produced by actinobacteria (Purves et al. 2016). Actinobacterial metabolites have been extensively used in medicine, industry and agriculture (Barka et al. 2016).They have been considered as treatment agents for various diseases, including inflammation (Kwiatkowska and Maślińska 2012), cancer (Schneider et al. 2008; Manivasagan et al. 2014), Alzheimer's disease(Eftekharzadeh et al. 2010), (Cai and Yan 2013), antiobesity (Birari and Bhutani 2007) and also as memory booster (Zhang et al. 1996).Iran has diverse ecological habitats of deserts with much less-explored biotechnological potentials (Wink and Mohammadipanah 2015). In this study, soil samples from different ecological areas of Iran have been investigated as a supply of actinobacteria. The fermentation broth extractsof the actinobacteria (FBEAs) have been screened against human umbilical vein endothelia the Sahara, one of the most extreme environments on Earth, constitutes an unexplored source of alkalitolerant actinobacteria. In this work, we studied the diversity of alkalitolerant actinobacteria in various soils collected from different regions of the Algerian Sahara. A total of 29 alkalitolerant actinobacterial strains were isolated by usinga complex agar medium. The diversity of these actinobacteria was evaluated using a polyphasic approach, which included morphological, chemotaxonomic, physiological (numerical taxonomy) and 16S rRNA gene analyses.The isolates which were assigned to the genus Nocardiopsis, shared relatively low 16S rRNA gene sequences similarities

compared to closely related species suggesting that they belonged to putatively new species. All of the strains were tested for antibiotic activity against a broad range of microorganisms and screened for genes encoding polyketide synthases and non- ribosomal peptide synthetases and found to have the potential to produce secondary metabolites. Consequently, the study supports the view that extreme environments contain many novel actinobacteria, which represent an unexplored source for the discovery of biologically active compounds.Actinobacteria are a prolific source of antibiotics. Since the rate of discovery of novel antibiotics is decreasing, actinobacteria from unique environments need to be explored. In particular, actinobacterial biocontrol strains from medicinal plants need to be studied as they can be a source of potent antibiotics. We combined culture-dependent and culture-independent methods in analyzing the actinobacterial diversity in the rhizosphere of seven traditional medicinal plant species from Panxi, China, and assessed the antimicrobial activity of the isolates. Each of the plant species hosted a unique set of actinobacterial strains. Out of the 64 morphologically distinct isolates, half were Streptomyces sp., eight were Micromonospora sp., and the rest were members of 18 actinobacterial genera.

In particular, Ainsliaea henryi Diels. hosted a diverse selection of actinobacteria, although the 16S ribosomal RNA (rRNA) sequence identity ranges of the isolates and of the 16S rRNA gene clone library were not congruent. In the clone library, 40% of the sequences were related to uncultured actinobacteria, emphasizing the need to develop isolation methods to assess the full potential of the actinobacteria. All Streptomyces isolates showed antimicrobial activity. While the antimicrobial activities of the rare actinobacteria were limited, the growth of Escherichia coli, Verticillium dahliae, and Fusarium oxysporum were inhibited only by rare actinobacteria, and strains relatedto Saccharopolyspora shandongensis and Streptosporangium roseum showed broad antimicrobial activity.Actinobateria belong to a distinct group of bacteria widely distributedin nature. At the present time (Holt et al. 1994) they comprise eight groups with 48 genera, and the special attention given to them in biotechnological applications is a natural resultof their great metabolic diversity (Piret and Demain 1988). They are the best common source of novel antibiotics (Okami and Hotta 1988) and more recently have been shownto be a promising source of a wide range of enzymes, enzymeinhibitors, immunomodifiersand vitamins (PeczynskaCzoch and Mordarski 1988). In nature, they play an important role in the cycling of organic compounds and have also been associated with soil organicmatter production, owing to their black pigments called melanins, which are related, in some respects, to soil humic acid (Szegi and Gulyas 1971;

Huntjens 1972; Coelho and Linhares 1993; Gomes et at. 1996).Streptomyces is the most common actinomycete genus in soils, corresponding to up to 90% of the isolates. However, new approaches forthe isolation of soil actinobateria have revealed that other genera are also significant, andmany new species have been isolated, most of them also able to produce novel secondary metabolites (O'Donnell 1988). Although the first antibiotic from an actinomycete has been reported more than 50 years ago , and since then more than 4000new bioactive compounds have been obtained, the search for new actinobateria of interest to biotechnology is still important (Schatz et at. 1944). Actinomycete taxonomy isextremely complex, and classification, using only the traditional methods based on a fewmorphological and physiological characteristics has led to very heterogeneous suprageneric groups. More recently, three main approaches have been suggested: chemotaxonomy, numerical taxonomy and molecular systematics Brazilian tropical soils have an enormous biodiversity potential. Some of them have been described as habitatsof high biological activity (Bull et at. 1992). Nevertheless, they have not been extensivelyexplored for search and discovery of novel actinomycetes. In the present paper, an attempt has been made to isolate new actinobateria from Brazilian tropical soils. Emphasis has been given to tdispersion and differential centrifugation (DOC) technique aiming at a maximum dissociation of actinobateria propagules from soil aggregates. Some of the isolates, promisiing for use in biotechnology, were tentatively identified (Hopkins et al. 1991).

OBJECTIVE

1. Isolation of actinobacteria from soil.

2. Morphological screening of actinobacteria.

3. Biochemical characterization of isolated strains.

4. Antibacterial screening of bioactive compounds of Actinobacteria against pathogenic bacteria.

METHODOLOGY

MATERIALS AND METHODS

Chemicals and media

Culture media and chemicals will use in this study such as Starch Casein agar (SCA), Actinobacteria isolation agar (AIA), Mueller Hilton agar, nutrient broth, starch casein broth, glucose phosphate broth, peptone water, simmons citrate agar, triple sugar iron agar, dimethylsulfoxide, nalidixic acid, actidione etc.

Sample collection

Soil samples will collect from 5-25 cm depth in sterile plastic bags and transportaseptically to the Microbiology laboratory. Soil sample will directly transfer into polyethylene bags to minimize moisture losses during transportation. Clinical strains of bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus* etc.

Pre-treatment of soil samples

Pre-treatment of soil with 1% of calcium carbonate to reduce the number of vegetative bacterial cells and allowing maximum actinobacteria spores cell to survive.

Isolation of actinobacteria

The samples will dry in air for one week at room temperature. Isolation and enumeration of Actinobacteria will be done by serial dilution and spread plate technique. One gram of soil will suspend in 9ml of sterile double distill water and then the dilution will carry up to 10-5 dilutions. Aliquots (0.1 ml) of 10-2, 10-3, 10-4 and 10-5 will spread on the actinobacteria isolation agar.

To minimize the bacterial and fungal growth, nalidixic acid 100 mg/land actidione 20 mg/l will add respectively. Then the plates will incubated at 30 ° C for 10 d. The plates will observe intermittently for the actinobacteria growth during incubation. After incubation, actinobacteria colonies which are morphologically distinct will pick from the actinobacteria isolation agar plates and further purify by repeated streak plate method. Once the pure colonies will obtain, each colony will further identify base on its characteristics such as earthy like the smell, colony morphology, the color of hyphae and the presence or absence of aerial and substrate mycelium. Then, selected and identified colonies of actinobacteria will transfer to starch casein agar slant and will incubated at 27 °C for their growth.

Screening of actinobacteria and extraction of antibacterial compound

The isolates of actinobacteria will screen for antibacterial activity against these four clinical isolates using nutrient agar by cross streak method. Each nutrient agar plate will streak with each actinobacteria isolate at the center of the plate and incubated at 37 °C for 6-7 d. Then, 24-hour subcultures of bacteria will streak perpendicular to the actinobacteria isolates and the plates will incubate at 37°C for 1 d. After incubation, the zone of inhibition of the test organisms will indicate that the actinobacteria isolate will has antibacterial activity. The actinobacteria isolates that show potent antibacterial activity will select for extraction of the crude antibacterial compound by submerged culture technique.

Characterization and Identification of actinobacteria

Characterization of potent actinobacteria isolates will carried out by morphological and biochemical and physiological studies.

Morphological Identification

Gram staining and lactophenol blue staining will done to study the morphology of the actinobacteria cells and spore chain morphology will study coverslip culture technique with a light microscope.

Physiological and cultural characterization

Colony morphology of the isolates of actinobacteria will study under a high power magnifying lens by observing color of the colony, nature of the mycelium, spore surface and felling the consistency with a sterile loop

Biochemical characterization

Actinobacteria isolates will characterized by various biochemical tests such as indole test, methyl red test, vogus-proskauer test, citrate utilization test, triple sugar iron test, nitrate reduction test, starch hydrolysis test, catalase test, mannitol and sucrose utilization tests.

Antagonistic activity by agar well diffusion method will use to determine the antimicrobial activity of isolates. The supernatant broth containing compounds of actinobacteria will test for their antibacterial activity against four different clinical isolates namely, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus. The bacterial test isolates will inoculate in test tubes containing nutrient broths separatelyand labeled. Then they are incubated at 37 °C for overnight for obtaining broth cultures. After overnight incubation, bacterial cultures will swab in solidified Muller Hinton agar plates and 6 mm diameter wells will punch in the plate. The plates will incubate at 37 °C for 24 h. After incubation, the diameter of the zone of inhibition around the wells will measure and record. The antibiotic ciprofloxacin is used as a control and dimethylsulfoxide without antibacterial compound is use as a blank. Inhibition zones produce by crude extracts will show significant differences when compare with control ciprofloxacin test against different test organisms.

Production of bio active compound-

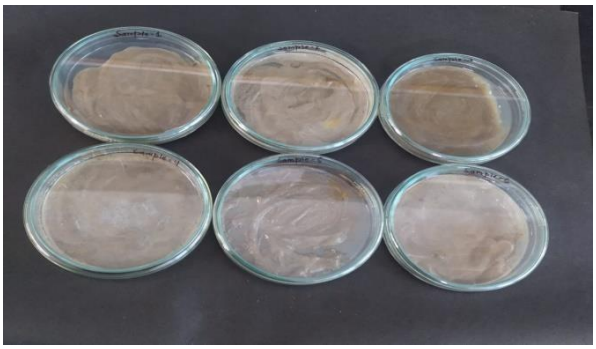
The ethyal acetate crude extract was derived by inoculating actinobacterial strain in starch casein broth and incubated in ashaker at 28 ± 2 C, 200 rpm for 4–8 days. A single-centre streak was made by the potent actinobacteria isolates on nutrient agar medium plates and incubated at 28 ± 2 C for 4 days. The potent actinobacteria strains were inoculated into the 250 mL Erlenmeyer flasks containing Starch casein broth and incubated in an orbital shaker at 200 rpm at 28 ± 2 C for 7 days. After incubation, cultures were subjected to centrifugation at 10,000 rpm for 10 min. The supernatant was aseptically collected, and their antimicrobial efficacy (900 lg/mL) was tested by employing agar well diffusion assay, the crude obtained from all the isolates was carried on against

the test pathogens namely *E. coli, S.aureus, P.aeruginosa, S.abony* and *K.pneumonia* Muller Hinton agar plates were prepared and lawn culture of bacteria was made. The nutrient broth was cultivated on bacterial sample organisms for 24hr. For the preparation of the bacterial lawn, a 100 mg cultivation of each bacterial species was used. Agar wells of 6 mm diameter were prepared with the help of a sterile cork borer. The wells were loaded with crude extract 900 mg/mL, along with 30 mg/mL of as a positive control. The plates were incubated 24 hr at 37 C and the zone of inhibition was calculated (Dhanasekaran et al., 2008).

RESULTS

Sample collection

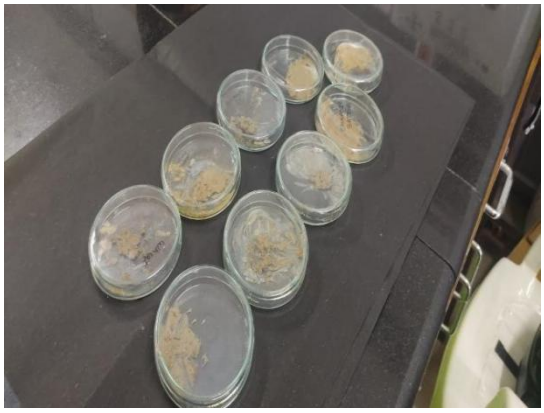
Soil samples will collect from 5-25 cm depth in sterile plastic bags and transportaseptically to the Microbiology laboratory. Soil sample will directly transfer into polyethylene bags to minimize moisture losses during transportation**.**



Air drying of soil sample at room temperature.

Pre-treatment of soil samples

Pre-treatment of soil with 1% of calcium carbonate to reduce the number of vegetative bacterial cells and allowing maximum actinobacteria spores cell to survive.



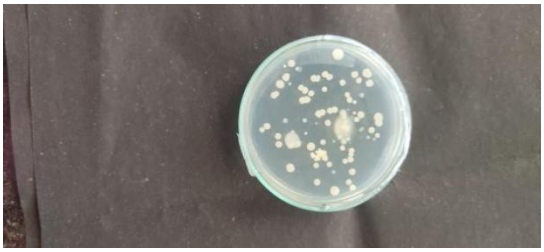
Air dried soil sample were crushed with 1% caco3 and incubated 30 celsius for 48 hoursin closed sterile petriplate.

Humidity was maintained in Petri dish with 1 ml distilled water.

1 gm of soil sample was suspended in 100 ml of sterile distilled water and were kept inrotatory shaker at 150 rpm for 30 minutes.

Serial dilution were carried up to 10^9 concentration in distilled water.

After serial dilution spreading on petriplates and incubate for 4 to 7 days.



Colonies are sub-cultured in new agar plates to obtain pure colonies.

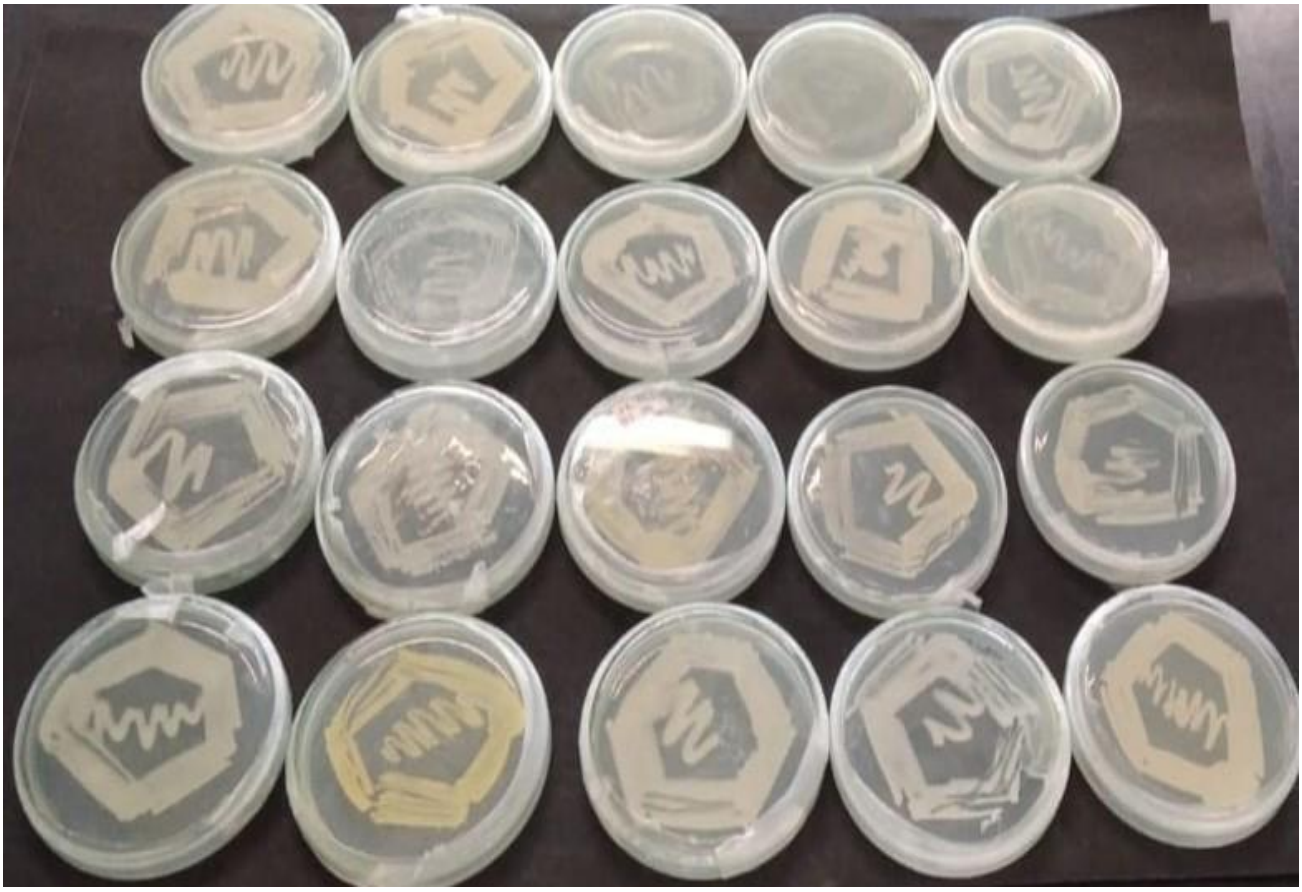
Pure colonies are found.

The actinobacteria isolation was based on the morphological characters like colony.

Positive isolates of actinobacteria from soil samples.

Location of soil	Sourceof soil samples collected	No. of soil sample collected	No. of positive bacteria isolates	Percentage of positive isolates
Integral university	Wheat soil Potato soil Mustard soil Guava soil Banana soil	05	01	20%
Barabanki	Mango soil Rose soil(red) Rose (white) Mint soil Aloevera soil	05	02	40%
Barabanki	Rose soil Wheat soil Lemon soil Mustard soil Mango soil	05	02	40%
Dubba, Sitapur	Lantanas soil Cannabis sativa Poppy soil Drumstick soil	05	01	20%

Total 06 positive Actinobacteria were obtained on the Actinomycetes isolation Agar.



Morphological characteristics of actinobacteria.

No of postive isolates	Coding	Growth	Color	Pigmentation	Elevation	Surface	Margin
1	M(A)	+++	White	White	Raised	Rough	Filamentous
2	L(B)	++	Light yello	light yellow	Flat	Smooth	Slightly curved
3	B(B)	++	Grey	Grey	Flat	Smooth	Slightly curved
4	P(B)	+++	Dark yellow	Dark yellow	Raised	Rough	Filamentous
5	G(A)	++	White	White	Flat	smooth	Slightly curved
6	D(A)	+	White	White	Flat	Smooth	
7	G(A)	+++	White	White	Lightly raised	Wrinkled	Filamentous
8	W(B)	++	White	White	Flat	Smooth	Slightly curved

9	G(B)	+++	White	White	Flat	Smooth	Filamentous
10	D(B)	++	Grey	Grey	Raised	Rough	Slightly Curved
11	B(A)	++	Light yellow	Light yellow	Raised	Rough	Slightly Curved
12	M(B)	++	White	White	Raised	Rough	Slightly Curved
13	PO(A)	+++	Light yellow	Light yellow	Flat	Smooth	Filamentous
14	D(B)	++	White	White	Flat, smooth	Smooth	Slightly Curved
15	D(A)	++	White	White	Raised, Rough	Rough	Slightly Curved
16	PO(B)	++	White	White	Raised, Rough	Rough	Slightly Curved
17	G(B)	++	Grey	Grey	Flat	Smooth	Rough
18	W(A)	++	Dark yellow	White	Raised	Wrinked	Slightly Curved
19	L(A)	+++	White	Dark yellow	Raised	Rough	Filamentous
20	D	+	White	White	Flat	Wrinked	Rough

Primary Antibacterial Screening

Primary Screening for evaluating the antimicrobial potential of the axenic culture was performed by perpendicular method.

Isolated actinobacteria were cross streaked as a single line on AIA.

Incubate for 5-6 days at 28 celsius

Test organism were cross streaked perpendicular to the original streak

06 isolates shows antibacterial activity against selected bacteria

Microbial Strain showing moderate to ‘good’ inhibition.

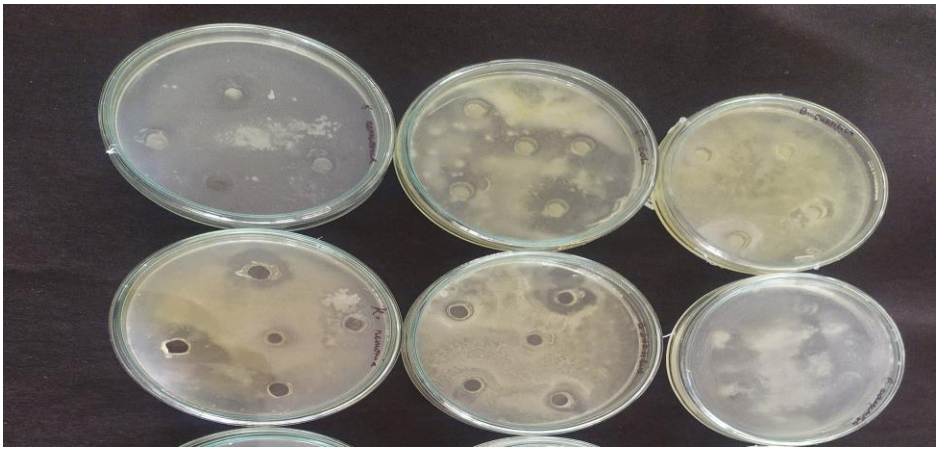
Primary antibacterial screening of Actinobacteria

Name of actinobacteria	*E.coli*	*S.aureus*	*P.aeruginosa*	*S.abony*	*K.pneumonia*
Po(B)	+	+	+	+	+
B(A)	+	+	+	+	+
G(A)	+	+	++	++	+
M(B)	+++	+	+	+	++
D	-	-	-	-	-
D(B)	-	-	-	-	-
P(B)	-	-	-	-	+
D(A2)	+	-	-	+	+
WB	-	-	-	-	-
DA2	+	-	-	-	+
GB2	+	+	+++	+	+
DB	++	+	-	+	+
MA	-	-	++	+++	+++
WA	-	-	+	+	++
LA	+	-	-	-	+++
L(B)	++	+++	+	+	+++
G(A)	+++	++	+++	+++	+++
Po(A)	+++	+++	+++	+++	+++
GB	-	+	-	+	+
BB	+	++	++	+	-

+++:Good, ++:Moderate, +:Weak , -:No activity.

Secondary Antibacterial screening of Actinobacteria

The agar diffusion test is used to qualitatively assess the efficacy of textiles treated with diffusible biocides. Samples are placed in the centre of nutrient agar plates which have been inoculated with the test bacteria. The samples are incubated at 37°C for 18-24 hours. The evaluation of this test is based on the level of growth both under and around the sample. The 'zone of inhibition' around the test material is measured and any growth present underneath the sample is scored.



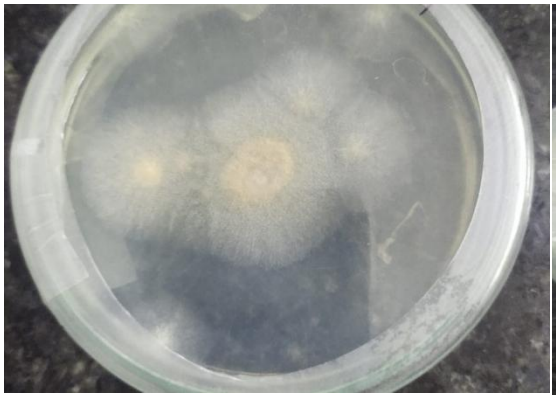
Secondary Antibacterial activity

Bacteria	**M(B)**	**G(B2)**	**M A**	**L(B)**	**G(A)**	**PoA**
B.*abony*	10	11	10	14	13	13
E. *coli*	15	18	14	12	20	15
K.*pneumonia*	10	12	15	13	12	14
S.*subtilis*	12	14	17	12	13	14
P.*aeruginosa*	12	13	13	11	10	12
S.*aureus*	13	14	13	18	22	12

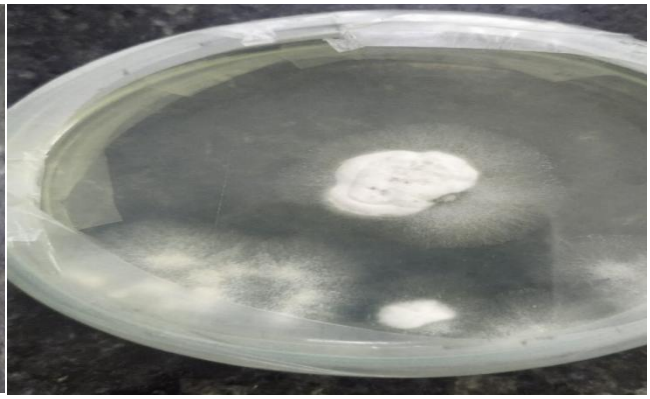
Secondary Antibacterial screening of Actinobacteria by well diffusion method

Antifungal Screening of Actinobacteria

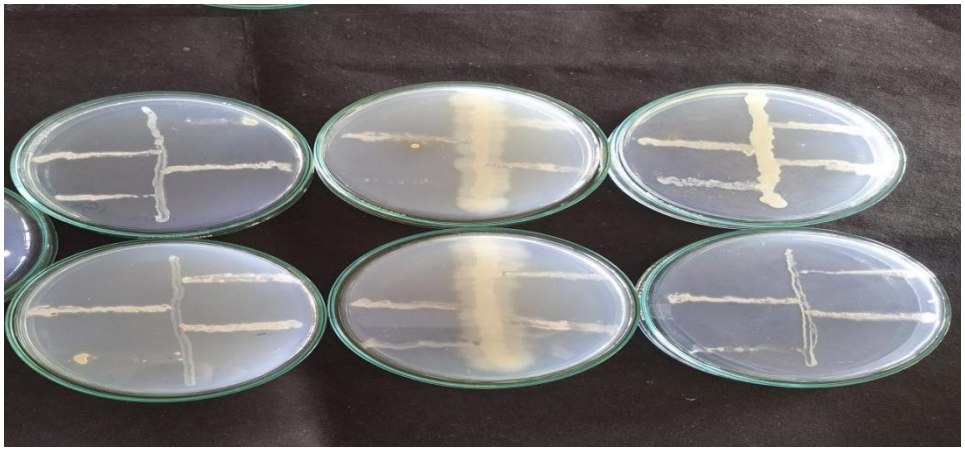
The pathogens were maintained on fresh potato dextrose agar at 4 °C. The fungi on PDA were incubated at 25 °C for seven days.After 4 days streaking isolated actinobacteria by perpendicular method then incubate 2 days at 25 °C. The diameter of each colony was measured, and the inhibition rate was calculated after 5 days.



T.*Viride*



A.*fumigatus*



Antifungal activity

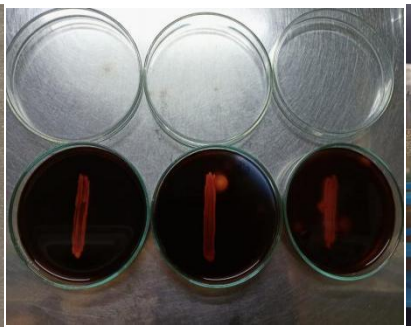
Fungal	**M(B)**	**GB2**	**MA**	**L(B)**	**G(A)**	**Po(A)**
T.*Viride*	+++	++	+	++	+	++
A.*fumigatus*	+	+	++	+	++	+++
A.*flavus*	++	+	-	+	++	
A.*niger*	+	+++	++	-	++	-

+++: Good, ++: Moderate, +: Weak, -: No activity

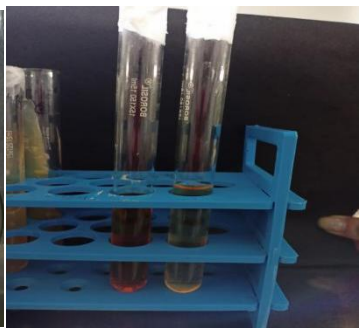
Results of Biochemical Test

S. No	Isolated strain	IMVP Indole,VR,MR	Urease	Catalase	Starch hydrolysis
1	M(B)	- + -	+	-	+
2	G(B)2	- - +	+	-	-
3	M(A)	- + +	-	+	-
4	L(B)	- + -	+	-	+
5	G(A)	- + -	+	+	-
6	Po(A)	- + -	+	-	+

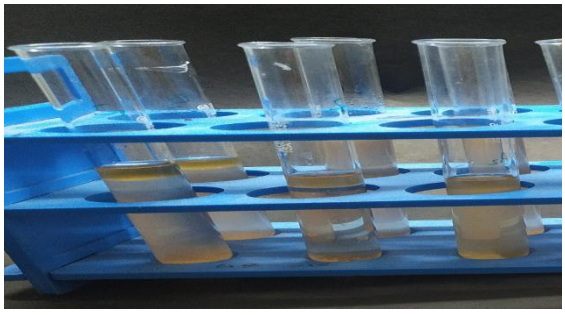
Catalase



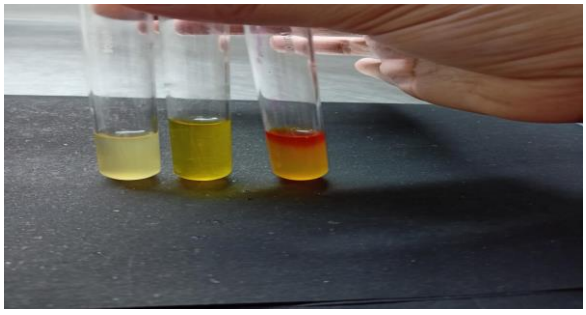
Starch hydrolysis



Ureas



Indole test



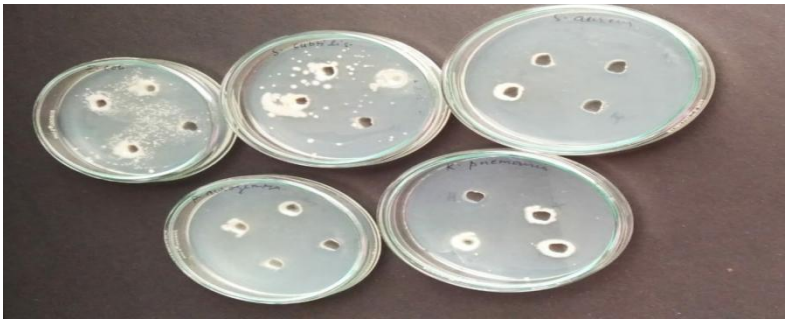
Methyl Red

Bioactive compounds of Actinobacteria against MDR Strain of pathogenic bacteria

After 20 days incubation period

ISP2 Media incubated 28°C, 150 rpm

For 20 days



Bioactive compounds against pathogenic bacteria

S.N.	Bioactive compound of Isolated strain	*E.coli*	*S.aureus*	*P.aeruginosa*	*S.abony*	*K.pneumonia*
1	G(B)2	19	17	18	20	25
2	G(A)	12	15	15	21	18
3	M(A)	19	12	12	18	19
4	L(B)	12	10	13	12	21

Discussion

Actinobacteria produce the different types of bioactive compounds, which contain antibiotic, anticancer, and antimicrobial activities (Elsayed et al., 2020; Bukhari et al., 2021). Actinobacteria are omnipresent in the earth's solid constituents, such as humus,dung, litter, and soils. Actinobacteria are found in the air in which their hyphal biomass is dispersed through fragmentation and are carried by air and water and grow at the extent of the organic residues. Actinobacteria have a significant property in digestion an production of certain components such as proteins in the form of keratin and certain vitamins (Lechevalier, 1981). A large majority of antibiotics that have been isolated in the numerous tested programs of new chemotherapic agents against various bacteria (Waksman et al., 1952). Two-third of the naturally available antibiotics were produced from Actinobacteria (Tanaka and Omura,1990). Streptomyces are specifically prolific and can make many biologically active secondary metabolites and antibiotics. In the current study, Actinobacteria isolate was found to develop a flexuous spore chain with a smooth surface, this is the main characteristic feature of Streptomyces, these study was supported by Atalan et al. (2000). The composition of the medium is an essential aspect of the morphology of the microorganisms. The Actinobacteria isolates were grown on different culture media like yeast extract malt extract agar, oatmeal agar, glycerol asparagine agar, tyrosine agar medium and starch casein agar (Ouchari et al., 2019), starch casein agar was best suitable than the other media tested. For the characterization of Actinobacteria isolates metabolites, various physiological tests were carried out (Abbas et al., 2019). In the current study the S. felleus (BHPL-KSKU5) showed positive results in carbon utilization to dextrose, L-arabinose, maltose, starch, D-arabinose, fructose, mannose, and lactose but it unable to utilize mannitol, sucrose, xylose, and D-galactose. The utilization of different nitrogen sources in strain BHPL-KSKU5 showed positive results to sodium nitrate and asparagine, whereas it did not utilize ammonium sulphate, ammonium nitrate but utilized potassium nitrate, and calcium nitrate. The utilization of amino acids was also tested. Among the tested amino acids, namely DL- 2 amino-N-butric acid, L-tyrosine, DL-tryptophan, DL-ornithine, L-lysine, DL-leucine, L-arginine, DL-alanine and L leucine were utilized. Whereas it did not utilize L-glutamic acid, L-cystine, and DL-aspartic acid, these results could be utilized as a taxonomic criterion at genus level identification. In identifying actinomycetes 16S rDNA gene sequence played a vital role which is evident by many workers (Al-Ansari et al., 2020; Daigham and Mahfouz, 2020).

Conclusion

Actinobacteria could be found in different environments such as soil, husk, and other than the source and our preferred site of soils are more tolerant of their growing and novel antibiotics and significant secondary metabolites. In present study totally 24 soil samples were collected from different area in Lucknow. The strain G(B)2 and G(A)was identified and characterized which contain more antibacterial and antifungal activity. Further, this study may be helpful to develop potential antibacterial and antifungal agents against different pathogens.

References

1. Kim E. S. (2021). Recent Advances of Actinomycetes. *Biomolecules*, *11*(2), 134. https://doi.org/10.3390/biom11020134.
2. Chaudhary, H. S., Yadav, J., Shrivastava, A. R., Singh, S., Singh, A. K., & Gopalan, N. (2013). Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). *Journal of advanced pharmaceutical technology & research*, *4*(2), 118–123.
3. Bérdy J. Bioactive microbial metabolites: A personal view. *J Antibiot (Tokyo)* 2005;58:1–26.
4. Goodfellow M, O'Donnell AG. Search and discovery of industrially significant Actinomycetes. In: Baumberg S, Hunter IS, Rhodes PM, editors. *Microbial Products: New Approaches, Society for General Microbiology Symposium No. 44.* Cambridge: Cambridge University Press; 1989. pp. 343–83.
5. Wang Y, Zhang ZS, Ruar TS, Wang YM, Ali SM. Investigation of Actinomycetes diversity in the tropical rainforests of Singapore. *J Ind Microbiol*
6. *Biotechnol.* 1999;23:178–87. 6. Okami Y, Okazaki T. Studies on marine microorganisms isolation from the sea. *J Antibiot.* 1972;25:456–60.
7. Baltz RH. Renaissance in antibacterial discovery from actinomycetes. *Curr Opin Pharmacol.* 2008;8:557–63.
8. Takizawa M, Colwell RR, Hill RT. Isolation and diversity of actinomycetes in the chasapeake bay. *Appl Environ Microbiol.* 1993;59:997–1002.
9. Edwards C. Isolation, properties and potential applications of thermophilic actinomycetes. *Appl Biochem Biotechnol.* 1992;42:161–79.
10 Selim, M., Abdelhamid, S. A., & Mohamed, S. S. (2021). Secondary metabolites and biodiversity of actinomycetes. *Journal, genetic engineering & biotechnology*, *19*(1), 72.

11..Berdy J. Thoughts and facts about antibiotics: where we are now and where we heading. *J Antibiot.* 2012;65(8):385–395. doi: 10.1038/ja.2012.27.

12. Mohan KD, Rajamanickam U. Biodiversity of actinomycetes and secondary metabolites. *Inn Orig Inter J Sci.* 2018;5(1):21–27.

13. Dewi TK, Agustiani D, Antonius S (2017) Secondary metabolites production by actinomycetes and their antifungal activity. Kn E Life Sci:256–264. 10.18502/kls.v3i4.713.

14. Mincer TJ, Jensen PR, Kauffman CA, Fenical W. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl Environ Microbiol.* 2002;68(10):5005–5011. doi: 10.1128/AEM.68.10.5005-5011.2002.

15. Bull AT, Ward AC, Goodfellow M. Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol Mol Biol Rev.* 2002;64:573–606. doi: 10.1128/mmbr.64.3.573-606.2000.

16. Dhanasekaran D, Rajkumar G, Sivamani P, Selvamani S, Panneerselvam A, Thajuddin N. Screening of salt pans actinomycetes for antibacterial agents. *Inter J Microbio.* 2005;2:62–66.

17.Salwana R, Sharmab V. Molecular and biotechnological aspects of secondary metabolites in actinobacteria. *Microbiol Res.* 2020;231:126374. doi: 10.1016/j.micres.2019.126374.

18. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of *Musa* sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. *Asian Pac J Trop Biomed.* 2013;3(9):737–742. doi: 10.1016/S2221- 1691(13)60148-3.

19. Goodfellow M, Williams T. Ecology of actinomycetes. *Annu Rev Microbiol.* 1983;37(1):189–216. doi: 10.1146/annurev.mi.37.100183.001201.

20. Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., & Pellegrini, M. (2022). Actinomycetes from Caves: An Overview of Their Diversity, Biotechnological Properties, and Insights for Their Use in Soil Environments. *Microorganisms*, *10*(2), 453.

21. B. Mukhopadhyay and N. K. Ganguly, “Tuberculosis research in India,” *Current Science*, vol. 105, no. 5, pp. 594–596, 2013.

22. J. Bérdy, “Thoughts and facts about antibiotics: where we are now and where we are heading,” *Journal of Antibiotics*, vol. 65, no. 8, pp. 385–395, 2012.

23. Q. Wang, F. Song, X. Xiao et al., “Abyssomicins from the South China Sea deep-sea sediment verrucosispora sp.: natural thioether michael addition adducts as antitubercular prodrugs,” *Angewandte Chemie—International Edition*, vol. 52, no. 4, pp. 1231–1234, 2013.

24. Abdel-Fattah YR and Olama ZA 2002. L-asparaginase production by Pseudomonas aeruginosa in solidstate culture: evaluation and optimization of culture conditions using factorial designs. Process Biochemistry, 38(1): 115–122.

25. Alexander, M. 1977. Introduction to soil microbiology, 2nd éd. Krieger Publishing Company, Malabar, FL. 467 pp.

26. Baltz, R.H. Renaissance in antibacterial discovery from actinomycetes. Curr. Opin. Pharmacol. 2008, 8, 557-563.

27. Barakate, M., Y. Ouhdouch, Y. Oufdou, and C. Beaulieu. 2002. Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. World J. Microbiol. Biotechnol. 18: 49-54.

28. Bashan, Y., Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Appl. Environ. Microbiol. 1986, 51, 1089–1098.

29. Berdy, J. Bioactive microbial metabolites. J. Antibiot. 2005, 58, 1-26.

30. Fenical, W.; Sethna, K.M.; Lloyd, G.K. Marine microorganisms as a developing resource for drug discovery. Pharm. News 2002, 9, 489-494.

31. Gardner, J.M., J.L. Chandler, and A.W. Feldman. 1984. Growth promotion and inhibition by antibioticproducing fluorescent pseudomonads on citrus roots. Plant Soil 77: 103-113.

32. Godden, B., A. S. Ball, P. Helvenstein, A. J. McCarthy, and M. J. Penninckx. 1992. towards elucidation of the lignin degradation pathway in actinomycetes. J. Gen. Microbiol. 138:2441–2448.

33. Goodfellow M, Williams ST. Ecology of actinomycetes. Annu Rev Microbiol. 1983;**37**:189–216.

34. Jensen PR, Dwight R, Fenical W. Distribution of actinomycetes in near-shoretropical marine sediments. Appl Environ Microbiol. 1991 Apr;**57**(4):1102–1108.

35. Goodfellow, M. and S.T. Williams, 1983. Ecology of actinomycetes. Annual Review of Microbiol. 37: 189-2.

36. Haeder, S., R. Wirth, H. Herz, and D. Spiteller, 2009. “Candicidinproducing Streptomyces support leafcutting ants to protect their fungus garden against the pathogenic fungus Escovopsis,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 12, pp. 4742– 474616.

37. Janssen, P.H.; Yates, P.S.; Grinton, B.E.; Taylor, P.M.; Sait, M. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. Appl. Environ. Microbiol. 2002, 68, 2391- 2396.

38. Johnsen, A.R., A. Winding, U. Karlson and P. Roslev, 2002. Linking of microorganisms to phenanthrene metabolism in soil by analysis of -labaled cell lipids. Applied and Environmental Microbiol. 68: 6106-6113.

39. Hussain, A.A., S.A. Mostafa, S.A. Ghazal and S.Y. Ibrahim, 2002. Studies on antifungal antibiotic and bioinsecticidal activities of some actinomycete isolates. African J. Mycol. Biotechnol., 10: 63–80.

40. Kim, T.K.; Fuerst, J.A. Diversity of polyketide synthase genes from bacteria associated with the marine sponge Pseudoceratina clavata: culturedependent and culture-independent approaches. Environ. Microbiol. 2006, 8, 1460-1470.

41. KIoepper, J.W. 1996. Host specificity in microbemicrobe interactions. BioScience 46: 406- 409.

42. Long, P.F. and G.E. Amphlett, 1996. A super lytic actinophage system as a pre–treatment in the isolation of non–streptomycete actinomycetes from soil. Lett.. Appl. Microbiol., 22: 62–5.

43. Magot, M., B. Ollivier, and B. K. C. Patel, “Microbiology of petroleum reservoirs,” Antonie van Leeuwenhoek, vol. 77, no. 2, pp. 103–116, and 2000.

44. Maldonado, L.A.; Fragoso-Yáñez, D.; Pérez-García, A.; Rosellón-Druker, J.; Quintana, E.T. Actinobacterial diversity from marine sediments collected in México. Antonie van Leeuwenhoek 2009, 95, 111-120.

45. Lechevalier, M.P. 1988. Actinomycetes in agriculture and forestry. Pages 327-358 in M. Goodfellow, S.T. Williams, and M. Mordarski (eds.), Actinomycetes in biotechnology. Académie Press, New York.

46. Lomovskaya, N.D., K.F. Chater and N.M. Mkrtumian, 1980. Genetics and molecular biology of Streptomyces bacteriophages. Microbiol. Rev., 44: 206–29.

47. McCarthy, A.J.; Williams, S.T. Actinomycetes as agents of biodegradation in the environment-a review. Gene 1992, 115, 189-192.

48. Pozzi, R.; Simone, M.; Mazzetti, C.; Maffioli, S.; Monciardini, P.; Cavaletti, L.; Bamonte, R.; Sosio, M.; Donadio, S. The genus Actinoallomurus and some of its metabolites. J. Antibiot. 2011, 64, 133–139. [Google Scholar] [CrossRef] [PubMed]

49. Pozzi, R.; Simone, M.; Mazzetti, C.; Maffioli, S.; Monciardini, P.; Cavaletti, L.; Bamonte, R.; Sosio, M.; Donadio, S. The genus Actinoallomurus and some of its metabolites. J. Antibiot. 2011, 64, 133–139. [Google Scholar] [CrossRef] [PubMed]

50. Pozzi, R.; Simone, M.; Mazzetti, C.; Maffioli, S.; Monciardini, P.; Cavaletti, L.; Bamonte, R.; Sosio, M.; Donadio, S. The genus Actinoallomurus and some of its metabolites. J. Antibiot. 2011, 64, 133–139. [Google Scholar] [CrossRef] [PubMed]