A DISSERTATION ON

Screening of selected bacterial isolates for abiotic stress tolerance and plant growth promoting traits

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Certificate

This is to certify that dissertation entitled **"Screening of selected bacterial isolates for abiotic stress tolerance and plant growth promoting traits"** submitted by Ms. **Suman Kumari** in partial fulfilment of the requirement for the Degree of Master of Science in Biotechnology is a bonafide record of the authentic work carried out by her under my supervision at Institute of Environment & Sustainable Development, Banaras Hindu University, Varanasi, India.

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TO WHOM IT MAY CONCERN

This is to certify that **Ms.Suman kumari**, a student of M. Sc. Biotechnology (II Year, IV semester), Integral University has completed her four months dissertation work entitled **Screening of selected bacterial isolates for abiotic stress tolerance and plant growth promoting traits successfully**. She has completed this work from Institute of Environment and Sustainable Development, Banaras Hindu University is a record of original work undertaken by me under the guidance of Dr. Vishal Prasad, Assistant Professor,Instituteof Environment and Sustainable Development, Banaras Hindu University, Varanasi.

The dissertation was a compulsory part of her M. Sc. degree. I wish her good luck and future.

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DECLARATION

I hereby declare that the dissertation entitled "***Screening of selected bacterial isolates for abiotic stress tolerance and plant growth promoting traits being*** submitted to the partial fulfillment of the award of the degree of Master of Sciences in Biotechnology, Integral University Lucknow**.**

It is an authentic record of work carried out under the supervision of **Dr. Vishal Prasad**, Assistant Professor,Instituteof Environment and Sustainable Development, Banaras Hindu University, Varanasi,U.P.

I Suman kumari hereby declare that the work embodied that the work is not been submitted to any other University / Institution.

Date **Suman Kumari**

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PREFACE

Global food security is the most concerned agenda in the 21st century as there is a rapid surge in population. Cultivable land is declining and due to the intense use of fertilizers, there is poor soil quality being developed. Cultivation in such soil will never allow us to meet the need of feeding a huge population and increasing the productivity of the crop. There are a lot of potential solutions to this issue, Plant Growth-Promoting Microorganism is one such solution to this problem. The major objectives of the present study were to identify the plant growth-promoting traits of microorganisms and their adaptability at various salinity levels. This work is an attempt to understand how much the test bacterial isolates are beneficial for soil health and crop productivity. Chapter 1 deals with an overview of salinity and its impact on agriculture, the importance of PGPMs, and Objectives of the study. Chapter 2 deals with the background of the study such as the emergence of salinity, various factors involved in crop productivity, and a brief of various mechanisms performed by PGPMs. Chapter 3 deals with the materials and methodology adopted to perform the experiments. Chapter 4 deals with the results obtained after performing experiments, which show that PGPMs are potential biofertilizers. Chapter 5 discusses the results and concludes the study. This whole work centers around the concept that PGPMs are potential biofertilizers that can benefit plant growth and crop productivity and their mechanisms to tolerate abiotic stressors. PGPMs may be an important tool for sustainable agriculture and soil management practices in near future

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INTRODUCTION

Plants are the ideal sources of food, on which an entire human population is dependent directly or indirectly. During their development, they are exposed to different types of environmental challenges which include biotic and abiotic factors due to rapid variations in climatic conditions (Islam et al., 2015[i]). which result in more and more extensive use of chemical fertilizers.these agent are costly and creat environmental problem. Environmental stresses such as salinity, drought, heat, cold, flooding and heavy metal toxicity are major threat to the agricultural productivworldwide (Gaafar *et al*., 20120) crop yields are reduced, and reduce soil fertility. Today several matter, of human, disorders,diseases, malformation and malfunction of organs due to metal toxicity have been reported 8.

Concentration of these toxic metals has accelerated dramatically since the beginning of the industrial revolution (Ana *et al*., 2009) thus posing problems to health and environment (Nriagu, 1979). The stresses have a negative impact on thegrowth and development of plant, decrease crop yield and also reduce soil fertility (Nadeem et al., Glick et al., 2014) On the other hand, an ever- increasing world population and climatic variabilityare expected to severaly enhance the worldwide appeal for farmable land , a resource that is already in high demand (Coleman –Derr and Tringe, 2014) The necessity of providing food for the world sburgeoning population while repelling abiotic stresses is a bigger challengetoday , and it has given an imperative significance in plant and soil productivity research (Dimpka et al., 2009;Glick, 2014;Nadeemet al., 2014)This led to the application of chemical fertilizers and this resulted in land degradation, deterioration of soil health, heavy metal accumulation, groundwater contamination and many other environmental problems. Crop improvement includes a lot of factors; both biotic as well as biotic factors do contribute.These environmental factors cause huge losses of agricultural productivity worldwide. Among abiotic stresses, salinity, high temperature,UV-radiations, drought, mineral deficiency, pesticides and heavy metal cantamination, , fertilizer apllication , soil pH salinity (Ali and Alqurainy, 2006).

Among all abiotic factors, salinity is a matter of concern because it reducescrop productivity and soil health. Global climate change is also rapidly increasing the landscapes salinity. Saline soil is defined as soil that has electrical conductivity (EC) of the saturation extract (ECe) in the root zone of more than 4 dS/m (approximately 0.4 M NaCl) at 25℃ and exchangeable sodium (ESP) of 15% (Shrivastava & Kumar, 2015). Salinity affects almost all aspects of crop production of plant development including seed germination, vegetative growth, and reproductive. Soil salinity impose ion toxicity ,osmatic stress, and nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), zinc (Zn), iron (Fe) deficiency inthe plants (Shrivastava & Kumar, 2015). Salinity also induces water stress, cytotoxicity by increasing the uptake of ions- Na^+ and Cl^- , and nutrient imbalance (Isayenkov & Maathuis, 2019).such situation, it needs appropriatetechnology to improve crop productivity and soil health throughinteractions of soil microorganisms and plant roots under stressful conditions(Egamberdieva, 2015). Even though variousapproaches have been tested for the mitigation of abiotic stresses on plantgrowth, adoption of biological methods: containing soil rhizobacteriahas been confirmed to be efficient for the mitigation of divergent stresses(Dimpka et al., 2009; Nadeem et al., 2014).

Fungici Diverse kindsof soil bacteria are attracted by the exudates of plant roots. They occupythe rhizosphere of many plant species and provide benefit to the plants byenhancing plant growth and lowering disease development. Rhizobacteriaare primarily used for enhancing crop yield and preserving soil productivity(Azcon et. al., 2013; Glick, 2014). They arenot only useful in agriculture but they also have potential to solve environmentalproblems including abiotic stresses. Plant-associated microbial communities have appreciable capabilities tonegotiate many of the abiotic stress effects on plants (Mayak et al., 2004;Coleman-Derr and Tringe, 2014). Entireplants and almost all tissues within the plant are populated by a diversityof microorganisms, many of which endeavor benefits to the host, enhancinguptake of nutrients, protecting from pathogen attack, and enhancinggrowth of plants under unfavorable environmental conditions. In return, these microorganisms secure shelter from the ambience environmentand connection to a carbon-rich food supply (Yang et al., 2009).

Soil cantains different group of microorganism such as bacteria , fungi and algae that affect the physical, chemical, and biological properties of soil (Shahzad et al., 2012; Marek-Kozaczuk et al., 2013). The narrow zone of soil spread around the root sytam is called rhizosphere. The rhizosphere, valume of soil surrounding roots influenced physically, chemically, biologically by plant root, in the rhizosphere, impotant and intensive interaction occur among the plant, and micoorganisms, which can considerably influence plant growth and crop yields by producing growth regulators, inducing root exudation the availability of nutrients to plant, besides controlling soil borne plant pathogens(Tahir et al., 2013). The means plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can enhance plant growth by wide varity of mechanisms like phosphate solubilization , siderophore production, nitrogen fixation, increasing the availability of nutrient in the rhizosphere, increasing root surface area and enhancing other beneficial symbiosis of the host (Akhtar et al., 2012).

Sail salinity, erosion and land degradation problems not only crumble the quality of crop production but also affect the land and they be further used for cultivation (Miransari, 2012; Bhattacharyya et al., 2015) plants causing negative effects on photosynthetic apparatus, initiate senescence and reactive oxygen species (ROS) production, deactivates enzymes and inhibits overall growth and development of plants (Maleva et al., 2009; Kumar et al., 2012; Islam et al., 2015). Seed germination, and seedling growth are mostly affected by salinity. Salinity stress destroys the field and they also creat other type of stresses like oxidative stress. In most of the salt sensitive crop plant, Na^+ imposes osmotic stress and producing deteterious protein that causes growth inhibition cell death (Nadeem et.al., 2014). In the saline condition high level of Na^+ not only hinders the uptake of other nutrient but also causes peculiar ion toxicity (Ashraf and Wu, 1994). A high ratio of K^+/Na^+ is very essential in plant for tolerance against salinity and maintenance of osmotic potential (Hamdia et al.,2004).

Heavy metals contaminate the ecosystem Heavy metal contaminants causing ecological problems are of global concern. They remain a potential threat for many years. Heavy metal contaminants causing ecological problems are of global concern. Heavy metals are significant environment pollutants (Berry, 1986). Plants

are susceptible to heavy metal toxicity and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. There are different sources of heavy metals in the environment such as:natural sources, agricultural sources,industrial sources, domestic effluents atmospheric sources. There is a two way relationships between the high concentration of heavy metals in the soil and the expression of toxicity.

Heavy metal contaminants causing ecological problems are of global concern. Heavy metals are significant environment pollutants (Berry, 1986). Plants are susceptible to heavy metal toxicity and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. There are different sources of heavy metals in the environment such as:natural sources, agricultural sources,industrial sources, domestic effluents atmospheric sources. There is a two way relationships between the high concentration of heavy metals in the soil and the expression of toxicity. Heavy metals compete with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plants (Clarkson and Luttge, 1989) and on the other hand after uptake by the plant it accumulates in plant tissue and cell compartments and hampers the general metabolism of the plant (Taylor, 1988, Turner, 1997; Hasan *et al*., 2009).). Their availability in soils depends on natural procedure, especially lithogenic and pedogenic soils and anthropogenic factors such as mining, urban waste disposal, soil runoff, combustion of fossils fuels metal, boating activity, and phosphate fertilizer application. An increase in heavy metals in the soils could also be attributed to factors such as soil properties or different agricultural practices eg., application of sludge to agricultural land (Foy *et al*., 1978).The resistance or tolerance by plants towards heavy metals depends on chain of physiological, biochemical and cellular mechanisms. The foremost mechanism possessed by plants is to elevate the expression levels of different genes and peptides responsible for detoxification of metals (Ali and Alqurainy, 2006). Plants also lead to Introduction 2 accumulation of different secondary metabolites that activate the antioxidative defense expression within them against heavy metal stress (Ovecka et al., 2014) Apart from metal toxicities, plants are exposed to biotic stresses such as, bacteria, virus, fungi, herbivores, insects and nematodes (Fujita et al., 2006). These pathogens infect

many plant species worldwide and tend to decrease their productivities (Poschenrieder et al., 2006). The zone around plant roots is known as rhizosphere where interactions among plants, microbes and pathogenic organisms take place. The associations among micro-organisms and plants are usually beneficial but this complex region is also surrounded by pathogens that that causes different abnormalities in plants (Bais et al., 2006; Razavi et al., 2017). Due to omnipresent behaviour of microbes, they can survive in soil, air, water, land, desert and even in extreme conditions (Vidali, 2001; Ullah et al., 2015). The micro-organisms present in rhizosphere are of utmost importance as they interact with plants in many direct and indirect ways through signaling mechanisms (Daniels et al., 2004; Hao et al., 2012). They play an essential role in improving the nutrient uptake and reducing the ill-effects of different metal ions and pathogens through detoxification, transformation of metal ions and secreting certain volatile organic compounds that inhibit the survival of pathogens near plants (Gadd, 2010). The rhizobacteria that possess plant growth promoting characteristics are called as plant growth promoting rhizobacteria (PGPR) that can live in symbiotic relationship, free living or as endophytes with plants (Glick et al., 2012). The mechanisms acquired by PGPR include efflux, immobilization, stabilization, complexation, volatilization, sequestration and detoxification of different heavy metal ions (Rajkumar et al., 2012; Pavel et al., 2013). In addition, they have also ability to secrete chelating agents, siderophores and phosphate solubilisation that ultimately affects the mobility of metal ions (Yang et al., 2009). They also possess different strategies such as exclusion, bioaccumulation, Introduction 6 biotransformation, biosorption, precipitation and enzymatic detoxification of metal ions in different compartments of cells (Rajkumar et al., 2009; Chen et al., 2014). The toxic levels of heavy metals change the pattern of biomass productivity, plant growth, photosynthetic pigments, protein, amino acids, starch, soluble sugars, and essential nutrients uptake. To manage the salinity and heavy metal stress, there is need to develop simple and low cost biological method incuding those of microbes. If you can exploit their unique property such as tolerance to salinity condition, synthesis of compatible solutes, production of plant growth promoting hormones, genetic diversity, biocontrol potential and their intraction with crop plant, they would prove to be helpful in the management of salinity stress. PGPR are heterogeneous group of bacteria that colonize root and promote the growth of plant. PGPR Promote

plant growth either by direct or by indirect mechanisms. The tolreance capacity and growh production in salinity challenged plant can be increased with the help of various mechanistic action of PGPR as show in **figure 1**

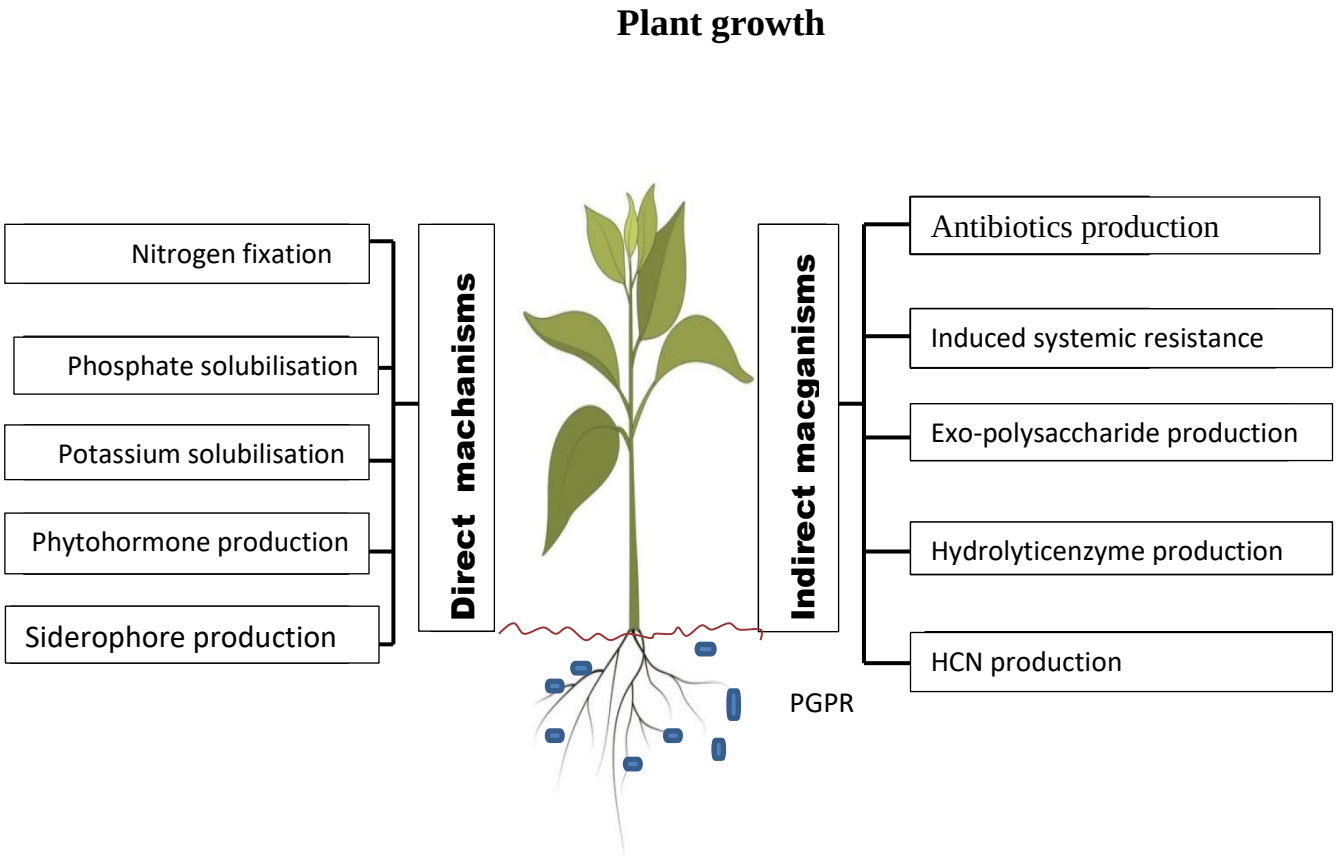


Figure 1 : PGPR mediated various direct and indirect mechanism of growth promotion

Direct mechanism: The direct mechanism of plant growth promotion by PGPR include production of metabolism (nitrogen fixing nitogen, solubilising phosphate, producing hormonces, siderophpore production). Or directly affrcting the plant metabolism incresing the uptake of water and minerals, the plant or helping other beneficial microorganism to enhance their action on the plant.

Indirect mechanism: PGPR can also promote the plant growth by suppressing plant pathagens. PGPR improve soil properties through various mechanisms regulating soil contaminations. In the sense of improving soil fertility and crop productivity the above abilities of the PGPR have great important ,thus redunicing the negative effect of chemical fertilizer on the environment, PGPR can comprte soil

microorganism or tremendous capacity for production of antifungal secondary metabolites.In the use of PGPR has increaseb rapidlyin different plant like sayabean , rice , beans , maize. In many like fertilizer, microbial and bio pesticides, PGPR has been absorved benificial effects. .Over 20 Psedomonas species are know for synthesize more than 100 aromatic antibiotic compounds(Feklistova and Marsimova;2008).the most widely group of rhizospheric bacteria with to the production of antibiotices is that of the fluorescent pseudomonads. Same well know antibiotics are 2,4 Diacetyl Phloroglucinol (DAPG), Phenazine-1-carboxamide (PCN), Phenazine-1-carboxylic acid (PCA), Butyrolactones, Kanosamine, OomycinA, Viscosinamide 2,4 Diacetyl Phloroglucinol (DAPG), Pyoluteorin (Plt), Pyrrolnitrin (Prn), OomycinA, Kanosamine, Zwittermycin (Liu,et al;2007).

Plant root influence the soil by physically chemically and biologically is provide for reproduction of microorganism that impact on soil fertility and plant health. PGPR elude soil acidification by increasing the pH and producing capsular envelope to protect itself. PGPR alters root exudates either directly or indirectly through other beneficial microbes like arbuscular mycorrhizal (AM) fungi, thereby facilitating root colonization. PGPR improve root colonization by undergoing phase variation.the intraction between plant PGPR for growth and crop yields occur by proveded growth regulation inducing exudation and enhancing the availability of nutrient to plant (Tahir et,al; 2013).Rhizobacteria utilize the nutrient released by plan root inculde amino acid, fatty acide , sugar pitrescine and vitamins, nucleoacides, organic acide phenolic plant growth regulator sterole. Bacteria also secrete certain metabolites into rhizosphere (Van Loon and Bakker;2003;Bavis et al;2004; Gray and Smith,2005;Kiely et al; 2005).Rhizobacteria have capability to multiply and colonize in all the ecological niches found on the root at all stage of plant growth, the presence of a competing micro flora (Malleswari and Bagyanarayana, 2013;Tahir et al; 2013). PGPR also show antagonistic effects by inhibition of the pathogen by antibiotics and surface active compound (bio surfacetants).

PGPRs are beneficial for plants directly as well as indirectly and studies have shown that most of them perform well in stress conditions i.e., salinity. For sustainable agricultural practice and environment protection by improving soil health, PGPR is an eco-friendly solution with lots of benefits

Objectives

1. Growth evaluation of bacterial isolates against various abiotic stressors
2. Evaluation of bacterial isolates for potassium solubilization and siderophore production

REVIEW OF LITERATURE

Soil salinity is one the major environmental problem in India. Plants including agricultural crops face continuous environmental threats from different abiotic factors, which have increased over time due to change in global climate pattern as well as human interference (Glick, 2014; Ilangumaran and Smith, 2017). These environmental stresses limit crop productivity and thereby pose an overall threat to food security. Extreme abiotic environmental stress conditions like salinity, prolonged drought, extreme flooding, high temperatures, frost and low temperatures are expected to become more stern in the future owing to continuously changing climate. This will significantly affect the health of plants and soil microorganisms leading to a decline in agricultural productivity as well as reduced microbial activity in soil (Ahemad and Kibret, 2014; Sorty et al., 2016). Stresses which are frequently experienced by plants are external environments that negatively affect growth, development and productivity. These stresses incomparably decrease crop productions and act as a barrier to the introduction of crop plants in areas which are not fit for crop cultivation. Depending on the crop variety every year the yield losses due to abiotic environmental stresses can reach 50% to 80 % (Saharan and Nehra, 2011).The Chemical impacts of these stresses on plant growth include physiological disorder such as epinasty, abscission and senescence, hormonal and nutritional imbalance, ion toxicity and perceptivity to diseases(Nadeem et al., 2014; Singh et al., 2015; Gupta and Pandey, 2019).

Salinity is considered as one of the most common environmental stress factors that negatively affects plant growth and crop production in cultivated areas worldwide. It is perceived that salinity influences approximately 1 billion hectares worldwide (Egamberdieva and Approximately 20% of irrigated land is salt affLugtenberg, 2014; Shrivastava et al., 2015). ected worldwide, with 2,500- 5,000 km2 of production loss every year due to salinity (Chakraborty et al., 2011). Soil salinity problem not only crumble the quality and quantity of crop production but also severely affects the land which further cannot be used for cultivation The salt affected areas refer to soils that are saline or sodic and having electrical conductivity ≥4 (Miransari, 2012; Bhattacharyya et al., 2015). dS m-1 (Egamberdieva and Lugtenberg, 2014).

Soil is mainly classified into three types depending on its salinity natural salinity; dryland salinity; and irrigation salinity. Natural salinity is also known as primary salinity, it is caused by natural means such as weathering of rocks, and salt accumulation from rainfall over multiple years. The dryland salinity is secondary salinity caused by rising groundwater levels and clearing the vegetation of drylands. Irrigation salinity is tertiary salinity that is caused by the reapplication of water over many cycles (Understanding Salinity,2022)

Table ; Levels of salinity with the salt concentration of soil water

Salt concentration in soil water (Saturation extract) (in g/L)	Salinity
0-3	Non saline
3-6	Slightly saline
6-12	Moderately saline
More than 12	Highly saline

The agricultural intensification together with unfavourable natural conditions has sped up soil salinity in many parts of the world. Soil salinity is particularly more severe in arid and semi-arid regions of India, because of insufficient rainfall to leach down the salts to deeper soil layers.. Soil salinity has harmful effects on soil physico-chemical properties (Singh et al., 2012a), biochemical and enzyme activities (Karlen et al., 2008), affects soil structure; reduces water permeability, disturbs microbial community, hampers organic matter decomposition and agricultural productivity (Guangming et al., 2017).In case of ion-excess toxicity, Na+ ion replaces K+ ion in biochemical reactions and there is a conformational change in protein due to Na+ and Clions (Shrivastava & Kumar, 2015). This can be cured with a lot of remedies and one of them is the incorporation of the microbial populations which are not only salt-tolerant but also promote the growth of plants and crops.

Plant Growth-Promoting Rhizobacteria

The term rhizospheric microorganisms are used to describe the populations of microbes living in close vicinity of the plants rhizospheric zone. The different species of bacteria which are generally found to have plant growth promoting abilities chiefly

belong to genera, of PGPR are Agrobacterium, Allorhizobium, Arthrobacter, Azobacter, Azospirillum, Bacillus, Pseudomonas, Brachybacterium, Bradyrhizobium, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Mesorhizobium, Pseudomonas, Rhizobium, and Serratia amongst others (Singh et al., 2015). They help and promote plant growth either directly or indirectly (Beneduzi et al., 2012). Direct benefits involve, biofertilization (improved nutrient acquisition), bioprotection (suppression of plant disease causing pathogens), biostimulation (phytohormone production), Singh et al., 2015; Sorty et al., 2016). Such as Indole-acetic acid (IAA), abscisic acid (ABA), gibberellic acid, and cytokinins, siderophore production, and solubilize nutrients such as N, P, K for plants uptake (Dobbelaere et al., 2003). Indirect benefits include antagonistic effects against pathoge

Negative impact on growth

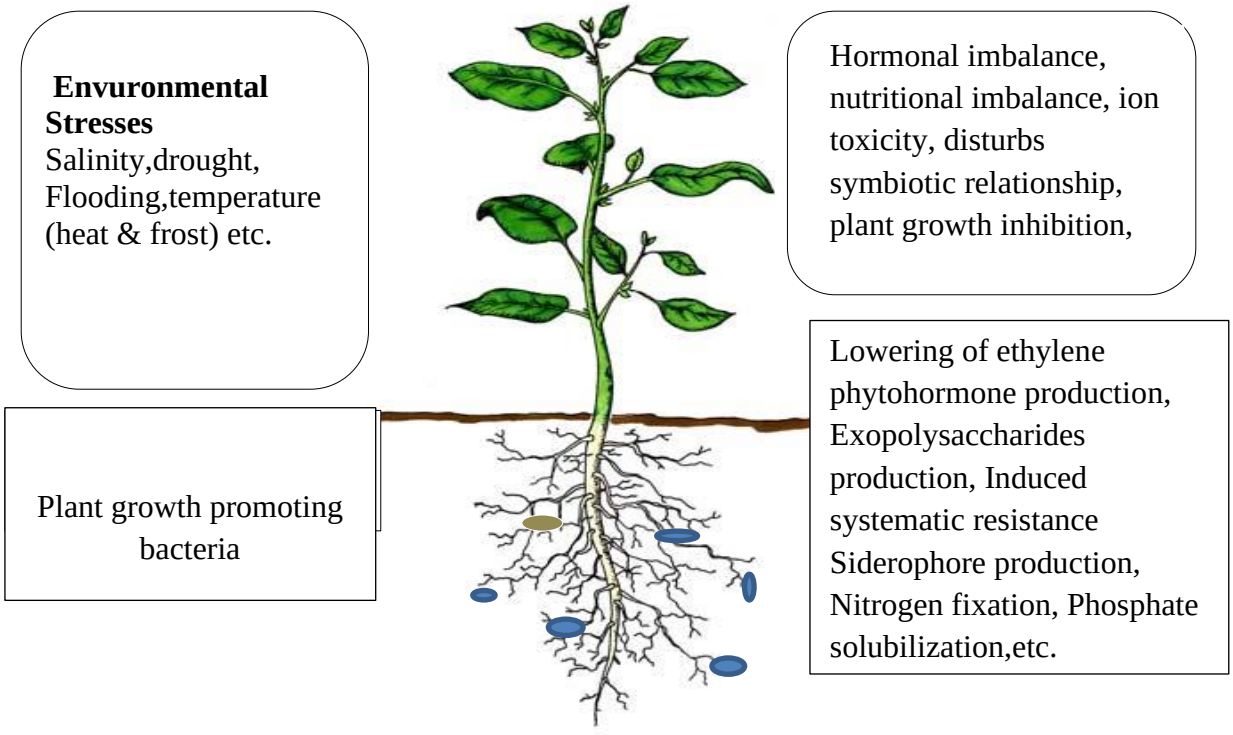


Figure : Various mechanisms used by PGPR for enhancing plant grwoth under stressful environmental condition.

Rhizobacteria can also boost plant resistance against diseases by changing host plants susceptibility, by the mechanism called induced systematic resistance and serve protection against pathogen attack (Nadeem et al., 2014;) The rhizobacterial inoculation has been provide a significant stimulatory effect on growth of plant in nutrient deficient soils (Bell et al., 2015). The abiotic environmental stresses is mainly due to phytohormones produced by rhizobacteria, these phytohormones stimulate plant growth either directly other bacterial secondary metabolites (Dimpka et al., 2009). such as, auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Patten and Glick, 1996; Dimkpa et al., 2009; Egamberdieva et al., 2015). Other compounds which are produced by PGPR include enzymes, nitric oxide, osmolytes, siderophore, organic acids and antibiotics. which are also responsible for plant growth by different mechanisms (Chakraborty et al., 2006; Dimkpa et al., 2009).

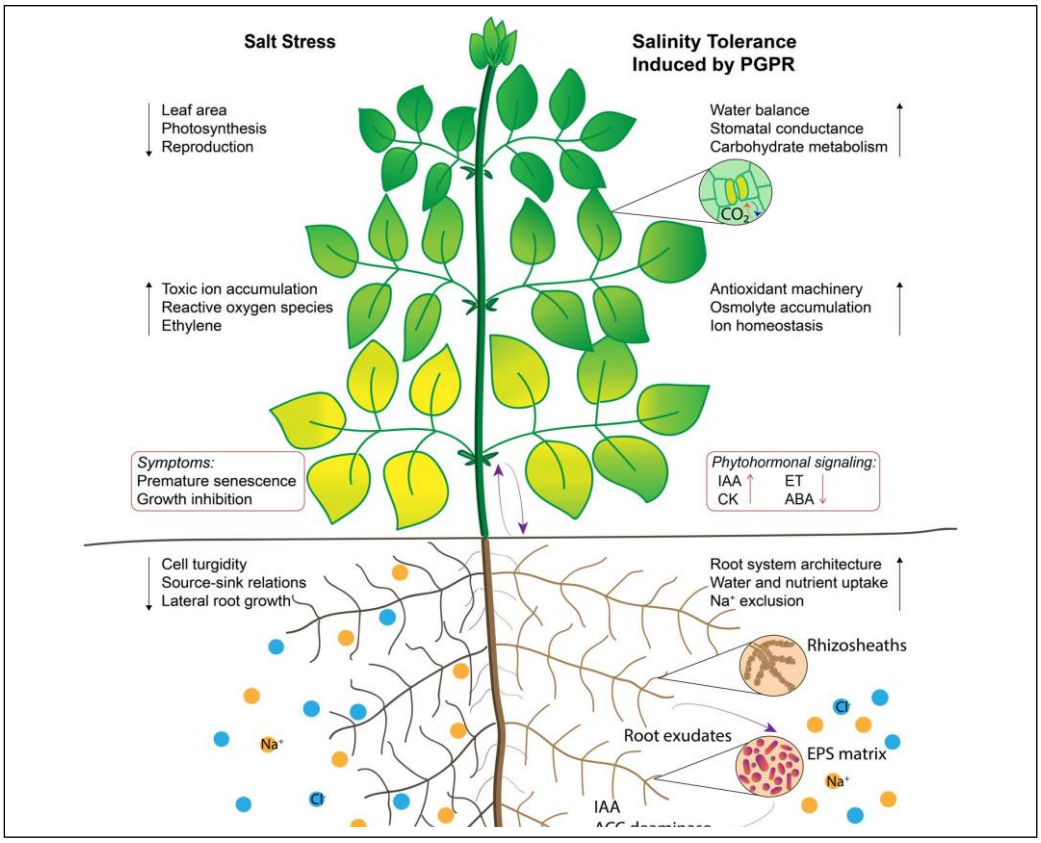


FIGURE ; Illustration of salt tolerance mechanisms induced by plant growth promoting rhizobacteria (PGPR)

PGPR not only promotes the growth of the plant in normal conditions but also in presence of various stress conditions such as salinity. Salt-tolerant PGPR such as Acinetobacter calcoaceticus, Burkholdera cepacia, Promicromonospora sp., etc. reduce the Na+ ions and increase K+ ions in the soil, improve water uptake of the plant, and improve membrane permeabilityto reduce the efflux of electrolytes present inside the cell (Kang et al.,2014). These salt-tolerant rhizobacteria grow under saline conditions. They are isolated from there and used to improve the growth of crops under salinity stress. Some of these bacteria are Brachybacterium sp., B. licheniformis,Exiguobacterium oxidotolerance,Pseudomonassp., and Hallobaclillussp.(Hartmann et al., 2016). IAA is the active form of auxin, a phytohormone that is responsible for the growth of plants by promoting cell growth, root elongation, and also the formation of lateral and adventitious roots hairs, seed and tuber germination, rate of xylem formation, etc. (Singh, 2015). Therefore improvement of root system of plant during stress condition improves water and nutrient uptake efficiency of plants thus helping in overall growth of plant (Singh et al., 2016; Ilangumaran and Smith, 2017, Numan et al., 2018). . Since IAA is required by the plant in low quantity, and if the amount is optimum then it is great for plants' health and growth. The presence of tryptophan as root exudates helps bacteria in IAA production as it is the precursor of IAA production and 80% of rhizobacteria are capable of IAA synthesis (Spaepen & Vanderleyden, 2011). Interaction of the In agricultural soil one of the important nutrient phosphorus (P) is present in organic and inorgnic forms but its inaccessibility to plant roots makes it a major limiting factor for plant chemical fertilizers contained rock phosphate in it. These organic fertilizers improved crop productivity but overuse of it resulted in soil degradation and pollution over time (McGrath et al., 2014). Under salinity condition to increased pH of soil free phosphate ion binds with Ca_2^+ and converted into calcium phosphate which further is not freely available to the plant. The rhizospheric microbes help plant by solubilising mineral P to soluble P (Prasad et al., 2018). When plant are grow under stress condition , the level of ethylene is incresed in cell and it damages the plant (Argueso et.al., 2007) .A low concentration of ethylene is essential for normal plant growth, but at high concentrations of ethylene can be negative impact on plant growth and development, because it induces defoliation and other cellular processes that may affect overall crop plant growth and development. 1-aminocylopropane-1-

carboxylate (ACC) is precursor of the ethylene. By production of enzyme ACC deaminase, many PGPR destroy ACC and plant growth and development by decreasing plant ethylene level. (Glick et.al.,2000).

Therefore, siderophore plays an important role in providing iron by solubilising iron from complex compound under the conditions of starvation or limitation. Siderophore is a low molecular weight iron-chelating molecule. It has a high affinity for ferric ions (Fe^{3+}). In iron-limiting conditions, bacteria release siderophore and chelate the iron which is present in an insoluble form (Ahemad, 2015).

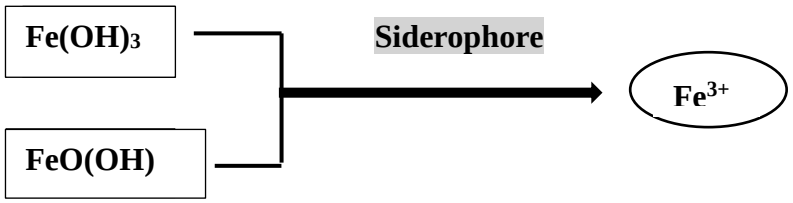


Figure : Mechanism of iron chelation by siderophore

Hundreds of siderophorevarieties are identified that arereleasedby cultivable microorganisms. Besides iron chelation, siderophore production by PGPR benefits plants by colonizing the root of the plant, sequestering dissolved iron, lowering dissolved iron in the surroundings, and excluding competitor microorganisms (Ramamoorthy et al., 2001). If the competitor microorganisms arepathogenic to plants, then itis a double benefit for plants. Bio-control ability of PGPR is another trait. PGPR releases chemicals such as antibiotics that inhibit the growth of plant pathogens or kill them. According to Haas and Defago (2005), there are six classes of antibiotic compounds- phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and hydrogen cyanide (Beneduzi et al., 2012)

Microorganisms when feel osmotic fluctuations in their surrounding environment accumulate osmolytes such as proline, glycine betaine etc.. The compatible solutes absorbed through plant root support in maintaining osmotic balance and further preventing cellular oxidative damage under saline condition (Qurashi and Sabri, 2013; Numan et al., 2018). Under saline conditions, a high level of Na^+ not only hinders the uptake of other nutrients but also causes peculiar ion 24 toxicity (Ashraf

and Wu, 1994). A high ratio of K^+ /Na^+ is very essential in a plant for tolerance against salinity and maintenance of osmotic potential (Hamdia et al., 2004). Some PGPR strains also have the capability to protect the plants from the deleterious effects of high Na^+ concentration in the saline soil. They do this by their ability to produce exopolysaccharides. The exopolysaccharides so produced eliminate Na^+ uptake in the plant by binding it and also by formation of biofilms (Khodair et al., 2008). The decreased availability of Na^+ results in reduced uptake of Na^+ thereby balancing high K^+ /Na^+ ratio that facilitate the plant to withstand better under salt stressed conditions (Ashraf et al., 2004; Khodair et al., 2008; Nadeem et al., 2014).

Materials and Methods

Methodology

Sub-culturing of rhizobacterial isolates

A total of 6 rhizobacterial isolates provided from the laboratory named (H-33, H-34, H-35, H36, H101, and S-4) were revived in Nutrient Broth media and the isolates were identified as gram-positive and gram-negative following Gram's staining method (Aneja, 2017). Gram's Staining For Gram's staining of isolates, freshly overnight grown isolates were used. Thin smears of bacterial isolateswere made on separate glass slides. Then the smears were air-driedand heatfixed. Smears were covered withcrystal violetfor 30 seconds, then it was washed with distilled water for a few seconds, using a wash bottle. Gram's iodine was used to cover each smear for 60 seconds, then washing was done with 95% ethyl alcohol, drop by drop until no colorflowed from the smear. Slides were washed with distilled water and drained, then safranin was added to the smears for 30 seconds. It was washed with distilled water and blot-dried with the absorbent paper then itwas air-dried, after which it wasready to observe under the microscope.

The were for Zinc solubilizing bacterial isolate by Bunt and Rovira medium amended with insoluble Zinc oxide (ZnO) as source of zinc.The solubilization potential of bacterial isolates was evaluated by plate assay.

S. No	Component	Amount
1	Glucose	10.0g
2	Ammonium sulphate ($(NH4)_2SO_4$)	1.0g
3	Potassium chloride (KCl)	0.2g
4	Potassium dihydrogen phosphate (K_2HPO_4)	0.1g
5	Magnesium sulphate ($MgSO_4.7H_2O$	0.2g
6	Agar	1.5%
7	Zinc oxide (ZnO)	0.1%

pH 7

Table No. 1: Composition of Bunt and Rovira medium

All the isolates were inoculated into bunt and Rovira medium dextrose: 10.0g; (NH4)2SO4: 1.0g; KCl: 0.2g; K2HPO4: 0.1g; MgSO4: 0.2g; pH: 7.0 and insoluble Zn compound (ZnO and ZnCO3: 0.1%; Agar: 15.0 g) and autoclaved at 121°C for 20 min. After autoclaving, it was again transferred to petri pates which were fully sterilized in a hot air oven. Actively growing fresh cultures of each strain were spot-inoculated (2 µL) onto the agar and plates were incubated at 30°C for 24 hours. After a day holo zones occurred on these bacteria inoculated plates. The diameter of the bacterial colony and halo zone around the colony was measured and the values were calculated using solubilizing index formula SI= (Colony diameter + Halozone diameter/colony diameter). where unable three bacteria and three bacteria isolated were able on based of diameter holo zone and named zinc solubilization bacteria (ZSB).

Table No. 1: Composition of Nutrient agar medium

S. No.	Chemicals	Amount
1	Yeast extract	0.3gm
2	Proteose peptone	0.5gm
3	Sodium chloride	0.5gm
4	Agar	1.5gm
5	Distilled water	100ml

pH-7

Salt tolerance test :

All the 6 bacteria isolates come frome isolation were used to check their salt tolerant capabilites. The salt NaCl was used impose salinity stress on the bacterial isolates under in vitro condition on a NA plates.The different 4 concentration of NaCl tested in the present were (250µM, 500µM, 750 µM, 1000 µM and 1M of NaCl)respectively.The plates were prepare with different concentration of NaCl were incubated after bacterial incolation at 30℃ for 24-48 hours. After the abservation for appearance of colonies the bacterial isolates were marked postive and negative for their to grow in different concentration of NaCl.Accoding to visual observation of the growth moderate growth, low growth , very low growth ,high growth, very high growth, and no growth on NA media with different concentration **.**

pH tolerance test

All 11 isolates were for pH tolerance at 10 different concentrations of pH (with (pH 3, pH 4 , pH 5, pH 6, pH 7, pH 8, pH 9, pH 10,)) by observing their growth behavior on media plates. The nutrient agar (NA) media was prepared, supplemented with different pH concentrations of use, pH value increase in use to a 1M NaOH solution and pH value in decrease in use to a 0.1N HCl solution, After the pH was measured, again agar solution were mixed after autoclaving separately, pour NA media into each plate , which were fully sterilized in a hot air oven. Actively growing fresh cultures of each strain were spot-inoculated (2 µL) onto the agar and plates were incubated at 30°C for 24 hours and again The plates were observed after 24 hours and the growth of bacteria was checked.

Assay for production of siderophore

Siderophore production is a biocontrol property of PGPR. For checking the siderophore production ability the overnight raised fresh cultures were spot inoculated onto CAS agar media (Schwyn and Neiland ,1987). It was again poured in petri pates which were fully sterilized in a hot air oven. The isolates were spot inoculated (2 µL) onto the CAS agar media plates and incubated for 5 days in incubated at 30°C for 24 hours.The plates were observed after every 24 hours 5 days and the growth of bacteria and size of orange color zone developed was measured using centrimeter scale.

CAS Agar media

A. Blue Dye solution (1000mL)		
	Chemicals	**Quantity**
Solution	CAS Distilled water	0.6 g 500 mL
Solution 2	$FeCl_3.6H_2O$ HCL (10Mm)	0.27 g 100 mL
Solution 3	CTAB Distilled water	0.73 g 400 mL

Mix Solution 1 with 90 ml of Solution 2, Then mix with Solution 3, Solution should now be a blue color.

B. Mixture solution		
Chemicals		**Quantity**
Minimal media (1000Ml)	KH_2PO_4 NH_4Cl NaCl	30 g 100 g 50 g 1000 g
20% Glucose Solution 1000mL	Glucose Distilled water	200 g 1000 g
Casamino acid (270 mL)	Casaminoacid solution Distilled water	30 g 270 g

For CAS agar preparation, 500 mL minimal media was added to 3750 mL distilled water in which 75 g agar was added and then autoclaved. Autoclaved media was cooled to 50℃. In this 150 mL of sterile casamino acid solution and 50mL of 20% glucose solution was added to minimal solution. Then 500 mL blue dye solution was added and mixed thoroughly. It was poured in petri plates 25-30 mL each.

Heavy metal tolerance Test

Determining heavy metal sensitivity and identifying the heavy metal tolerant isolates amongst the total population of salt tolerant rhizospheric bacterial isolates obtained from salinity test were inoculated on NA plate amended with using concentration of different type of heavy metal mentioned in under in vitro condition. The plates were prepared by mixing the desired amounts of heavy metal salt in NA media and then plates containing these amended media were incubated at 30°C for 24-48 hours. After the observation for appearance of colonies, the growth of bacteria was , measured using centrimeter scale, for their ability to grow in different concentration of cadmium(Cd), lead (Pd), Arsenic (Ar) and mercury (Hg). According to the visual observations of the growth obtained after 12 and 24 hours of incubation the bacterial isolates were graded for high growth, moderately high growth, low growth, very low

growth and no growth on NA media amended with various concentrations of Cd, Pb and Hg.

Serial No.	Metal salt used	Name of Heavy metal	concenttation
1	Cadmim Choride	Cd	100 µm
			200 µm
			300 µm
2	Lead	Hg	250 mM
			500 mM
			750 mM
			1000 mM
3	Arsenic	Ar	10 mM
			20 mM
			30 mM
4	Mercury Chloride	$HgCl_2$	20 µmL
			40 µmL
			80 µmL

Table : Name of metal salt with their concenttation

Fungicide tolerance test

To obtain fungicide- tolerance bacteria isolates and determine their fungicide sensitivity NA plate method supplemented with different concentration of fungicide was used. All the bacterial isolates come from isolation were used to check their fungicide tolerant capabilities. The fungicide was used to impose salinity stress on the bacterial isolates under in vitro condition on NA plates. The different concentration of fungicide tested in the present in investigation were 500µg, 1000µg, 2000µg, 3000µg, 5000µg and 0.M of fungicide respectively. The NA plates amended with different concentration of fungicide were incubated after bacteria inoculation at 4 day in 30ºC for 24-48 hours. The plates observed after 24 hours and the growth of bacteria was checked using centrimeter scale.

Results

All six bacterial isolates provided from the laboratory were cultured, characterized and tested for various plant growth promoting properties in presence and absence of salinity stress and the results obtained are tabulated below.

Gram's staining

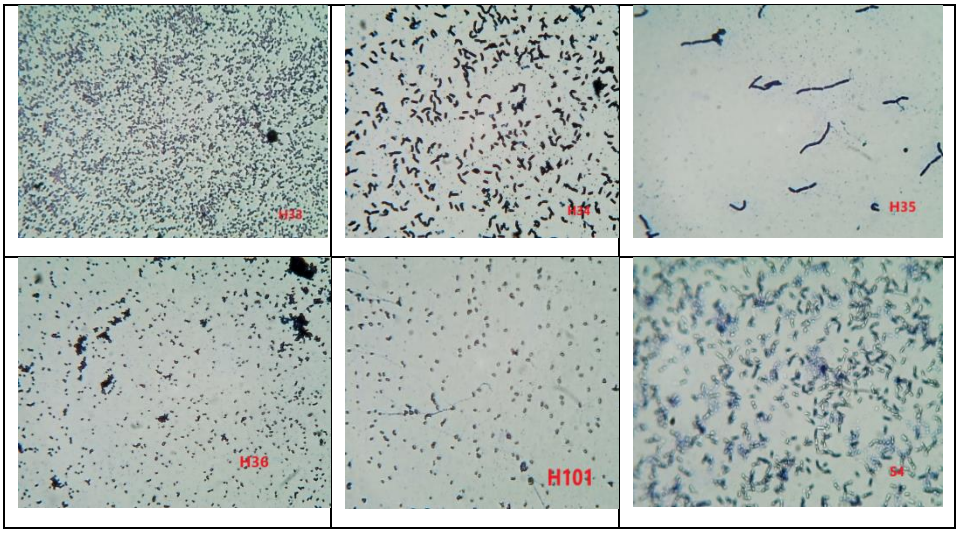


Figure 4.1. Gram's staining of bacteria isolates: (H-33, H-34, H-35, H36, H101, and S-4).

Nature and shape of bacteria isolate

Table 1: Nature and shape of bacteria isolate

Isolates	**Nature**	**Shape**
H-33	Gram positive	Round
H-34	Gram positive	Rod
H-35	Gram positive	Rod
H-36	Gram positive	Round
H-101	Gram positive	Comma
S-4	Gram positive	Rod

Zinc solubilization Test

Fig 1. Solubilization of insoluble zinc compound by zinc solubilizing bacteria

Table 2 : Shows the growth developed all the isolates of the rhizospheric

Isolate No	Colony diameter	Zone+colony	Zone diameter(cm)
H33	1.3	3	1.7
H34	1.8	2.8	1
H35	0	0	0
H36	0	0	0
H101	1.6	2.5	0.9
S4	0	0	0

Zinc solubilization was observed up to 5 day beyond which negligible change was observed in all the assay plate. The 6 bacteria isolates obtained were named as (H-33, H-34, H-35, H-36, H-101 and S-4), were unable to form halo zones. Only 3 were able to produce a clear zone around their colonies on the solid medium with zinc oxide ofter at 30°C**.** The diameter of the zone was also measured using a scale**.** The Largest solobilization zone volue were observed in H-33 (1.7cm) and maxium solobilization zone volue H-101 (1cm), Lowest diameter was recorded volue H-34 (0.9cm) .

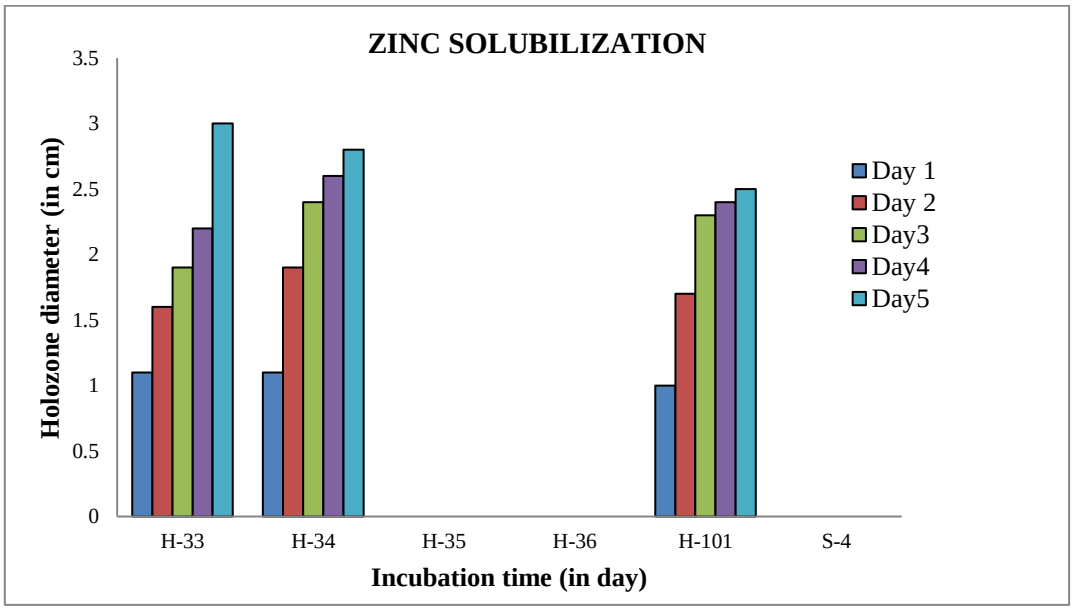


Figure 2 : **Shows the growth developed all the isolates of the rhizospheric**

Salt tolerance test

All the 6 culture isolates selected were tested for their salt tolerance potential on NA plates amended with (0.M, 250µM, 500µM, 750µM, 1000µM), concentration making it selective medium.Based after the visible growth of colonies, the isolates on these plates they were scored positive or negative for their ability to tolerate against salinity that particular various concentration of NaCl, after 24 hours of incubation.

The control plate where amendment of NaCl was made showed full growth. Based on plate visible growth pattern a hypothetical scale was developed to mark the growth and tolerance of the isolates against salinity.

Screening of the rhizobacterial isolates for salinity tolerance

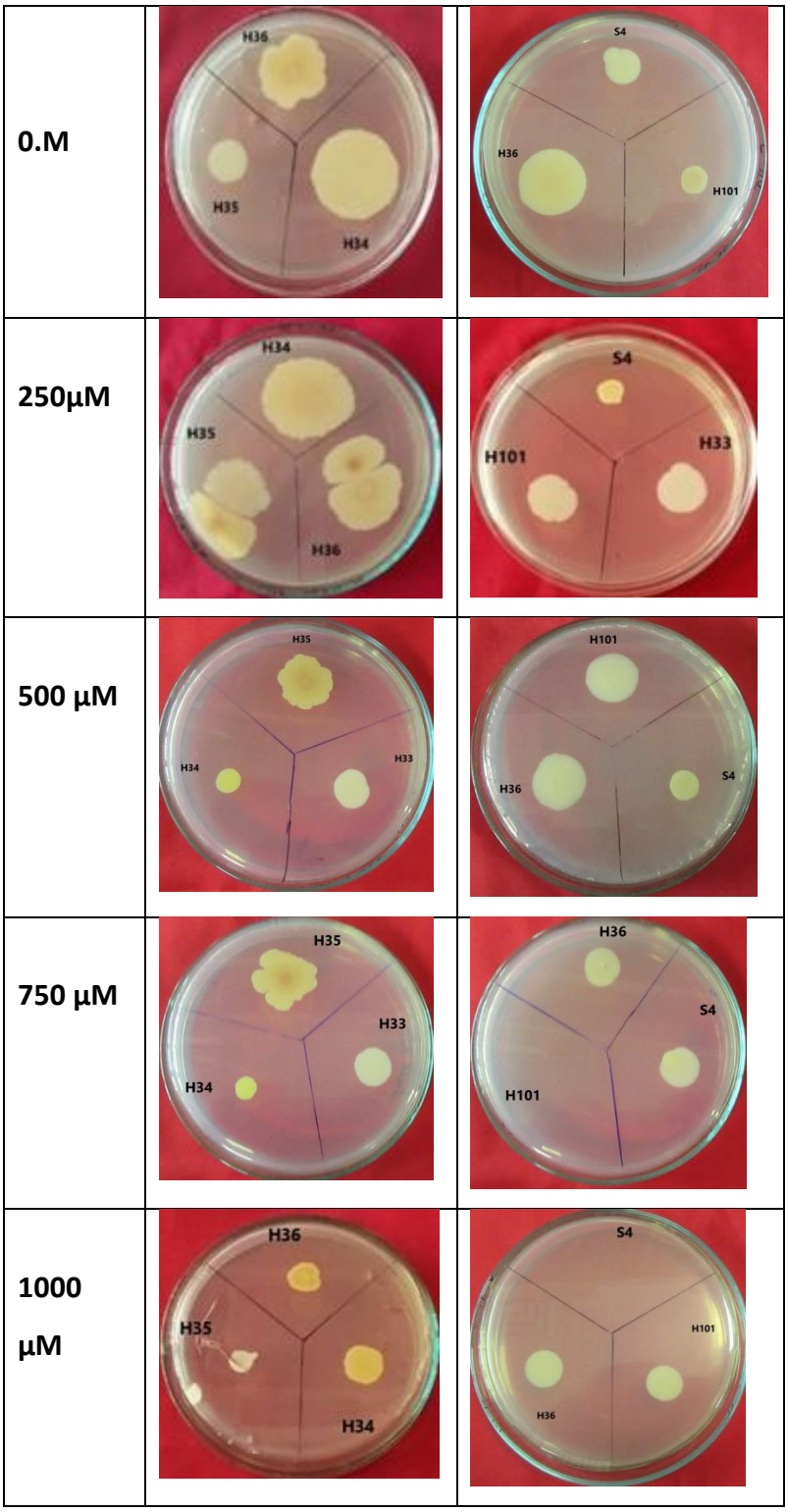


Figure 3 :Colonies of rhizobacterial isolates obtained on NA plates (control and NaCl) After 24 hours of 5 day incubation

Isolates H-33

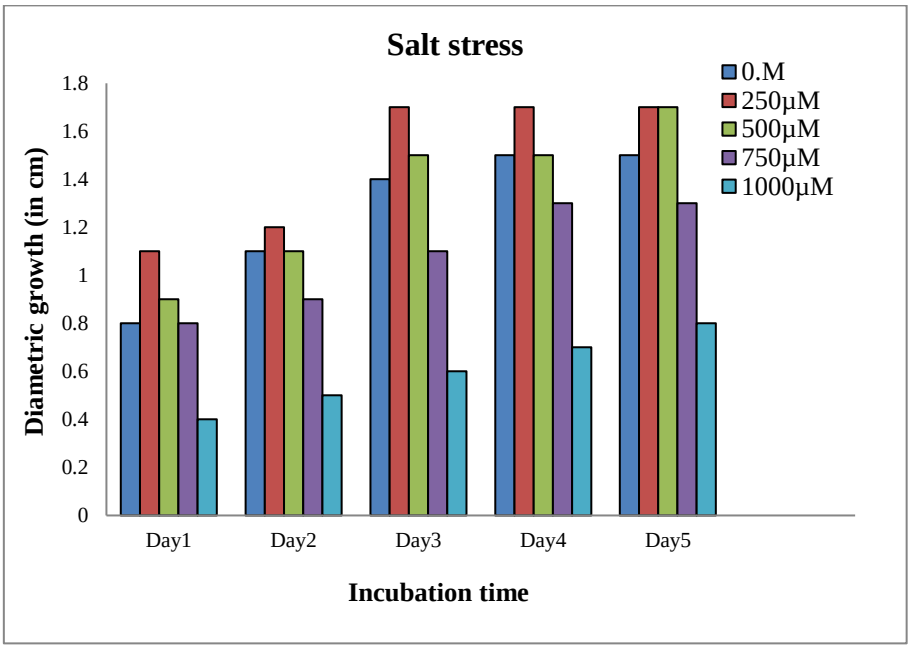


Fig 4: Growth bahavior of H-33 at various salinity levels

Isolate H-34

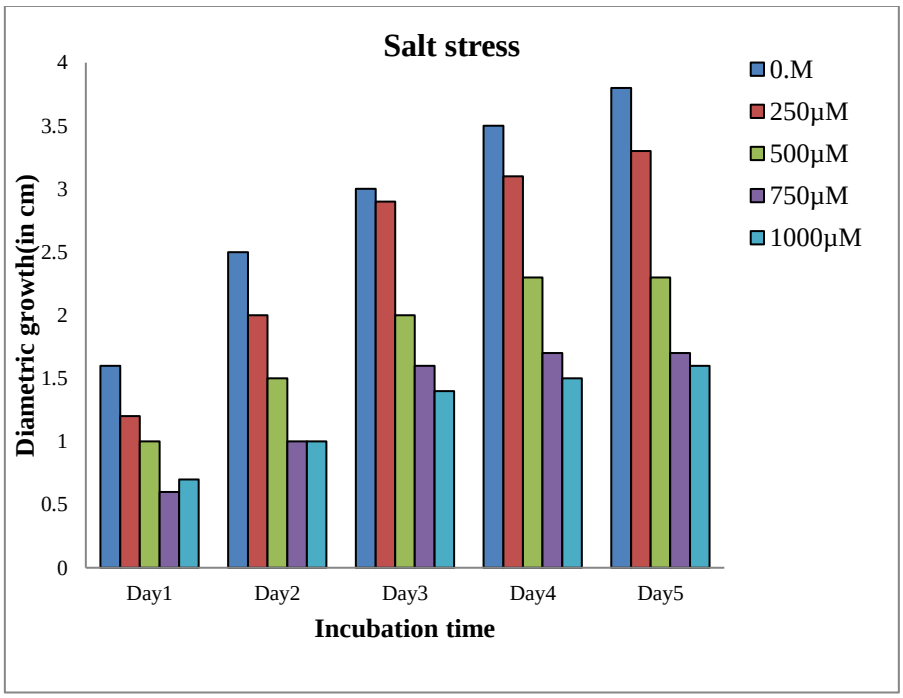


Fig 5: Growth bahavior of H-34 at various salinity levels

Isolates H-35

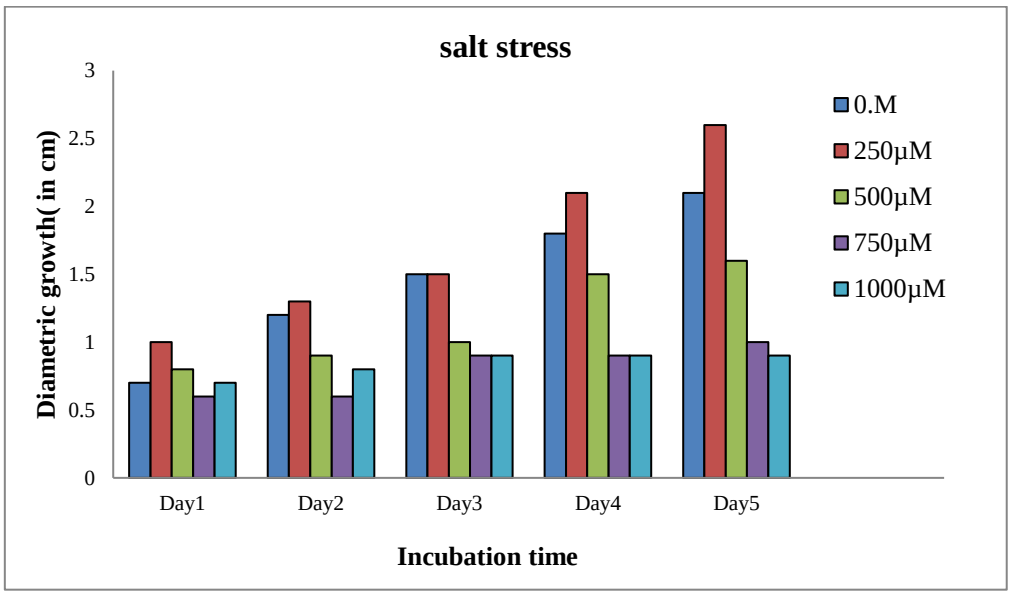


Fig 6: Growth bahavior of H-35 at various salinity levels

Isolate H-36:

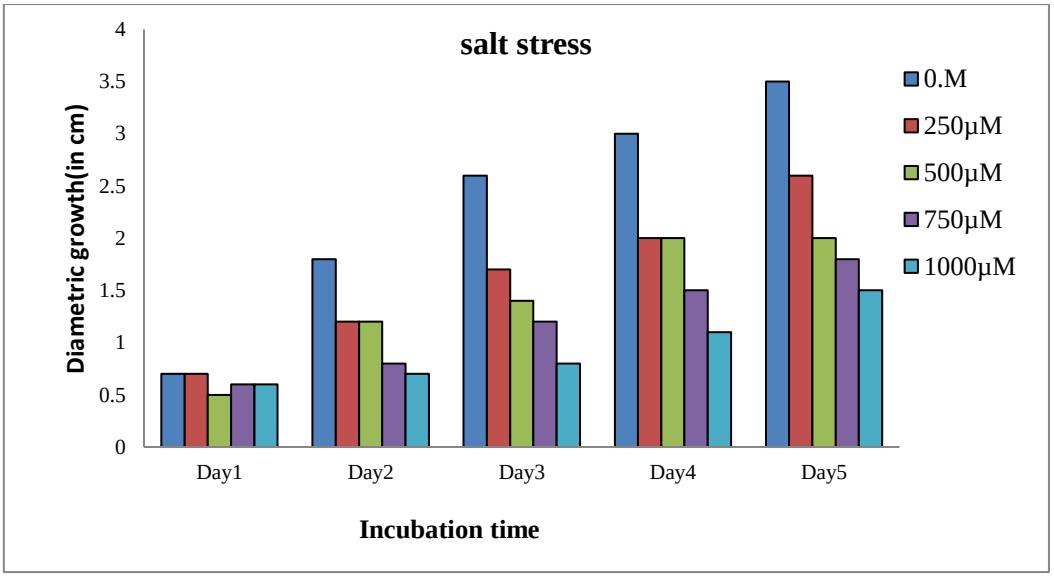


Fig7: Growth bahavior of H-36 at various salinity levels

Isolate H-101

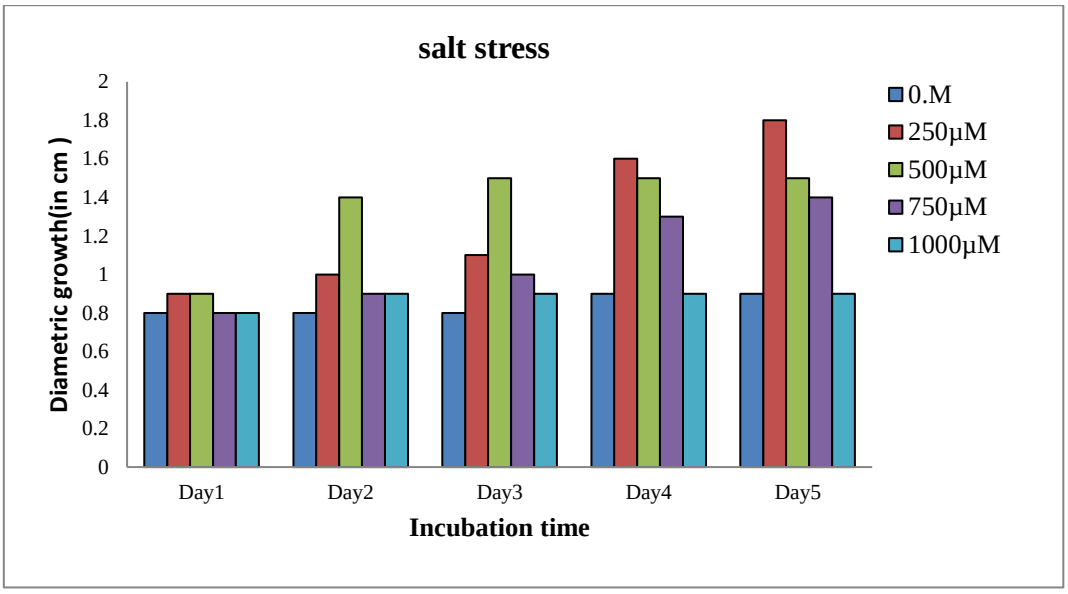


Fig 8: Growth bahavior of H-101 at various salinity levels

Isolate S-4

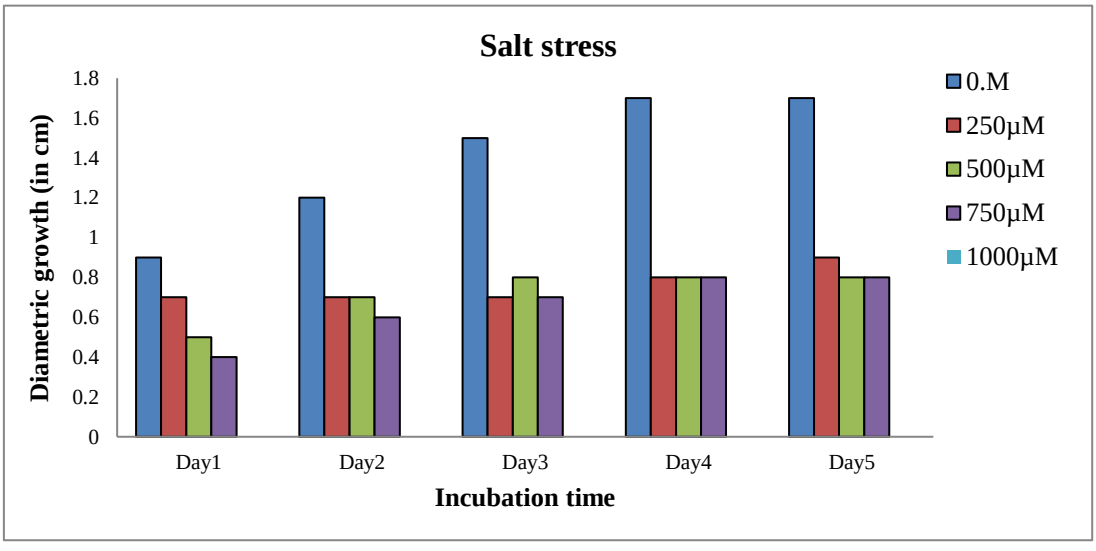
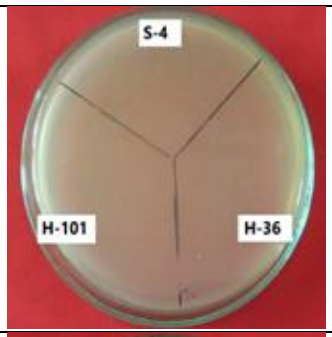
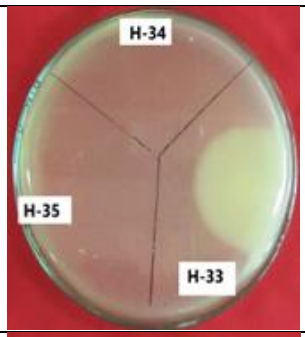
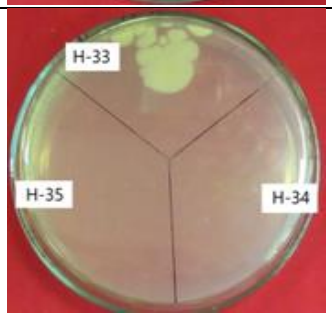
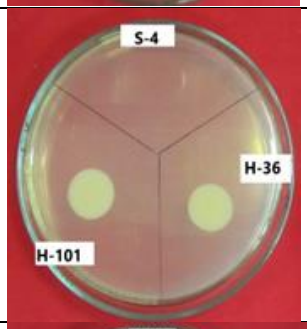
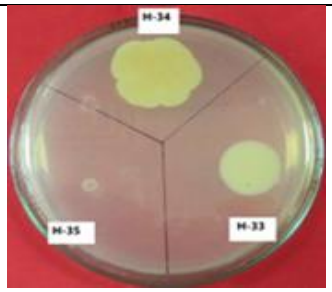
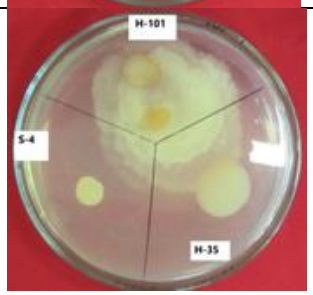
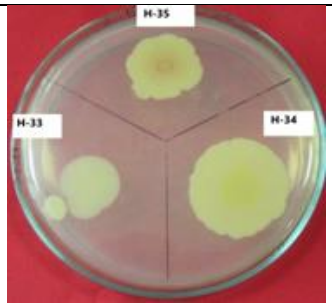
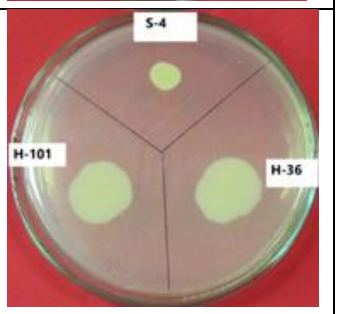
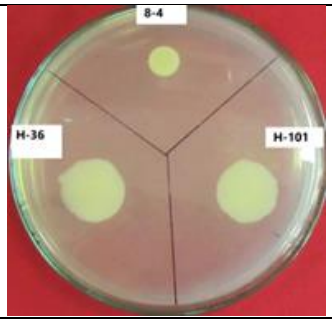
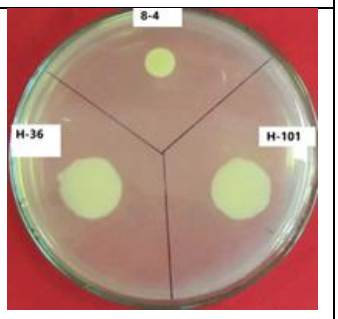


Fig 9: Growth bahavior of H-34 at various salinity levels

pH tolerance test

pH=4		
pH= 5		
pH=6		
pH=7		
pH=8		

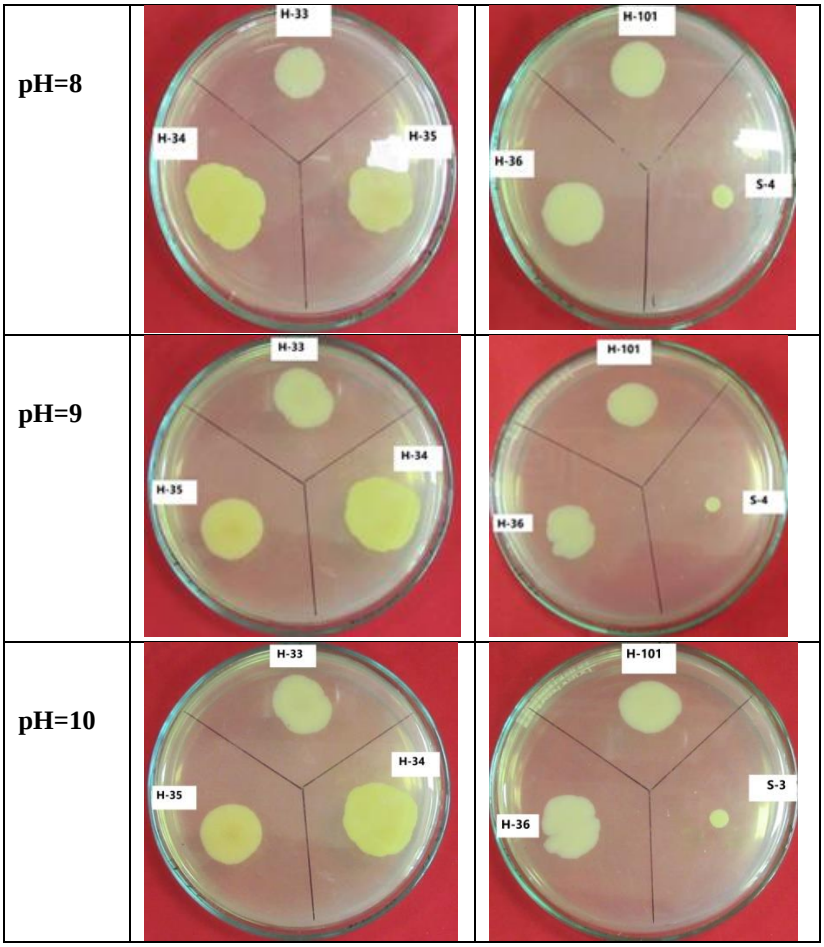


Fig 10:Colonies of rhizobacterial isolates obtained on NA plates different pH After 5 day of incubation

pH to tolerance of the rhizobacterial isolates

All the six isolates bacteria also tested for their pH tolerance potential on NA plate amended with (pH 3, pH 4 , pH 5, pH 6, pH 7, pH 8, pH 9, pH 10), concentration making it selective medium. Based on observation of visible growth of the isolates on these plates they were scored full growth and maximum tolerance, scored positive or negative for their ability to tolerate against salinity that particular various concentration of pH, after 24 hours of incubation. There is not isolates bacteria growth at pH 4, because NA media on pH 4 , was not solidified thoroughly. And pH 3, was not solidified at all on NA media, the pH 4, plate where amendment of pH was not solidfiled in, On plate growth pattern a hypothetical scale was developed to mark the growth and tolerance of the isolates against salinity.

Isolate H-33

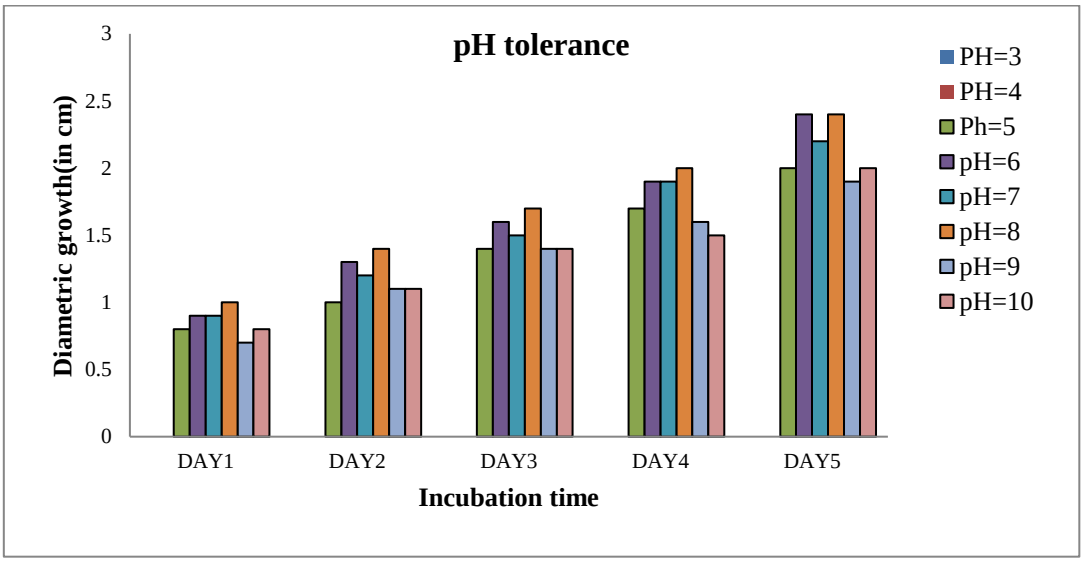


Fig 11: Growth bahavior of H-33 at various pH

Isolate H-34

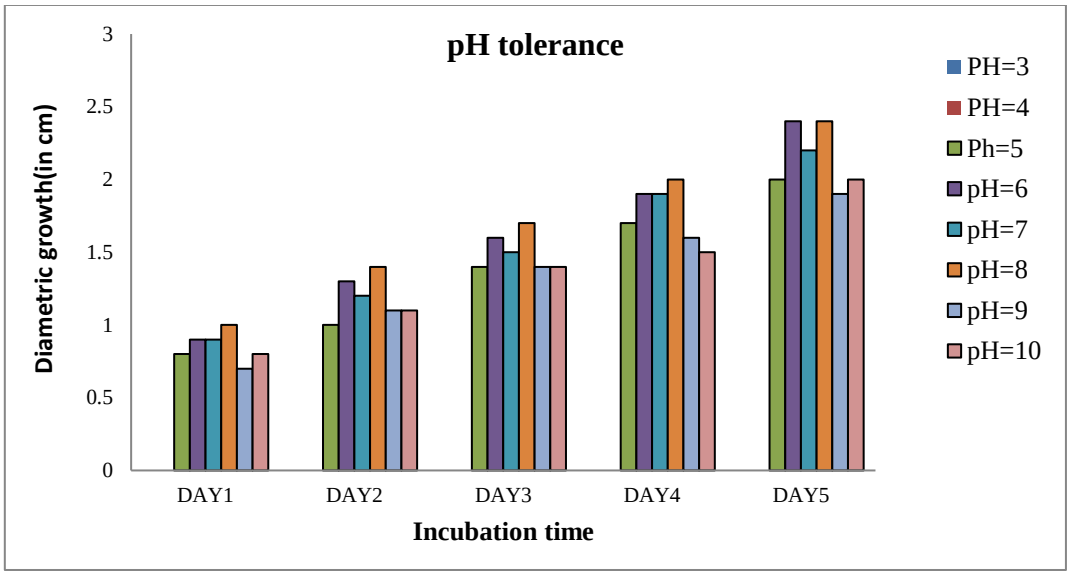


Fig 12: Growth bahavior of H-34 at various pH

Isolate H-35

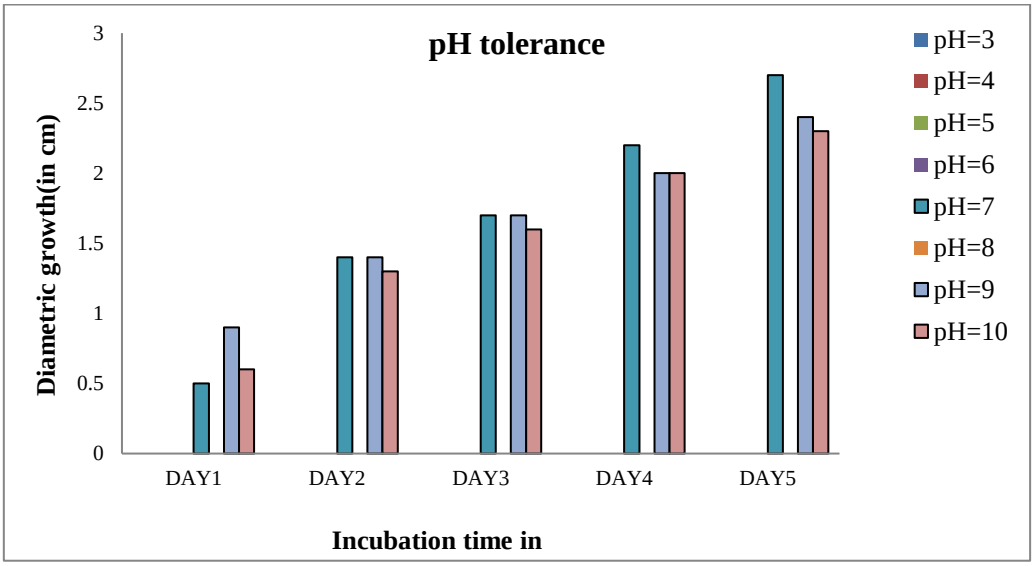


Fig 13: Growth bahavior of H-35 at various pH

Isolate H-36

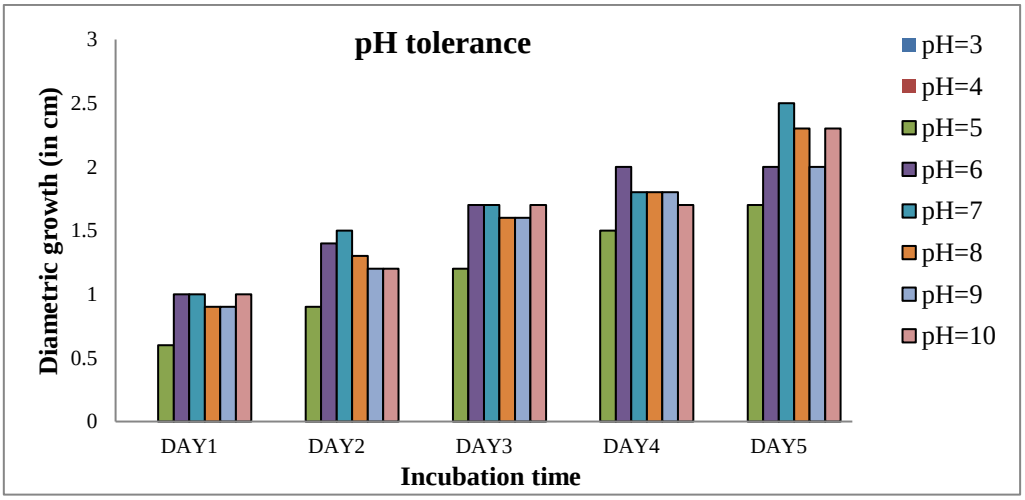


Fig 14: Growth bahavior of H-36 at various pH

Isolate H-101

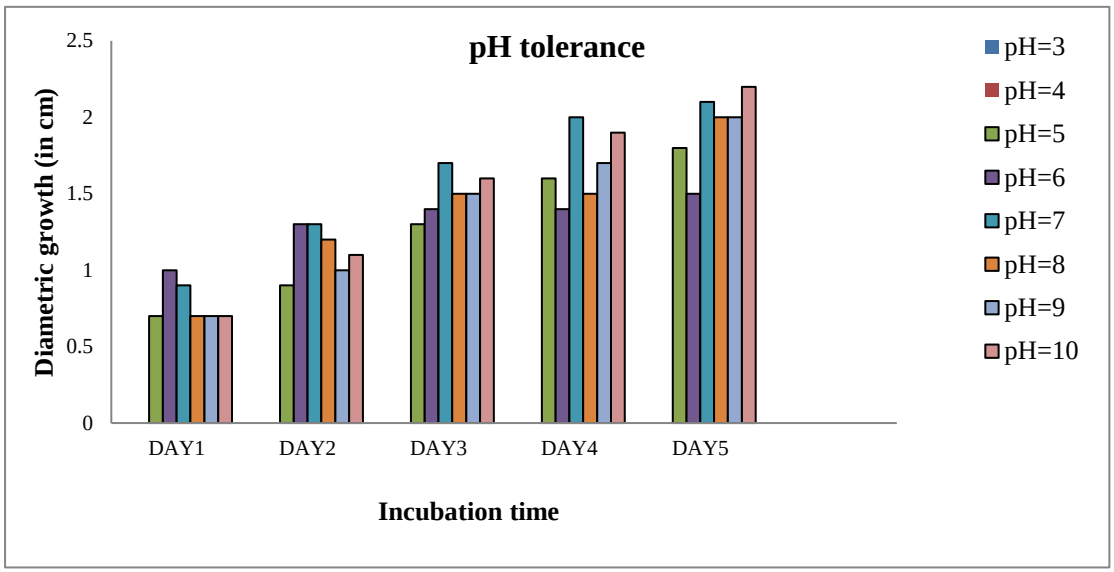


Fig 15: Growth bahavior of H-101 at various pH

Isolate S-4

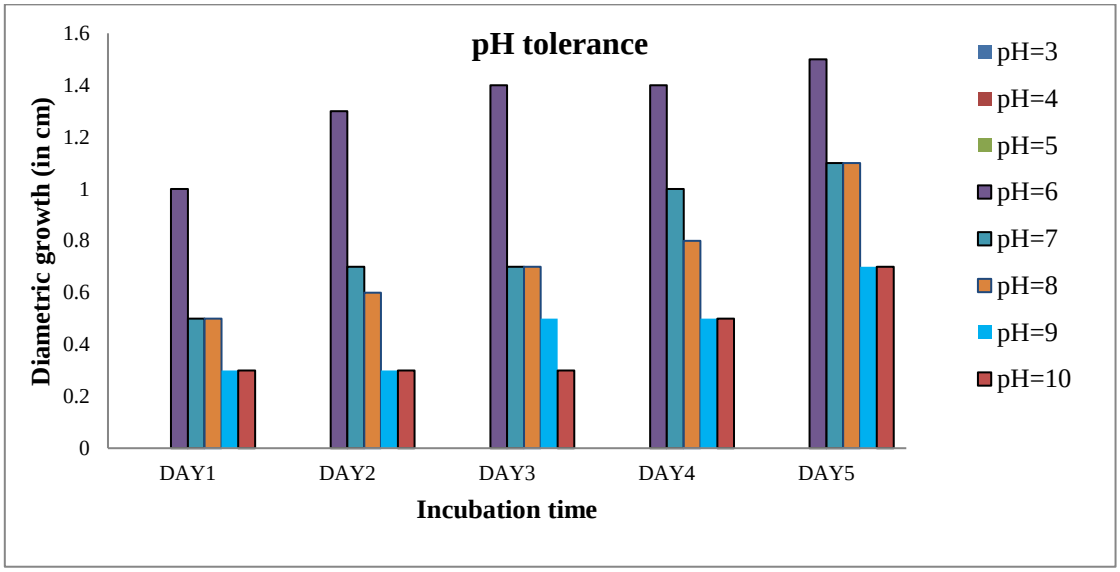


Fig 16: Growth bahavior of S-4 at various pH

Siderophore production by the bacterial isolates

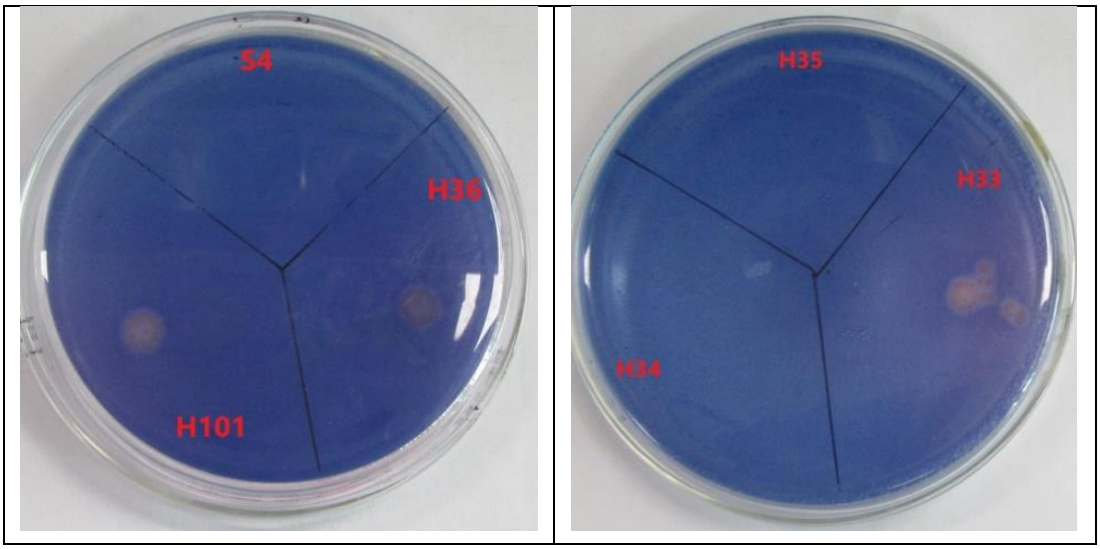


Figure 17: Colonies of the rhizobacterial isolates on CAS plates exhibiting siderophore production

Table 3: Siderophore production by bacterial isolates

bacterial isolates	colony +Halo zone diameter	colony diameter	halo zone diameter
H33	0.5 cm	0.8 cm	0.3 cm
H34	0	0	0
H35	0	0	0
H36	0.6 cm	0.8 cm	0.2 cm
H101	0.7 cm	1	0.3 cm
S4	0	0	0

All the 6 isolates were tested for production of siderophore on CAS agar plates media. While 3 isolates were not production siderophore , i.e formation of lighly orange color zone fig .The growth of 5 day. Diameter of zone was measured using a sacle For then the isolate bacteria all growth Only three were able, to the maximum zone was equal isolate bacteria were observed in H101(0.3) and (H33). The minimum diameter zone was recorded for isolate H36 (0.2).

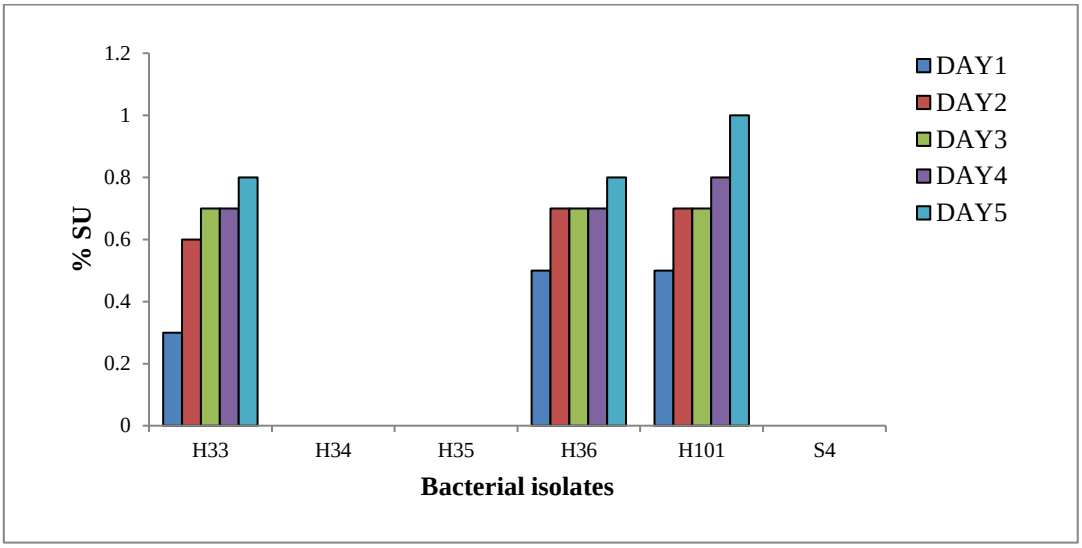


Figure 18: Siderophore production bacterial isolates after 48 hours of growth

There were 6 bacterial isolates, whose quantitative estimation was done for siderophore production figure. The result are presented in figure. All the isolates except isolates bacteria H-33, H-36, and H-101, were observed to posistive for siderophore production with zone of activity after 1 to 5 day. For then the isolate bacteria all growth Only three were able to the hightes growth of isolate bacteria were abserved in H-101 and medium growth H-33 (The lowest growth of isolates bactrial H-36.

Heavy metal tolerance of the rhizobacteria isolates

All the 6 isolates bacteria were also evaluated for tolerance against another important abiotic stress very prominently in soil .The heavy metals tested in this study were Cadmium (Cd), Lead (Pd) Mercury (Hg). Here again the heavy metal stress was imposed by amending the NA plates with specific concentration of different salt of the heavy metal tested and Actively tested and allowing the isolate to grow on at incubated condition 30°C for 24 hours.A control NA plate without any heavy metal amendment was also used for growing the isolates to have a comprative evaluation.

Here again the control plates of heavy metal was made showed full growth. Based on this visible growth pattern the diameter of zone was measured using a sacle , the growth and tolerance of the isolates against heavy metal.

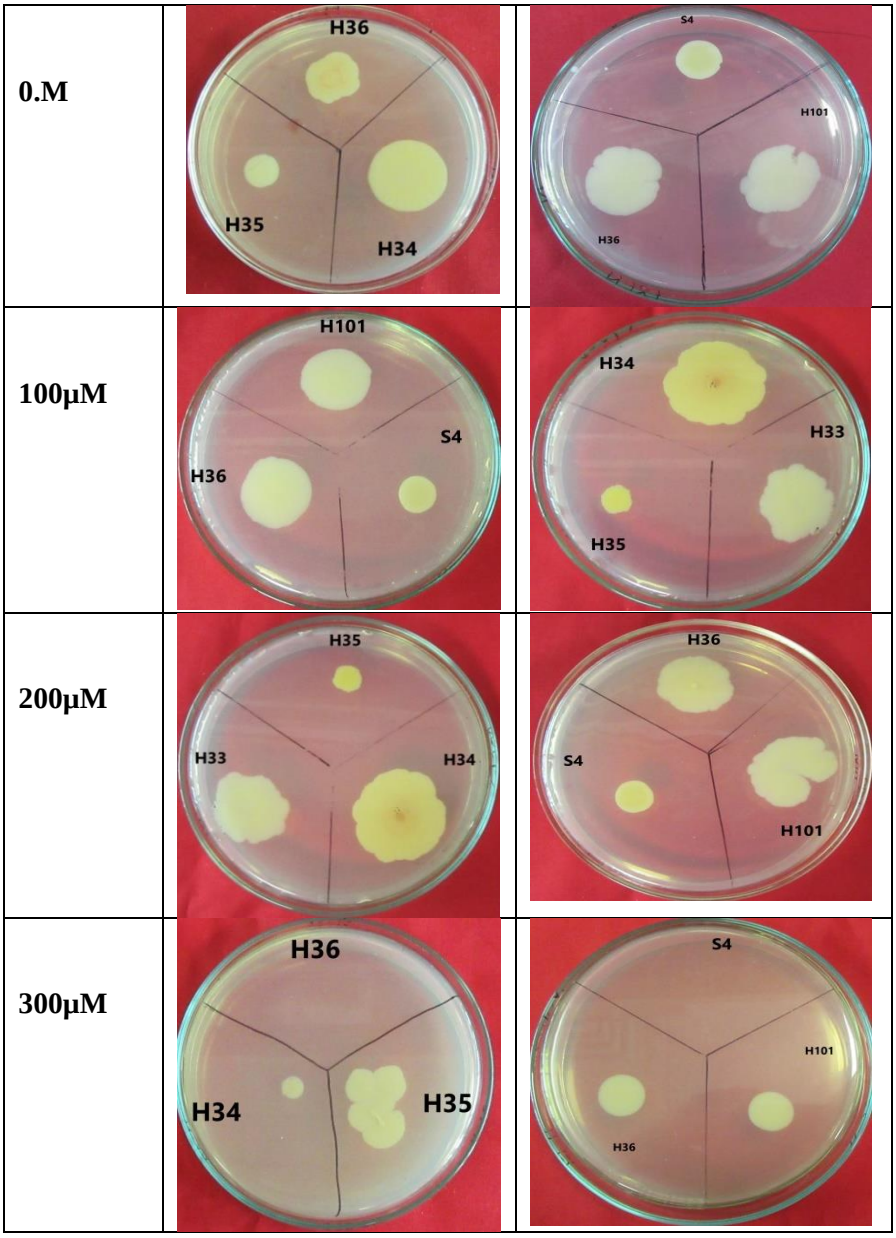


Figure 19: Colonies of rhizobacterial isolates obtained on NA plates (control and $CdCl_2$) after 24 hours incubation.

A total six isolates bacteria against Cd stress again two salt of Cd (Cadmium chloride – $CdCl_2$ at different concentration) were used. Cadmium chloride were tested at 10 µM, 20 µM, 30 µM, and 0.M concentration. Based on the observation of each after 24 hours of incubation it was observed that incase of Cadmium chloride

Isolates H-33

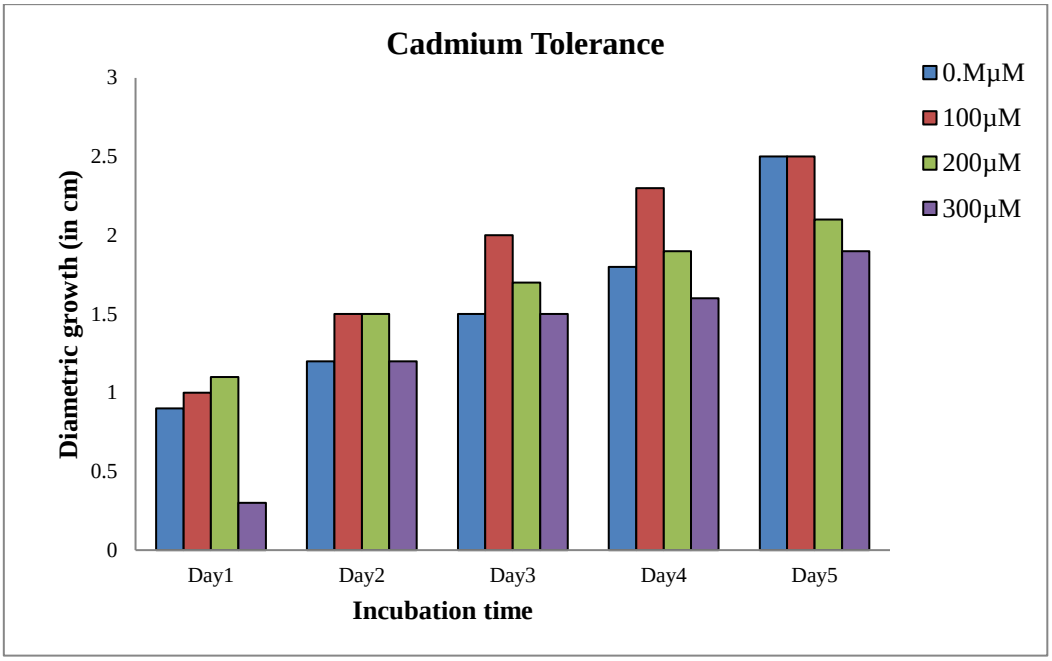


Fig 20: Growth bahavior of H-33 at various Cd levels

Isolates H-34

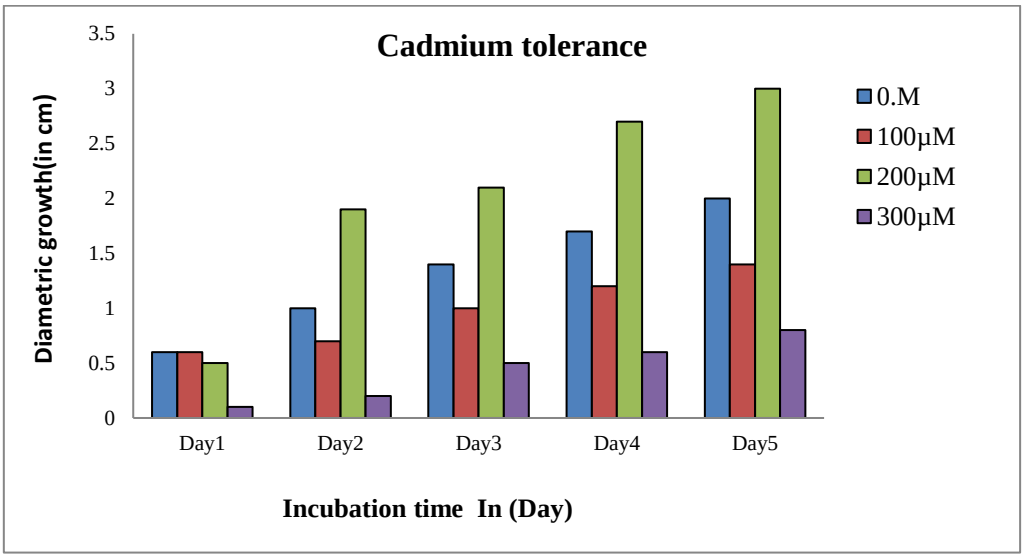


Fig 21: Growth bahavior of H-34 at various Cd levels

Isolates H-35

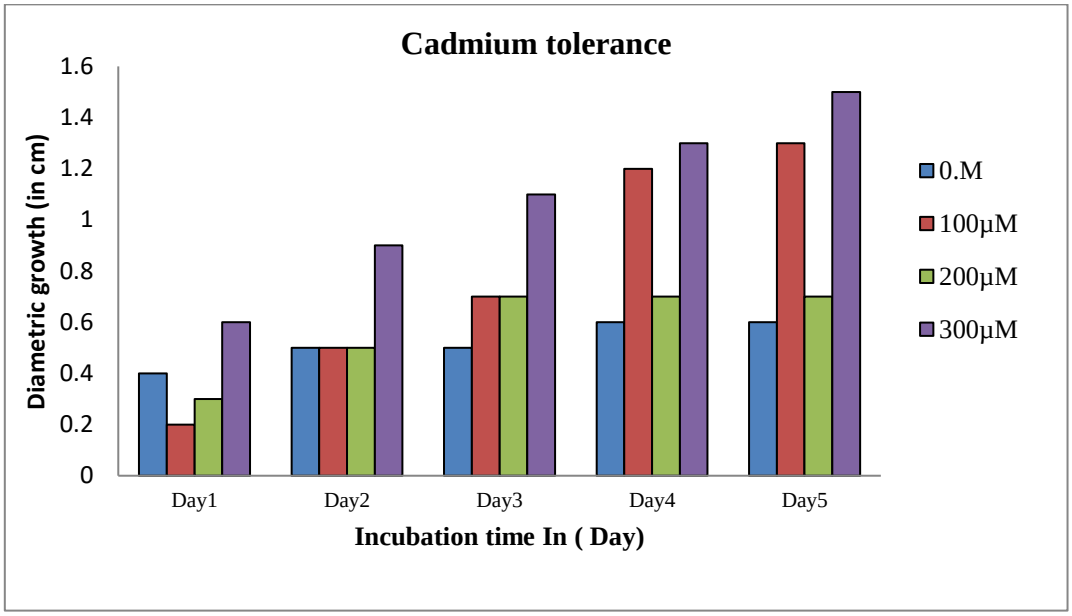


Fig 22: Growth bahavior of H-35 at various Cd levels

Isolates H-36

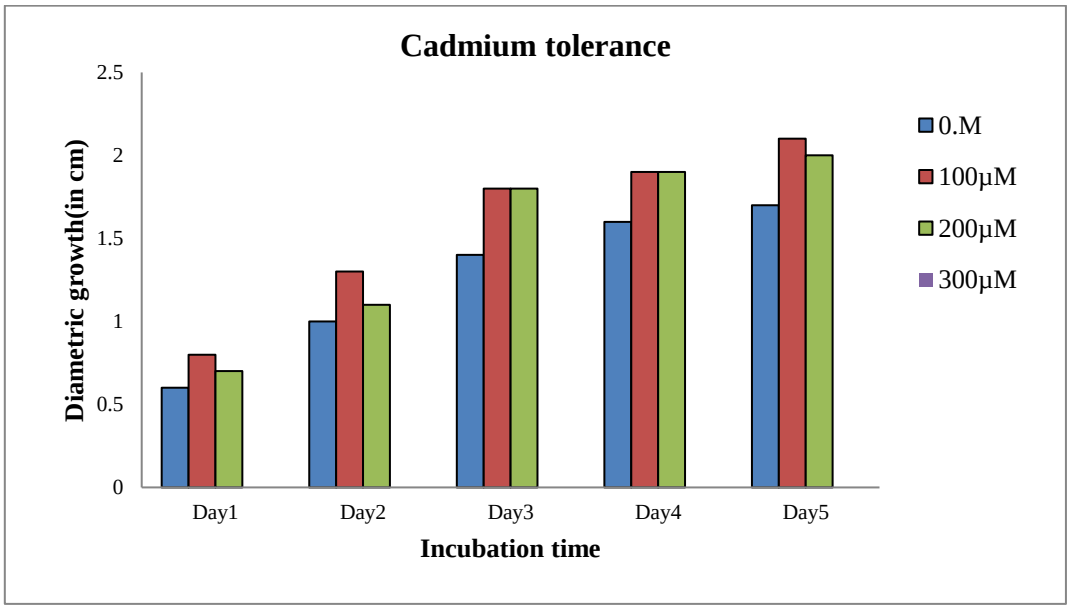


Fig 23: Growth bahavior of H-36 at various Cd levels

Isolates H-101

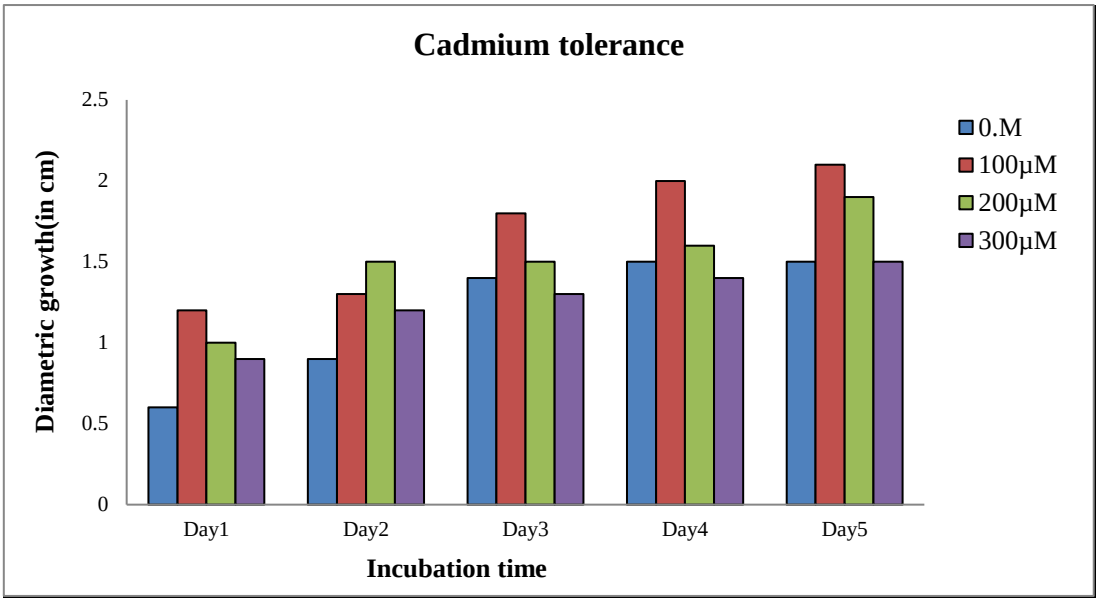


Fig 24: Growth bahavior of H-101 at various Cd levels

Isolates -S4

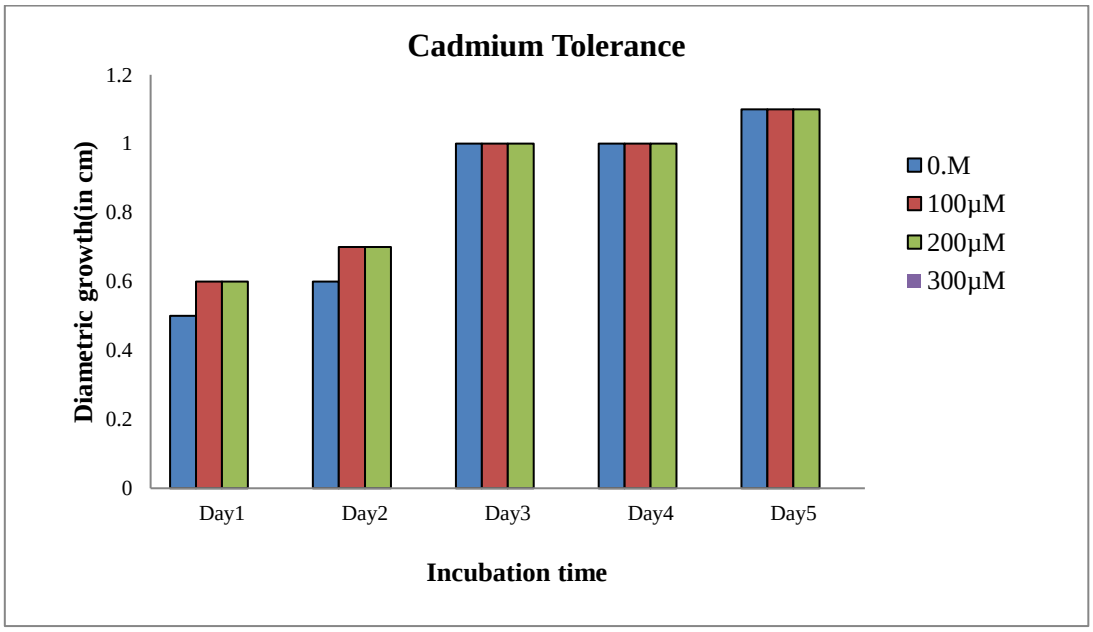


Fig 25: Growth bahavior of S-4 at various Cd levels

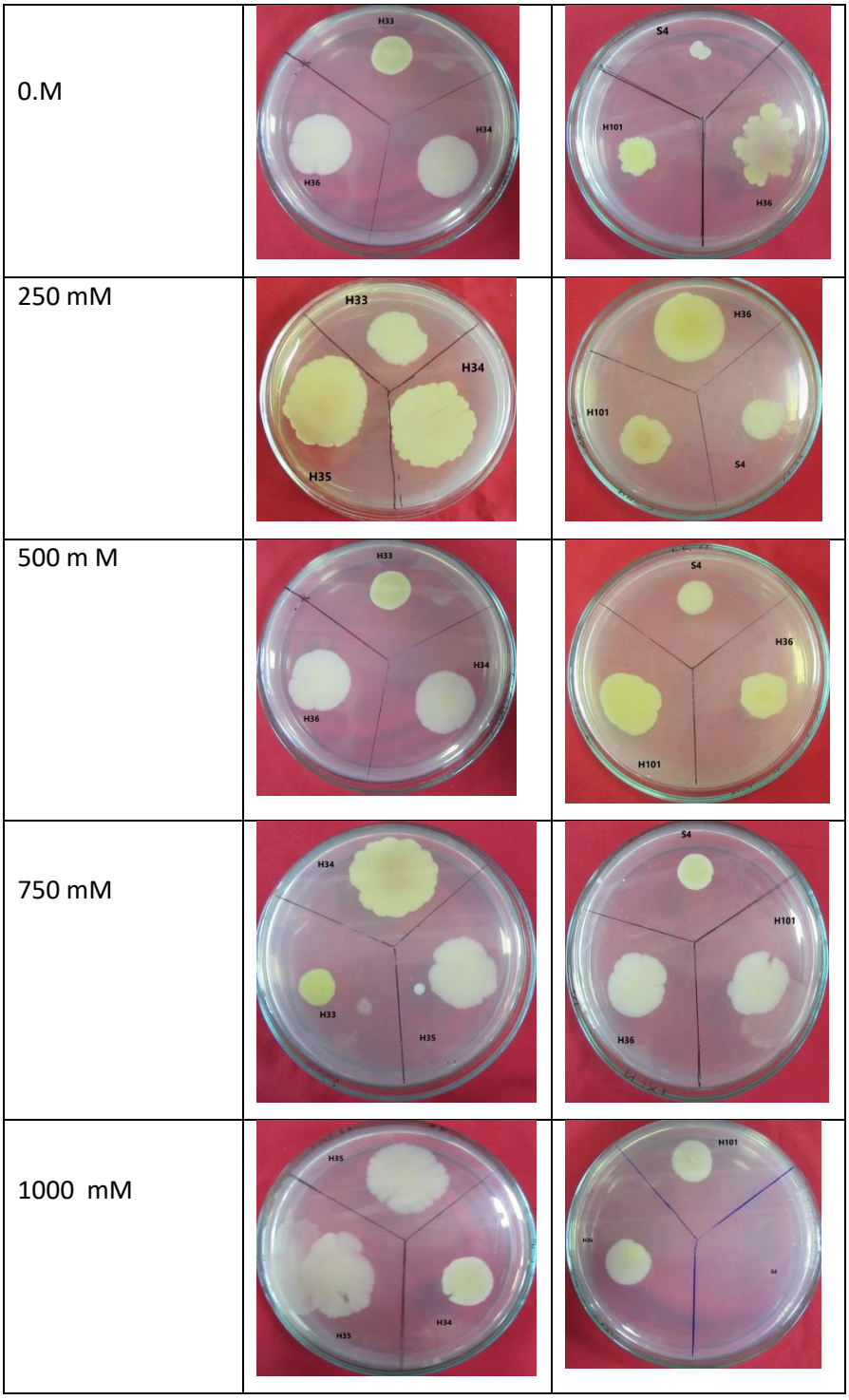


Figure 26: Colonies of rhizobacterial isolates obtained on NA plates (control and Pb) after 24 hours of incubation in growth of 5 day

A six isolates bacteria against Pb stress two salt of Pb (Lead nitrate –$Pb(NO)_2$ at different concentration) were used. Lead nitrate was tested at 0.M, 250 mM , 500 mM, 750 mM, 1000

mM, concentration respectively. Based on the observation of each after 24 hours of incubation it was observed that incase of lead nitrate all the six isolates exhibited moderate growth upto 200mm where at 250 isolates full growth show, ?

Isolates H-33

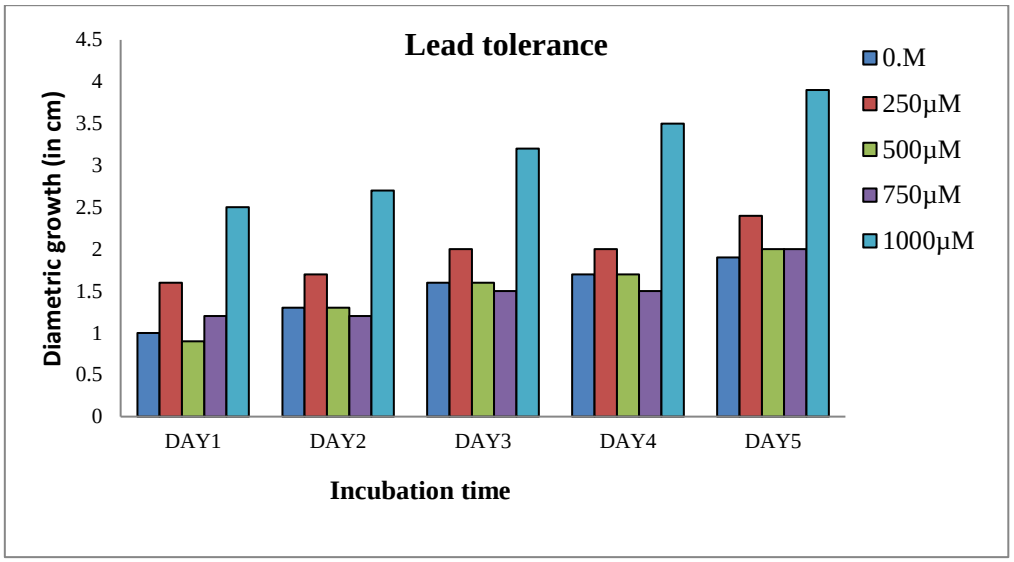


Fig 27: Growth bahavior of H-33 at various Pb levels

Isolates H-34

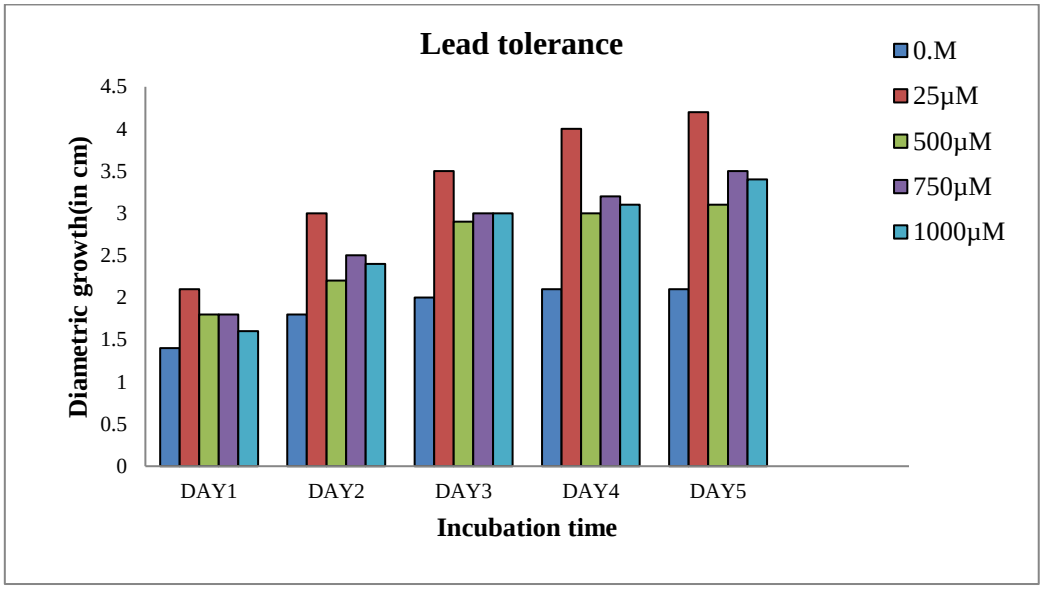


Fig 28: Growth bahavior of H-34 at various Pb levels

Isolates H-101

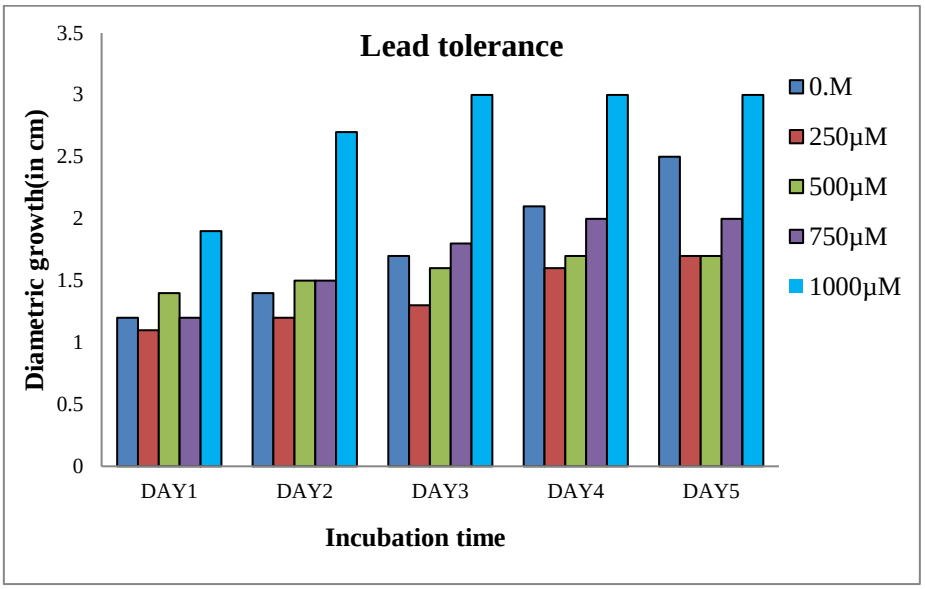


Fig 30: Growth bahavior of H-101 at various Pb levels

Isolates S-4

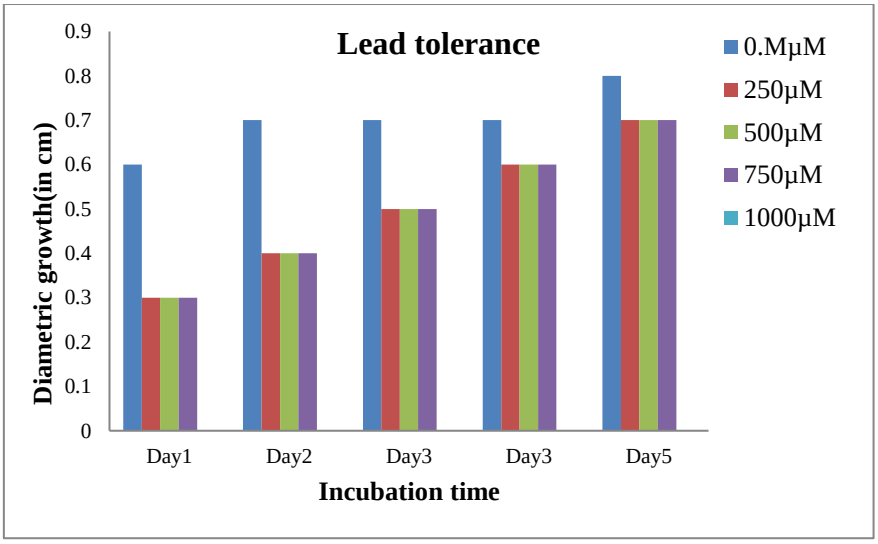
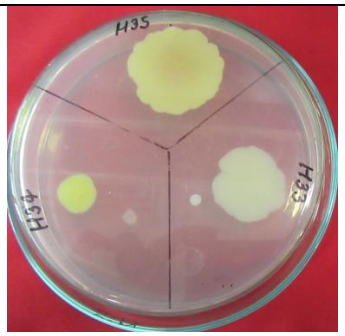
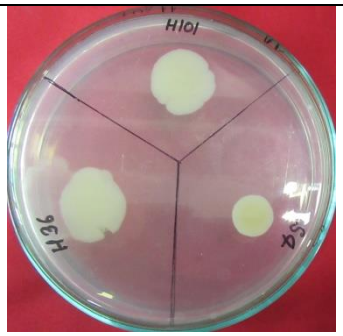
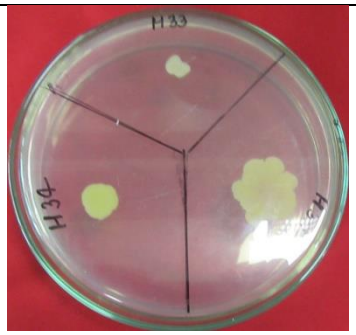
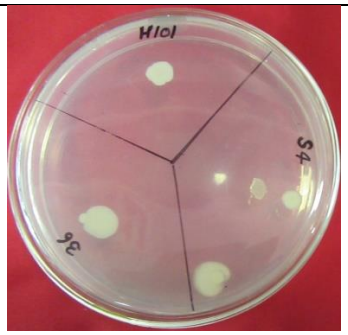
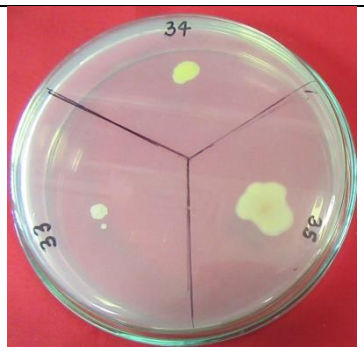
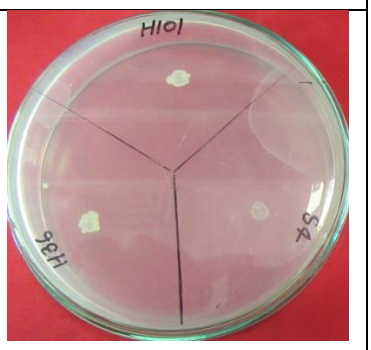
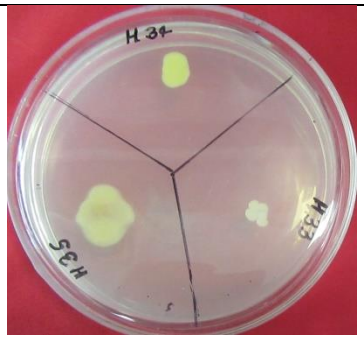
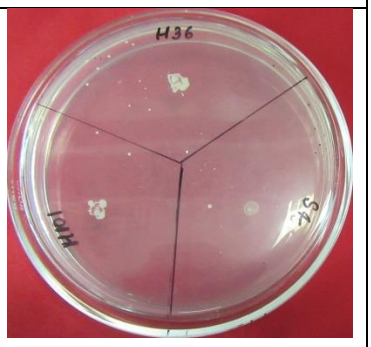


Fig 31: Growth bahavior of S-4 at various Pb levels

Screening of Arsenic tolerance -

Control		
10 µM		
20 µM		
30 µM		

Figture 32: Colonies of rhizobacterial isolates obtained on NA plates (control and As) after 24 hours of incubation in growth of five day

All the six isolates bacteria were tested against Ar stress using Arsenic (Ar). Arsenic was tested only at 10µM concentration. Based on the observations of growth after 24 hours of inculation it was observed that at 10µM out of the six isolates only 1 exhibited moderate growth while six isolates growth low growth wgereas 3 isolates showed no growth.

stress using Arsenic (Ar). Arsenic was tested only at 10µM concentration. Based on the observations of growth after 24 hours of inculation it was observed that at 10µM out of the six isolates only 1 exhibited moderate growth while six isolates growth low growth wgereas 3 isolates showed no growth.

Isolates H-33

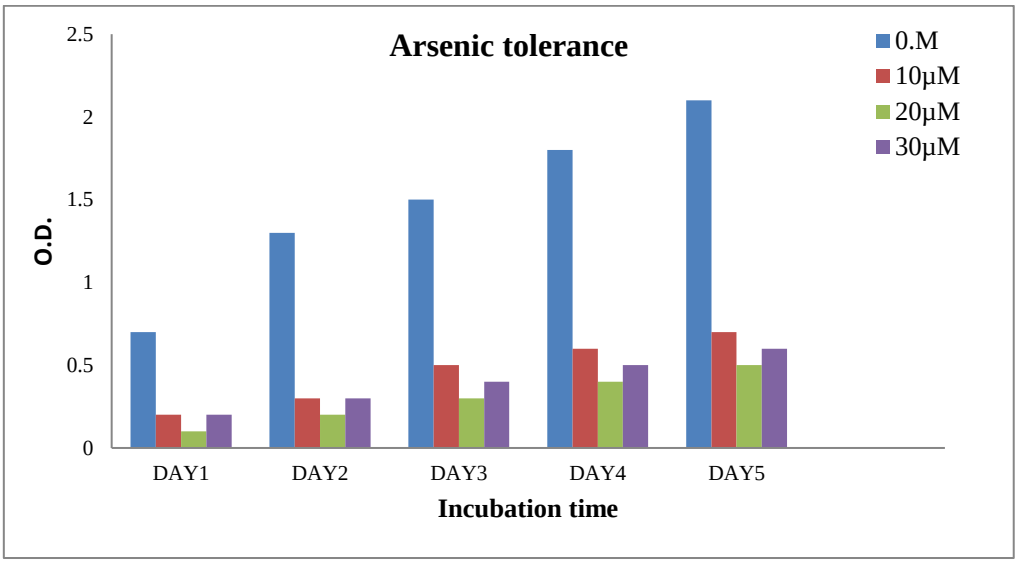


Fig 33: Growth behavior of isolates H-33 under various conc. of Arsenic

Isolates H-34

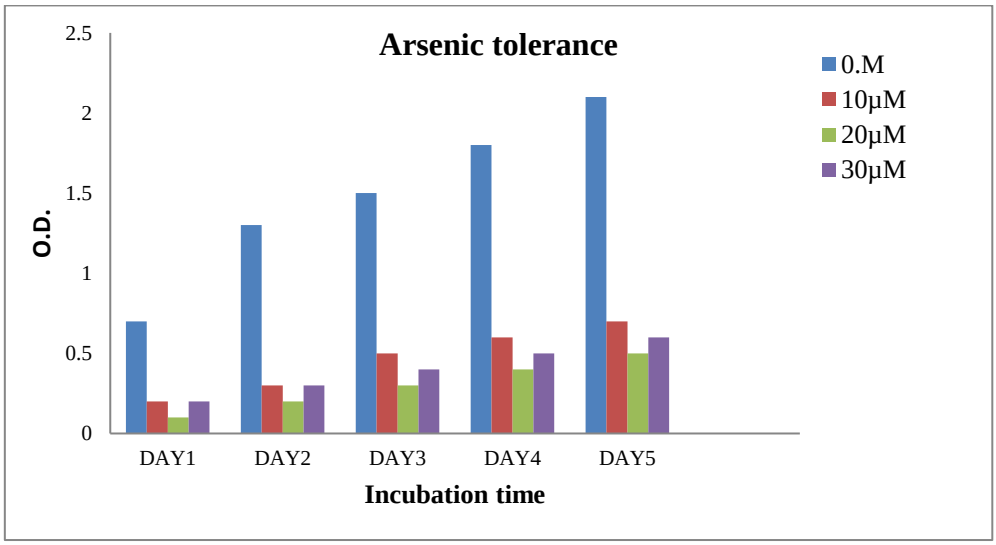


Fig 34: Growth behavior of isolates H-34 under various conc. of Arsenic

Isolates H- 35

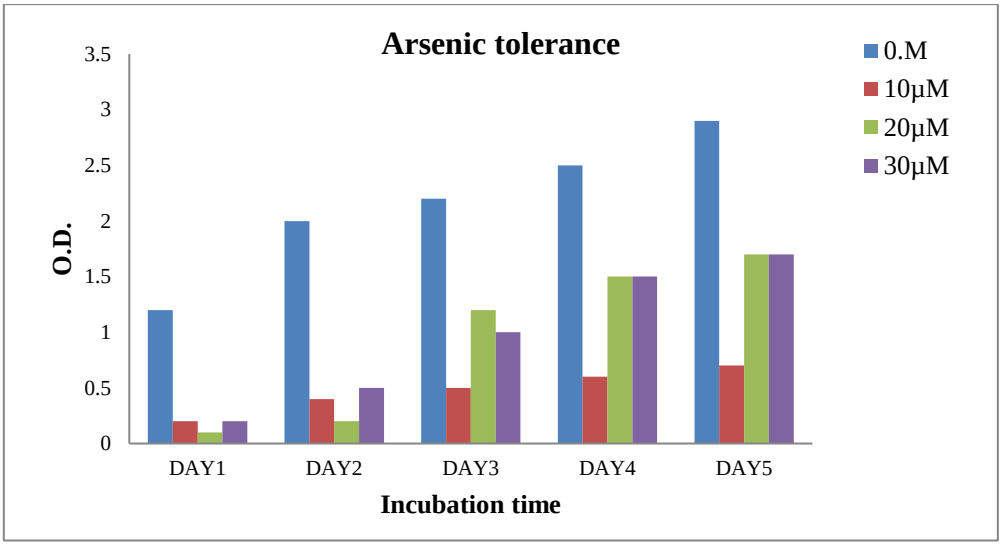


Fig 35: Growth behavior of isolates H-35 under various conc. of Arsenic

Isolates H-36

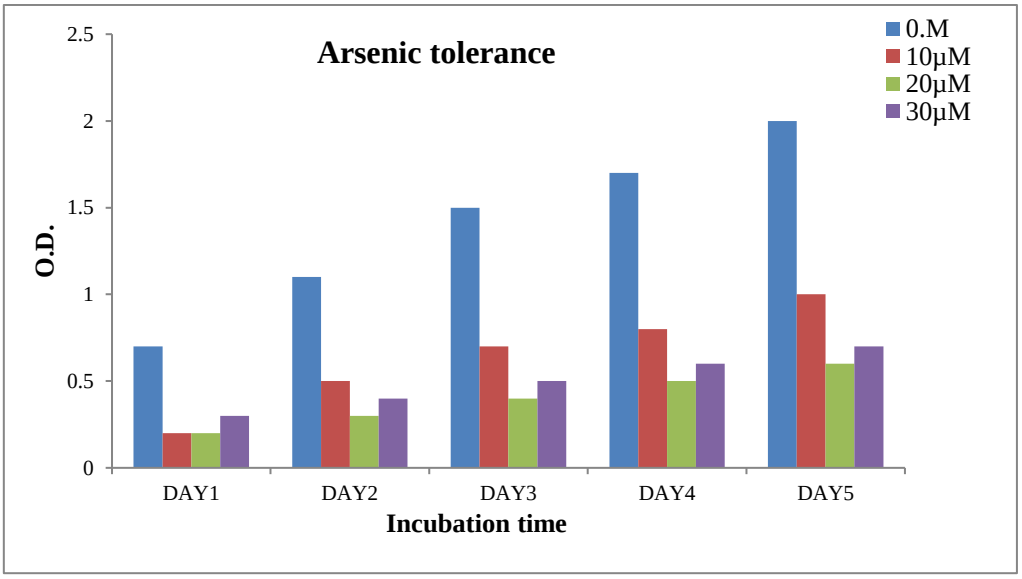


Fig 36: Growth behavior of isolates H-36 under various conc. of Arsenic

Isolate H-101

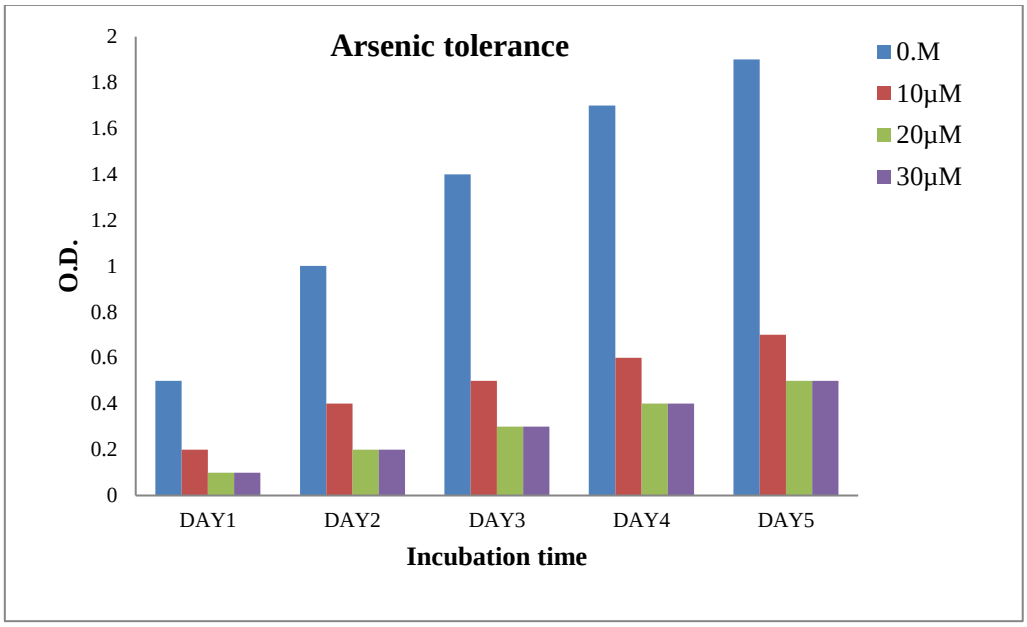


Fig 37: Growth behavior of isolates H-101 under various conc. of Arsenic

Isolates S-4

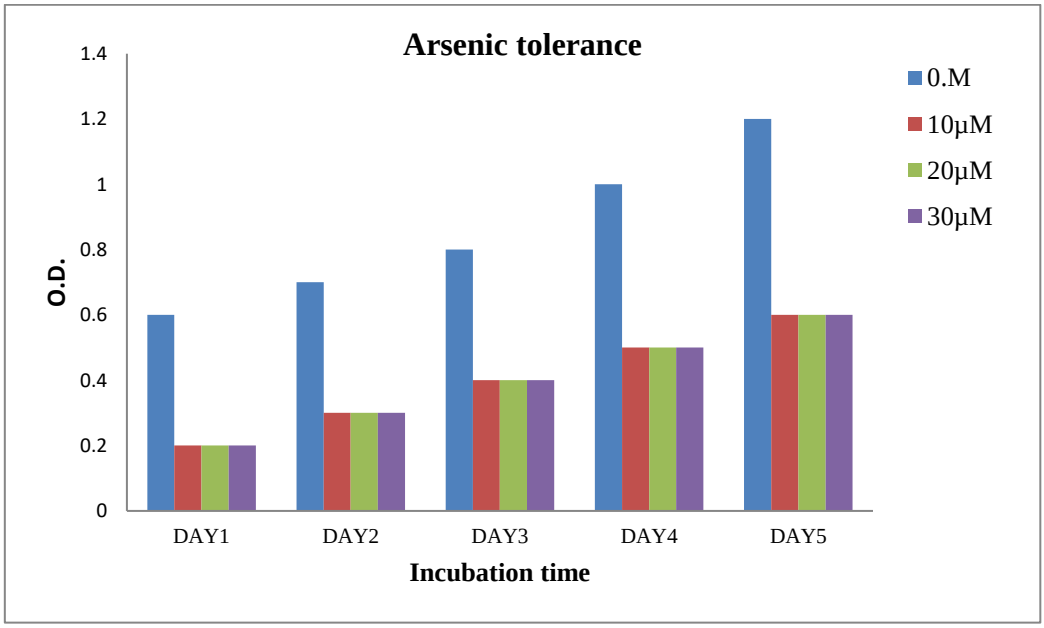


Fig 38: Growth behavior of isolates S-4 under various conc. of Arsenic

Screening of mercury tolerance .

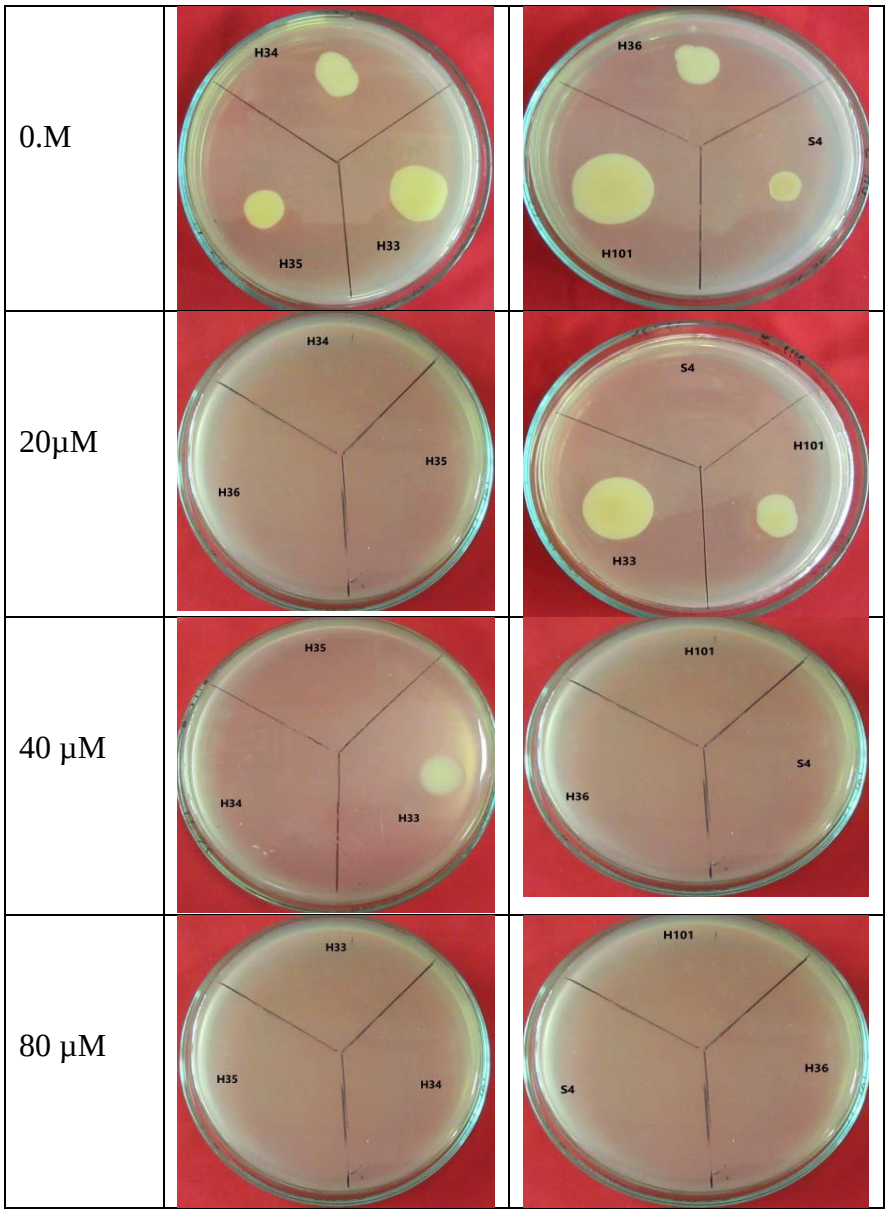


Figure 39: Colonies of rhizobacterial isolates obtained on NA plates (control and $HgCl_2$) after 24 hours of incubation in growth of five day

All the six isolates were tested against using Mercury chloride ($HgCl_2$). Mercury chloride was tested at 20 µM, 40µM, 80µM concentration . Based on the observations of growth obtained after 24 hours of incubation it was observed

that at 20 µM out of the 6 isolates only 2 isolates exhibited moderate growth. Whereas at 40 µM only 1 isolates showed growth, while 80 µM concentration no growth show.

Isolates H-33

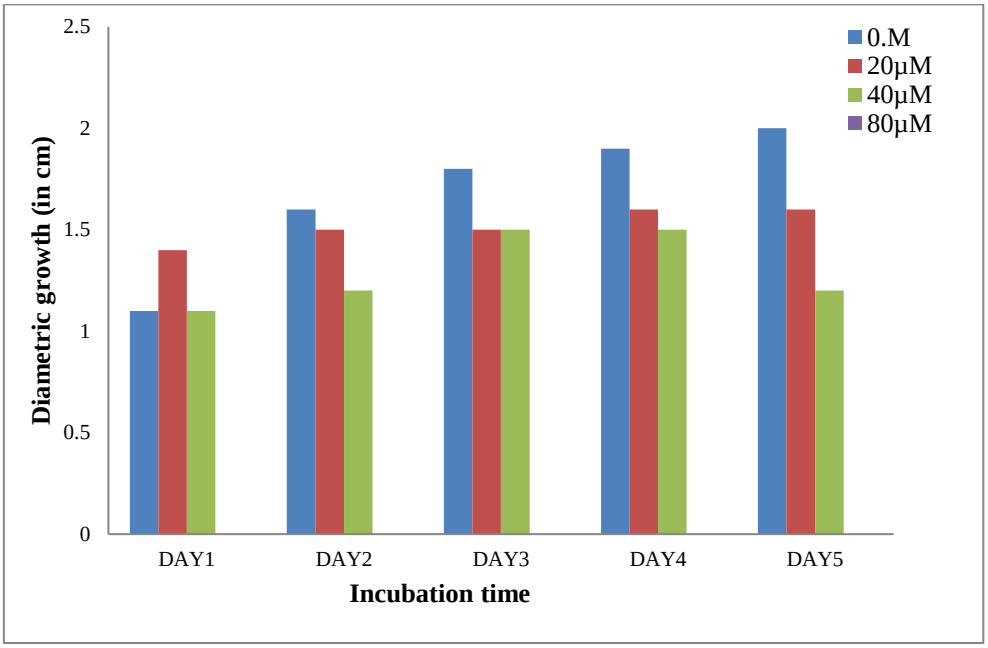


Fig 40: Growth behavior of isolates H-35 under various conc. of mercury

Isolates H-36

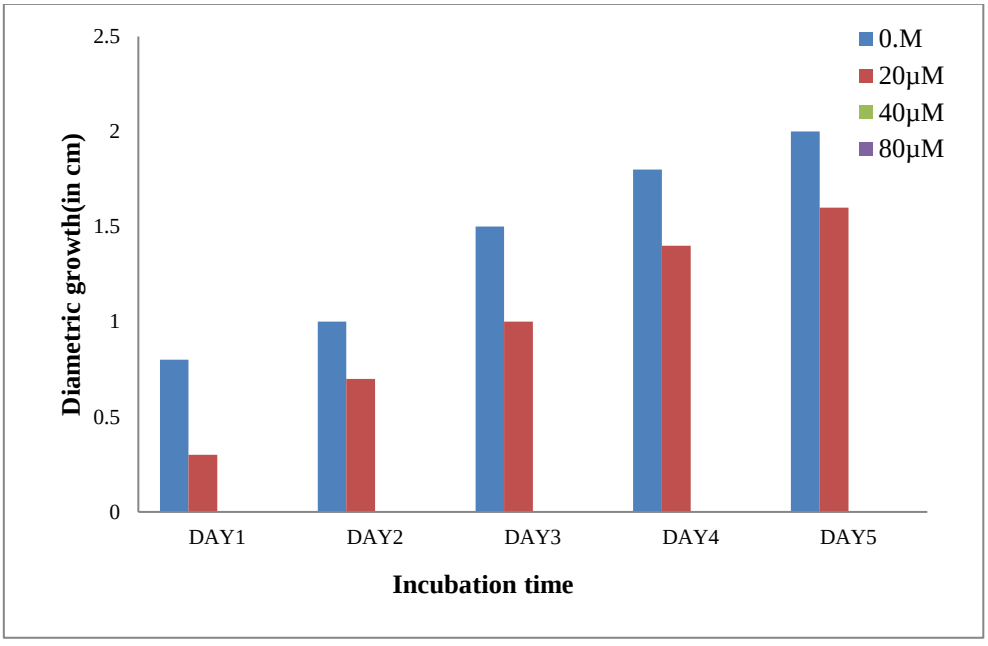


Fig 41: Growth behavior of isolates H-35 under various conc. of mercury

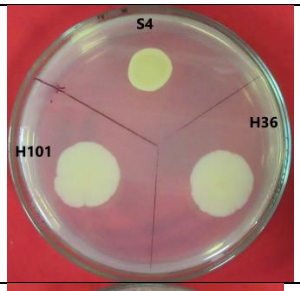
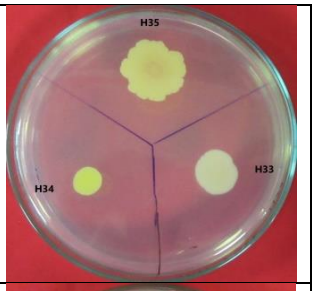
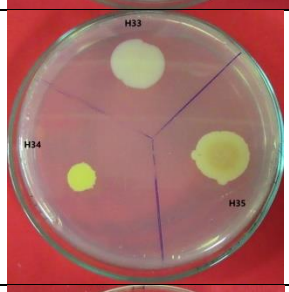
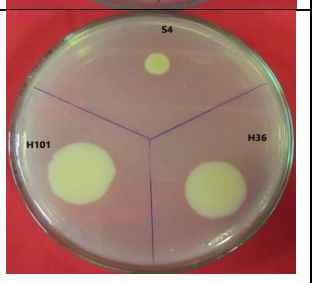
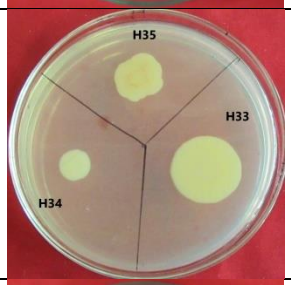
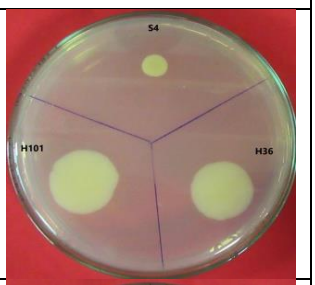
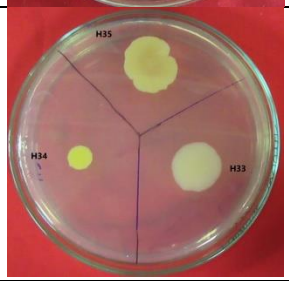
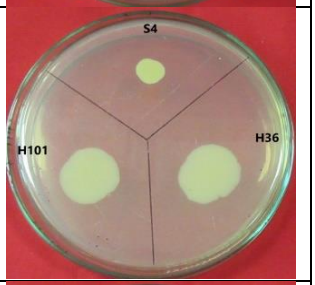
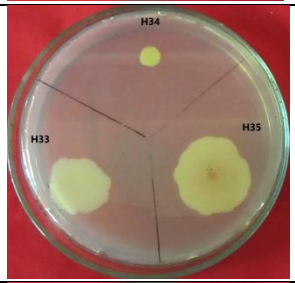
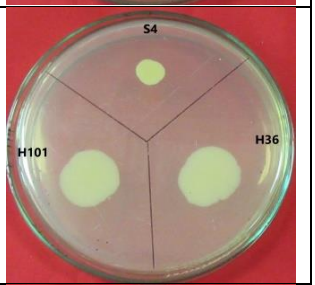
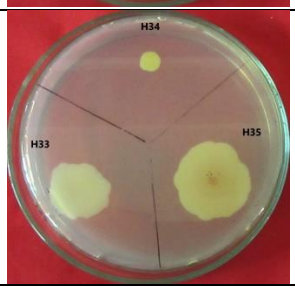
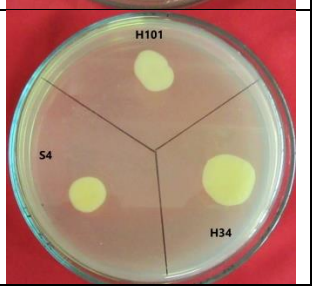
0.M		
500µg		
1000 µg		
2000 µg		
3000 µg		
5000 µg		

Fig 42: Fungicide tolerance behavior of bacterial isolates under various concentration of fungicide.

Isolates H-33

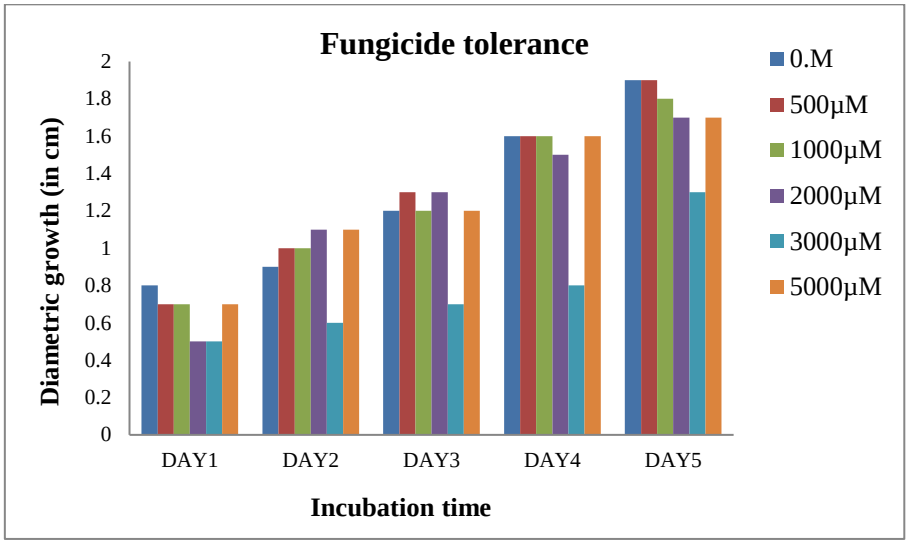


Fig 43: Fungicide tolerance behavior of H-33 isolate

Isolates H-34

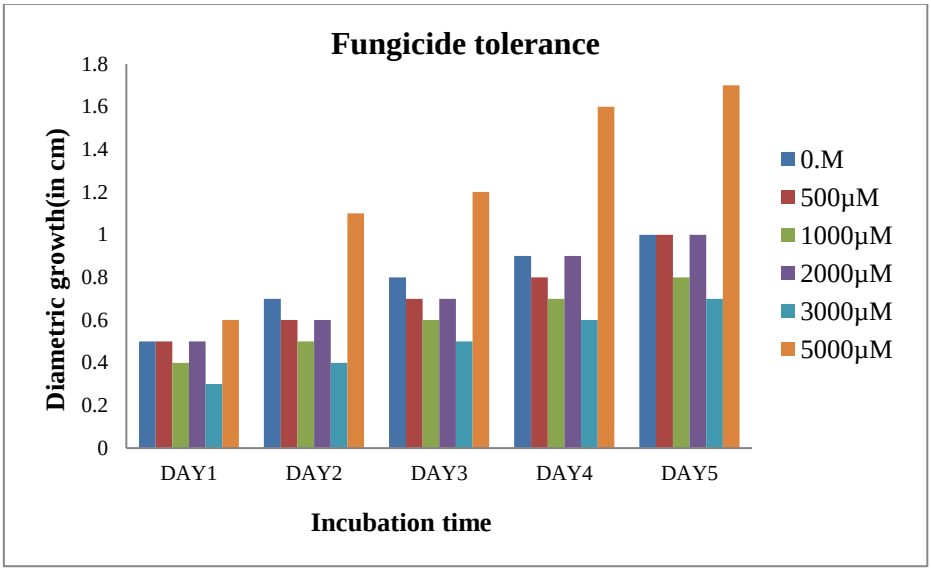


Fig 44: Fungicide tolerance behavior of H-34 isolate

Isolates H-35

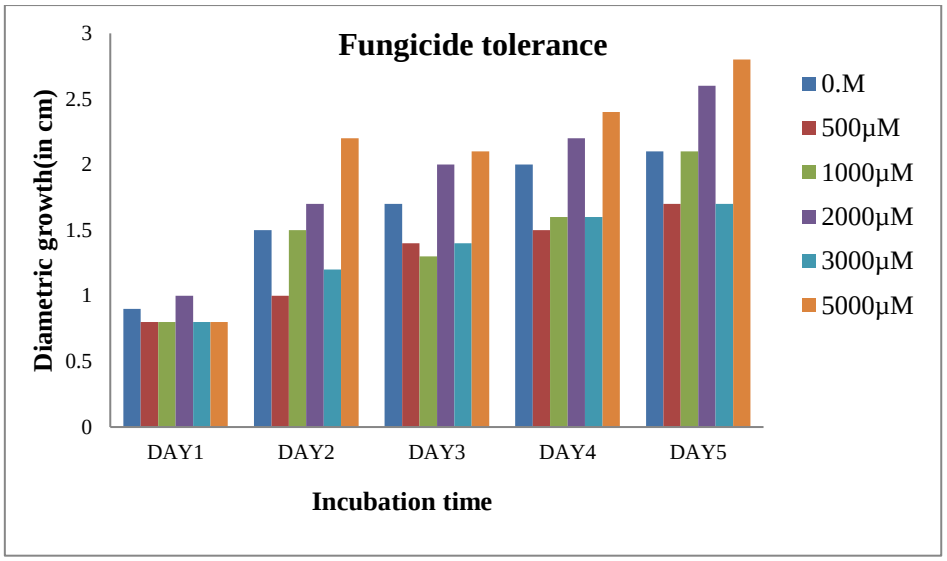


Fig 45: Fungicide tolerance behavior of H-35 isolate

Isolates H-36

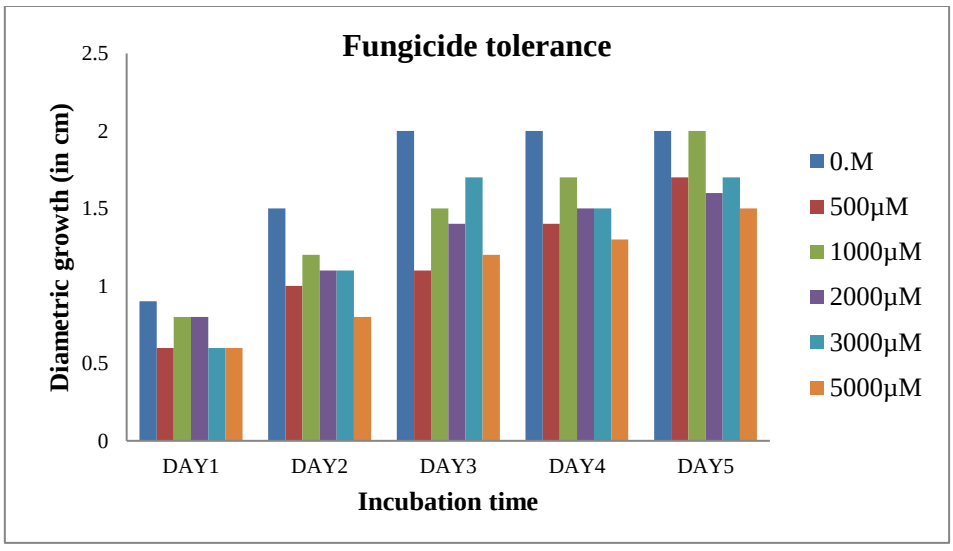


Fig 46: Fungicide tolerance behavior of H-36 isolate

Isolates H-101

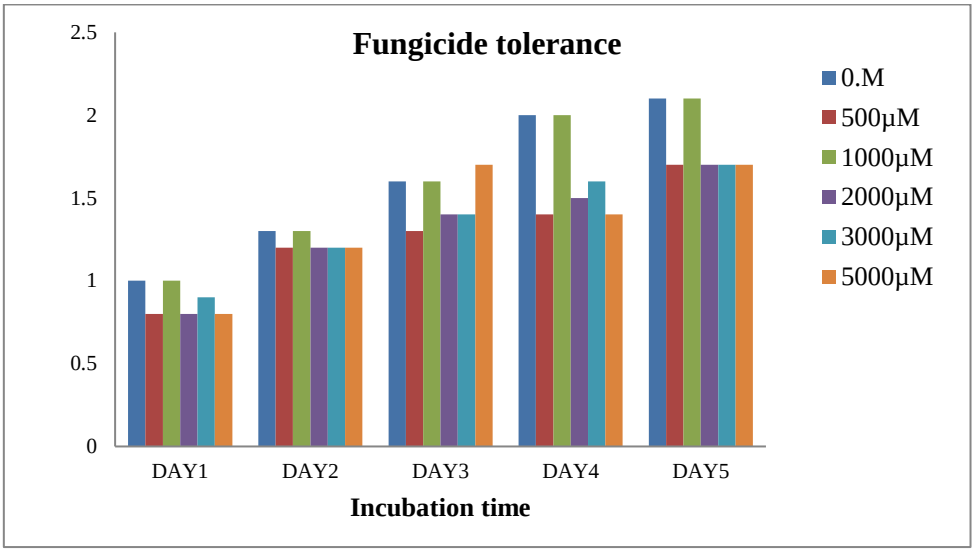


Fig 47: Fungicide tolerance behavior of H-101 isolate

Isolates S4

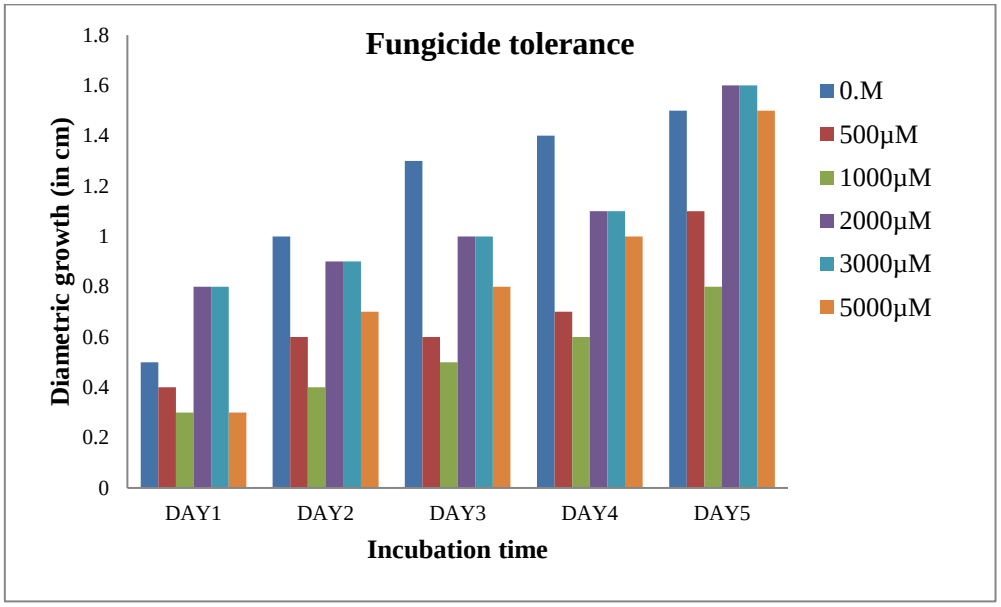


Fig 48: Fungicide tolerance behavior of S4 isolate

DISCUSSION

It is obvious from the above discussion that stressful environments can cause a damaging effect on plant growth and development by disturbing nutritional and hormonal balances. Abiotic stresses pose an unremitting threat to the growth and productivity of crop plants. However, PGPR provides a very economical and an environment friendly candidate for overcoming the adverse effects of several abiotic stresses such as salinity and heavy metal stress by conferring tolerance in plants as well as by promoting their growth and productivity. One feasible way is to search for soil microbial diversity having combination of plant growth promoting activities and well suited to the soil environment. They provide the potential to address numerous modes of action, different pathogens, and temporal or spatial variability. PGPR proposes an environmentally sustainable path to enhanced crop health and yield. Therefore, the obtained PGPR strains with the traits exemplified above such as production of zinc solubilization and siderophore and solubilization of phosphorus might be a good candidate to stimulate plant productivity under stressful environmental conditions. Continued efforts on research and development in this field have a lot to give and these simple creatures having potential for stress mitigation when combined with modern tools of biology can produce radical changes in crop productivity patterns and might be used as good alternatives of chemical fertilizer and other agricultural supplements. The present study was done to isolate rhizobacterial isolates which have capability to tolerate salinity and heavy metal stress. Isolating salt and heavy metal tolerant rhizobacteria along with PGP traits was the main focus of this study.

Further these bacterial isolates were also checked for their PGP attributes such as zinc solubilization efficiency, bacterial isolates spotted on Bunt Rovira agar medium plates which is being incubated at 30 ± 2ºc for 5 days. only 3 bacterial strains showed zinc solubilizaation activity with variable degrees of solubilisation as indicated by variation in diameter of halo zone formation. This is corroborated with earlier reports differ in their ability to solubilize

All 6 bacterial isolates were screened for siderophore production, out of which 3 isolates had shown the siderophore production.In our study, out of six isolates 3 were showed positive result for siderophore production.

These salinity tolerant rhizobacterial isolates along with PGP traits were also checked for their heavy metal tolerance capacity and we found that all bacterial isolates were tolerant to cadmium chloride metal salt up to 300µM and exhibited moderate to low growth. In case of mercury metal salt the bacterial isolates showed growth up to 100um while in case of cadmium metal salt isolates exhibited growth only at 10uM.

CONCLUSION

The functional screening of six bacterial isolates was done for salt-tolerance and plant growth-promoting traits. It was found that all the bacterial isolates were salt-tolerant. Plant growth-promoting traitssuch as phosphate solubilization, Zinc solobilization, pH tolerance, heavy metals, and siderophore were estimated quantitatively. It was found based on analysis that a total of six isolates –H33, H34, H35, H36, and S-4 were performing well under various salinity levels with siderophore production, pH tolerance, zinc solubilization and heavy metal tolerance. Among these six bacterial isolates, H33, was the best performing bacterial isolate asitwassolubilizing maximum phosphate, producing maximum siderophore, and zinc solubilization, Heavy metals, production was also above average. These salt-tolerant bacteriamay be potential PGPRs that can be used in salt degraded agricultural fields for soil improvement as well as crop productivity.

Refrereance

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