PLANT GROWTH PROMOTING ACTIVITY OF DIFFERENT SOIL BACTERIA

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CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the dissertation entitled **" Plant Growth Promoting activity of different soil bacteria"** in partial fulfillment of the requirements for the award of the degree of Master of Science in Microbiology, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is an authentic record of my own work during the period of six months from Jan 2013 to July 2013, under the supervision of Dr. Dinesh Goyal, Professor, Department of Biotechnology & Environmental Sciences, Thapar University. The report has not been submitted for the award of any other degree or certificate in this or any other university.

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CERTIFICATE

This is to certify that the thesis entitled submitted by Ms.Priya Thakur **"Plant growth promoting activity of different soil bacteria"** in partial fulfillment of the requirements for the award of degree of Master of Science in Microbiology to Thapar University, Patiala, is a record of student's own work carried out by her under her supervisor. The report has not been submitted for the award of any other degree or certificate in this or any other University or institute.

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LIST OF ABBREVIATIONS

PGPR	Plant growth promoting rhizobacteria
PGP	Plant growth promoting
PSB	Phosphate solubilising bacteria
PGPB	Plant growth promoting bacteria
PGB	Plant growth bacteria
HCN	Hydrogen cyanide
IAA	Indole-3- acetic acid
-L-TRP	withoutL-tryptophan
+L-TRP	with L-tryptophan
PKV	Pikovskaya medium
JM	Jensen medium
YEMA	Yeast extract mannitol agar
NA	Nutrient agar
DW	Distilled water
K_2HPO_4	Dipotassium hydrogen phosphate
NaCl	Sodium Chloride
$NaMoO_4$	Sodium molybdate
$FeSo_4$	Ferrous sulphate
$CaCo_3$	Calcium carbonate
h	Hours
gm	gram
rpm	rotations per minute

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that enhances plant growth by various attributes. Three Rhizobias, *Azotobacter* CBD 15*, Pseudomonas striata,, Paenibacillus, Bacillus subtilis*(T8), *Bacillus licheniformis*(T9), *Bacillus licheniformis* N14, *B*acillus *licheniformis*N15*, Lysinibacillus sphaericus* (DGA) were screened for various plant growth promoting activity. They were also checked for antifungal activity against phytopathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani*. The growth was optimized at different temperatures for different bacterial species like 28^0C for *Azotobacter* CBD 15, *Pseudomonas striata* and three Rhizobias and 37^0C for *Bacillus subtilis* (T8), *Bacillus licheniformis* (T9), *Bacillus licheniformis* N14, *Bacillus licheniformis* N15, *Lysinibacillussphaericus* (DGA) and *Paenibacillus*. Chickpea *rhizobacteria*, *Pseudomonas striata* and *Azotobacter* CBD 15 exhibited better production of indole acetic acid (IAA), whereas only Chickpea *rhizobacteria* and Pigeon pea *rhizobacteria* were also able to solubilise phosphorus. HCN production by *Bacillus subtilis* (T8), *Bacillus licheniformis*(T9), *Bacillus licheniformis* N15, *Bacillus licheniformis* N14 was positive. Ammonia production was observed by *Paenibacillus, Bacillus subtilis*(T8) and Pigeon pea *rhizobacteria*. The present study suggests that the use of bacteria as PGPR inoculants can be beneficial for crop cultivation as they can enhance plant growth due to the production of IAA, phosphate solubilisation and ammonia production. They can also have biocontrol activity as exhibited by HCN production and antifungal activity.

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefits plants by providing growth promotion. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticide, and supplements. A variety of bacterial traits components and specific genes contribute to this process, but only few of them have been identified. These include motility, chemotaxis to seed and root exudates, production of pili and fimbraie, production of specific cell components, ability to use specific components of root exudates, protein secretion, and quorum sensing. The plant rhizosphere is a remarkable ecological environment where numerous microorganisms colonize on, and around the roots of growing plants. Diverse groups of bacteria are associated with the root systems of all higher plants (Khalid *et al*., 2006). These bacteria are considered as efficient microbial competitors in the root zone, and the net effect of plant-microbe associations on plant growth could be positive, neutral or negative (Khalid *et al*., 2009). Such bacteria in close association with roots and capable of stimulating plant growth by any mechanism(s) of action are referred to as plant growth promoting rhizobacteria. The rhizosphere microflora hasbeen greatly affected and resulting decrease the soil productivity and nutrient use efficiency due to excessiveuse of chemical fertilizers.

Plant Growth PromotingRhizobacteria(PGPR) is alternative of chemical fertilizer because this has ability of phosphorus solubilization, production of plant growth hormones and biocontrol activity. PGPR represents alternative soil amendments promote plant growth and yield by several mechanism e.g., phytohormones production, provide available nitrogen by biological nitrogen fixation, available phosphorus by phosphate solubilization and suppression of phytopathogen (Akhtar *et al*., 2012). PGPR also protect plants against pathogens by direct antagonistic interactions between the biocontrol agent and the pathogen, as well as by induction of host resistance.

Phosphate solubilising bacteria has the ability to promote plant growth by synthesis of phytohormones (indole-3-acetic acid (IAA), gibberellins and cytokinins) and various other plant growth promoting substances (Verma *et al*., 2012). Phosphorus, the second most important nutrient after nitrogen, plays important role in development of root, stalk , flower and seed formation, crop maturity, plant disease resistant and biological nitrogen fixation. Part of the phosphorus added through fertilizers in soil is utilized by plants and remaining portion is converted into insoluble forms like iron and aluminium phosphate in the acidic and calcium phosphate in alkaline or normal soil (Gyansehwar *et al.,* 2002). Bacteria are able to solubilise organic and inorganic phosphorus from soil and made available to plant for growth and development. The PSB are well known soil bacteria in rhizosphere and non rhizosphere soil which is various types of bacteria like *Pseudomonas*, *Bacillus* spp. Bacteria solubilise insoluble inorganic phosphorous by secretion of organic acids, which through their hydroxyl and carboxyl groups chelate the cations (Al, Fe and Ca) bound to phosphate and decrease the pH of soil. The major organic acids produced by PSB are gluconic and keto gluconic acids which solubilise phosphorous by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil showed varying phosphate solubilising capacity under in vitro condition. The population of PSB were found maximum in rhizosphere soil of groundnut and minimum in rhizospheric soil of ragi, sorghum and maize.

In the present work, three different rhizobias, free living nitrogen fixing and phosphate solubilising bacteria and along with soil isolates such as *Bacillus* were investigated for Plant growth promoting activity in pure culture conditions for IAA production, phosphate solubilising, ammonia and HCN production and antifungal activity.

REVIEW OF LITERATURE

2.1 Plant growth promoting rhizobacteria (PGPR)

The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. Commercial applications of PGPR are being tested and are frequently successful; however, a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications (Burr *et al.,* 1984). PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant growth-promoting bacteria (PGPB) are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth. The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically (Joseph *et al.,* 2007). The plant growth stimulating efficiency of bacterial inoculants is affected by soil nutritional condition. The bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil. The simultaneous screening of rhizobacteria for growth promotion under gnotobiotic conditions and in production of auxins is a useful approach for selecting effective PGPR (Asghar *et al.,* 2004). The diazotroph bacterial inoculation significantly increases the seed cotton yield, plant height and microbial population in soil (Anjum *et al.,* 2007). The use of PGPR with P-enriched compost in an integrated manner improves the growth, yield and nodulation in Chickpea (Shahzad *et al.,* 2008).

2.2 Direct Mechanisms

2.2.1 Nitrogen fixation

In addition to *Rhizobia* spp., a number of free living bacteria, are also able to fix nitrogen and provide it to plants. However, it is generally believed that free-living bacteria provide only a small amount of what the fixed nitrogen that the bacterially-associated host plant requires (James *et al.*, 1997). Nitrogenase (nif) genes required for nitrogen fixation include structural genes involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme. In diazotropic (nitrogen fixing) bacteria, nif genes are typically found in a cluster of around 20-24kb with seven operons encoding 20 different proteins because of the complexibility of this system. Genetic strategies to improve nitrogen fixation have been elusive. The process of nitrogen fixation requires a large amount of energy in the form of ATP, it would be advantageous if bacterial carbon resources directed towards oxidative phosphorylation, which results in the synthesis of ATP, rather than glycogen synthesis, which results in the storage of energy in the form of glycogen (Marroqui *et al.,* 2001).

2.2.2 Phosphate solubilisation

Amount of phosphorus in the soil is generally quite high. Most of the phosphorous is insoluble and therefore not available to support plant growth. The insoluble phosphorus is present as an inorganic mineral such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonesters, and phosphotriesters (Khan *et al*., 2007). In addition, much of the soluble inorganic phosphorus that is used as chemical fertilizer is immobilised soon after it is applied so that it then becomes unavailable to plants and is therefore wasted.

The limited bioavailability of phosphorous from the soil combined with the fact that this element is essential for plant growth means that the inability to obtain sufficient phosphorous often limits plant growth (Feng *et al.,* 2004) Thus, solubilisation and mineralization of phosphorous by phosphate- solubilising bacteria is an important trait in Plant growth promoting Bacteria (Richardson, 2001). Typically, the solubilisation of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids such as gluconic and citric acid, both of which are synthesized by various soil bacteria (Rodriguez *et al.,* 2004).

2.2.3 Sequestering Iron

Despite the fact that iron is the fourth most abundant element on earth in aerobic soils, iron is not readily assimilated by either bacteria or plants because ferric ion or Fe+3, which is the predominant form in nature, is only sparingly soluble so that the amount of iron available for assimilation by living organisms is extremely low (Ma, 2005). Both microorganisms and plants require a high level of iron, and obtaining sufficient iron is even more problematic in the rhizosphere, where plant bacteria and fungi compete for iron (Loper *et al*., 1991).

The provision of iron to plants by soil bacteria is even more important when the plants are exposed to an environmental stress such as heavy metal pollution (Burd *et al.,* 2000). In this case, siderophores help to alleviate the stress imposed on plants by high soil levels of heavy metals. Plant iron nutrition can affect the structure of bacterial communities in the rhizosphere (Robin *et al.,* 2006).

2.2.4 Cytokinins and Gibberllins

Several studies have shown that many soil bacteria in general, and plant growth promoting bacteria in particular, can produce either cytokinins in the cell-free medium of some strains of *Azotobacter* spp., *Rhizobium* spp., *Bacillus subtilis*, and *Paenibacillus* (Yahalom *et al*., 1990) Moreover, plant growth promotion by some cytokinin- or gibberellins- producing plant growth promoting bacteria has been reported (Joo *et al.,* 2005). However, a detailed understanding of the role of bacterially–synthesized hormones and how the bacterial production of the plant hormones is regulated is not currently available. Thus, much of what we believe to be the role of bacterially-produced cytokinins and gibberellins is based on our knowledge of plant physiological studies following the exogenous addition of purified hormones to growing plants (Lorteau *et al*., 2001). Finally, some strains of phytopathogens can also synthesize cytokinins. However, it appears that plant growth promoting bacteria on plant growth is stimulatory while the effect of the cytokinins from pathogens is inhibitory (Kang *et al.*, 2009).

2.2.5 Modulating P.hytohormones levels

Plant hormones play key roles in plant growth and development and in the response of plants to their environment (Davies, 2004). Moreover, during its lifetime, a plant is often subjected to a number of non lethal stresses that can limit its growth until either the stress is removed or the plant is able to adjust its metabolism to overcome the effects of the stress (Glick *et al*., 2007). When plants encounter growth limiting environment conditions, the endogenous phtytohormones decrease the negative effects of the environmental stresses (Salamone *et al.*, 2005). While this strategy is sometimes successful, rhizosphere microorganisms may also produce or modulate phytohormones so that many PGPB can alter phytohormones levels and thereby affect the plant's hormonal balance and its response to stress (Glick *et al*., 2007).

2.2.6 Indoleacetic acid

Although several naturally occurring auxins have been described in the literature, indole -3-acetic acid (IAA) is by far the most common as well as the most studied auxin, and much of the scientific literature considers auxin (Patten *et al.*, 1996). IAA affects plant cell division, extension and differentiation; stimulates seed and tuber germination ; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation mediates responses to light, gravity and florescence affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions (Spaepen *et al*., 2011).

Plant responses to IAA vary from one type of plant to another, where some plants are more or less sensitive to IAA than other plants; according to the particular tissue involved. However, the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria. In this regard, the level of IAA synthesized by the plant is important in determining whether bacterial IAA stimulates or suppresses plant growth. In plant roots, endogenous IAA may be suboptimal or optimal for growth (Piet *et al.*, 1987) and additional IAA that is taken from bacteria could alter the IAA level either optimal or supraoptimal, resulting in plant growth promotion or inhibition, respectively. IAA synthesized by bacteria may be involved at different levels in plant-bacterial interactions. In particular, plant growth promotion and root nodulation are both affected by IAA (Patten *et al.*, 2002). This result was explained by the combined effect of auxin on growth promotion and inhibition of root elongation by ethylene

(Jackson *et al.*, 1991). The bacterial IAA that was incorporated by the plant stimulated the activity of the enzyme ACC synthase, resulting in increased synthesis of ACC and a subsequent rise in ethylene that inhibited root elongation (Riov *et al.*, 1989). Overall, bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. In addition, bacterial IAA looses plant cell walls and as a result facilitates an increasing amount of root exudation that an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Jackson *et al.*, 1991). Most *Rhizobium* strains that have been examined have been found to produce IAA (Badennoch-Jones *et al.*, 1984) and several studies have suggested that increases in auxin levels in the host plant are necessary for nodule formation (Mathesius *et al.*, 1998).

2.3 Indirect mechanisms

2.3.1PGPR as Biocontrol Agents

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. They can suppress a board spectrum of bacterial, fungal and nematode diseases. PGPR can also provide protection against viral diseases.The use of PGPR has become a common practice in many regions of the world. Although significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent. Recent progress in our understanding of their diversity, colonizing ability, and mechanism of action, formulation and application should facilitate their development as reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui *et al.*, 2006). The bacterial strains isolated from *Lolium* perene rhizosphere are capable of acting as plant growth promoting bacteria and as biocontrol agents as they show various plant promoting activities (Shoebitz *et al.*, 2006). A major group of rhizobacteria with potential for biological control is the *Pseudomonades* (Kremer *et al.*, 1996). *Pseudomonades* sp. is ubiquitous bacteria in agricultural soils. Tremendous progress has been made in characterizing the process of root colonization by pseudomonads, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence and the mechanisms of pathogen suppression (Weller *et al.*, 2007). *Pseudomonas* possesses many traits that make them well suited as biocontrol and growth-promoting agents (Weller *et al.,* 1988). These include the ability to (i) grow rapidly in vitro and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites i.e., antibiotics, siderophores, volatiles, and growth-promoting substances; (v) compete aggressively with other microorganisms; and (vi) adapt to environments stresses. In addition, pseudomonads are responsible for the natural suppressiveness of some soil to soil borne pathogens (Weller *et al*., 2002).

Cyanide production is one of the possible ways by which rhizobacteria may suppress plant growth in soil (Rudrappa *et al.,* 2008) elucidated the role of cyanide production in pseudomonad virulence affecting plant root growth and other rhizospheric processes. Growth inhibition of lettuce and barnyard grass by volatile metabolites of the cyanogenic rhizobacteria confirmed that HCN is major inhibitory compound produced (Kremer *et al.,*2001). Multitrophic interactions mediate the ability of fungal pathogens to cause plant disease and the ability of bacterial antagonists to suppress disease. A pathogen metabolite functions as a negative signal for bacterial antibiotic (HCN) biosynthesis, which can determine the relative importance of biological control mechanisms available to antagonists and which may also influence fungus-bacterium ecological interactions (Duffy *et al*., 2004). Positive correlations are found between HCN production in vitro and plant protection in the cucumber/Pythium ultimum and tomato/*Fusarium oxysporum* f.sp.radicis-lycopersici pathosystems (Ramette *et al*., 2003). *Bacillus subtilis* is also used as a biocontrol agent. This prevalent inhabitant of soil is widely recognized as a powerful biocontrol agent. In addition, due to its broad host range, its ability to form endospores and produce different biologically active compounds with a broad spectrum of activity, *Bacillus subtilis* as well as other *Bacilli* are potentially useful as biocontrol agents (Nagorska *et al*., 2007).

Microbial production of siderphores has been well documented by Selvakumar *et al*., 2009. Braud *et al*., 2009 found production and secretion of a major siderophore-Pyochelin (Pch) for iron assimilation by *Pseudomonas aeruginosa* (PAOI). Iron chelating ability through outer membrane transporter (FptA) in the extracellular medium and translocation into the cell by siderophores producing bacterium was also reported(Zhang *et a*l., 2009) studied and revealed the transcriptional activation and upregulation of iron by a growth promoting bacteria *Bacillus subtilis* (GB03) in Arbidiopsis. They stated that Fe-deficiency induced transcriptional factor is very crucial for bacterial strain-induction of ferric reductase and iron transporter IRT1. Additionally, *Bacillussubtilis* acidifies the root zone by increasing root proton release .They concluded that possible facilitation in the mobilization of iron is due to bacterial acidification. Enhanced iron uptake through microbially produced siderophores was previously noted by Crowley, 2006.

2.3.2 Antifungal activity

PGPR improve plant growth by preventing the proliferation of phytopathogens and thereby support plant growth. Some PGPR synthesize antifungal antibiotics, e.g .*Pseudomonas fluorescens* produces 2, 4- diacetylphloroglucinol which inhibits growth of phytopathogenic fungi (Nowak- Thomson *et al.*, 1994). Certain PGPR degrade fusaric acid produced by *Fusarium* sp. Causative agent of wilt and thus prevents the pathogenesis (Toyoda *et al*., 1991). Some PGPR can also produce enzymes that can lyse fungal cells. For example, *Pseudomonas stutzeri* produces extracellular chitinase and laminarinase which lyses the mycelia of *Fusarium solani* (Mauch *et al*., 1988). In recent years, *fluorescent Pseudomonas* has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black root-rot of tobacco (Voisard *et al.*, 1989) and root- rot of pea (Papavizas *et al*., 1974). Root-rot of wheat (Garagulia *et al*., 1988) damping-off of sugar beet (Fenton *et al.*,1992) and as the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents (Dowling*et al*., 1994). A concern is shown on the use of FLPs in crop plants as the antifungal substances released substances realeased by the bacterium, particularly 2, 4-diaacetylphloroglucinol(DAPG) could affect the arbuscur mycoohizal fungi (Andrade *et al*., 1992).

2.3.3 Induced systemic Resistance

Plant growth promoting bacteria can trigger a phenomenon in plants known as induced systemic resistance (ISR) that is phenotypically similar to the systemic acquired resistance (SAR) that occurs when plants activate their defense mechanisms in response to infection by a pathogenic agent (Pieterse *et al*., 2009). ISR-positive plants are said to be “primed” so that they react faster and more strongly to pathogen attack by inducing defense mechanisms, ISR does not target specific pathogens. Rather, it may be effective at controlling diseases caused by different pathogens. ISR involves jasmonate and ethylene signalling within the plant and these hormones stimulate the host plant’s defense responses to a range of pathogens (Verhagen *et al.*, 2004). Besides ethylene and jasmonate, other bacterial molecules such as the o-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide, flagellar proteins, pyoverdine, chitin, salicyclic acid have been reported to act as signals for the induction of systemic resistance (Bakker *et al.*, 2007).

Culture Methods – Bacterial cultures were maintained and grown routinely in respective media. Chickpea *rhizobacteria*, Pigeon pea *rhizobacteria* and Groundnut *rhizobacteria* were grown in Yeast extract mannitol broth (Subba Rao *et al*., 1977). *Azotobacter*CBD 15 was grown in Jensen Medium (Ranganayaki *et al.,* 1981) and *Pseudomonas striata* was grown in Pikovskaya medium (Subba Rao *et al*., 1977). Nutrient Broth was used for growing *Paenibacillus* and *Bacillus subtilis* (T8), *Bacillus licheniformis* (T9), *Bacillus licheniformis* (N15), *Bacillus licheniformis* (N14), *Lysinibacillus sphaericus* (DGA). Growth media were prepared using the distilled water and pH was maintained within the range of 7 to 7.4, and was sterilized at 121^0C at 15psi (1.06 kgm^2 pressure) for 20 mins. The glasswares were sterilized in hot air oven at 180^0 C for 1 hour and the optimal temperature required for growth of rhizobias, *Azotobacter*CBD 15, *Pseudomonas striata* was 28^0C whereas *Paenibacillus* and *Bacillus subtilis* (T8), *Bacillus licheniformis* (T9), *Bacillus licheniformis, Bacillus licheniformis* (N14), *Lysinibacillus sphaericus* (DGA) were grown at 37^0C.

List of 11 different 4 Gram positive and 5 Gram negative bacteria

Gram + bacteria	*Bacillus subtilis, Bacillus licheniformis, Paenibacillus, Lysinibacillus sphaericus*
Gram - bacteria	*Azotobacter, Pseudomonas*, chickpea *rhizobacteria*, pigeon pea *rhizobacteria*, Groundnut *rhizobacteria.*

3.1 Determination of phosphate solubilisation index in different bacteria (Sesdhari *et al.,* 2000)

Requirements

1) Pikovskaya media.

Procedure

1) The *in vitro* phosphate solubilization capacity of each strain was determined on NBRIP-BPB medium containing bromophenol as pH indicator.

2) Point inoculation (10μl) of each culture was done on Pikovskaya's agar plates containing bromophenol blue (0.025%).
3) Incubated the plates at 37^0C for 5 days, 7 days, 10 days.
4) Allowed the plates to stand for some time to absorb.
5) After that, incubated the plates at 28^0C and observed the zone of solubilisation at different intervals of time of 5, 7, 10 days.
6) Calculated the phosphate solubilisation index/efficiency according to the formula:

$$\text{PSI} = \frac{\text{zone diameter} + \text{colony diameter}}{\text{Colony diameter}}$$

3.2 Determination of ammonia production in different bacteria (Cappuccino and Sherman, 1992)

Requirements

1. Peptone broth.
2. Nessler's reagent

Procedure

1. The bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman, 1992.
2. Overnight grown bacterial cultures were inoculated in 10 ml peptone broth and incubated at 30±0.1^0C for 48 h at 120 rpm.
3. After incubation 0.5 ml of Nessler's reagent was added. The development of faint yellow to dark brown colour indicated the production of ammonia.

3.3Determination of HCN Production in different bacteria (Lorck, 1948)

Requirements

1) King's B medium, Nutrient medium, Jensen medium, Pikovskaya medium, Bushnell Haas medium (Allred *et al*., 1963)
2) Picric acid and sodium carbonate (0.5% picric acid in 2% sodium carbonate).
3) Whatman filter paper no.1.

Procedure

1) For the production of HCN,bacteria were streaked into King's B medium, nutrient medium, respective media, Bushnell haas medium supplemented with glycine (4.4g/l).
2) After this, petriplates were inverted and a piece of filter paper impregnated with 0.5% Picric acid and 2% Na carbonate was placed on the lid of the plate.
3) Petri plates were sealed with parafilm and incubated at 28^0 C for 96 h.
4) Discoloration of the filter paper form orange to brown after incubation was considered as microbial production of cyanide.

3.4 Determination of IAA in different bacteria(Yasmine *et al.*, 2009)

Requirements

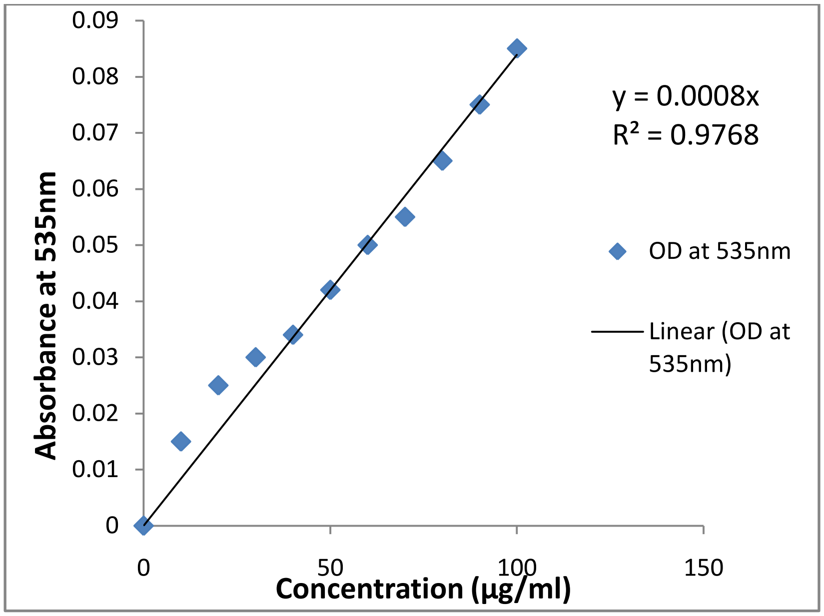
1) King's B medium.
2) L-Tryptophan (0.5% solution).
3) Salkowski reagent A (2% of 0.5M $FeCl_3$ in 35% perchloric acid).
4) IAA Stock (5mg/ml)

Procedure

1. Pure colonies of 7 isolates obtained from alkaline soil were grown in 50 ml conical flask containing 25 ml King's B (King *et al*., 1954) broth with and without L-TRP (0.5%) solution and incubated at 28 ± 2^0C for 24 h on a shaker.
2. The cultures were then centrifuged at 4000 rpm for 20 min.
3. One –milliliter culture supernatant was placed in a test tube and mixed with 2 ml Salkowski's reagent A (2% of 0.5M $FeCl_3$ in 35% perchloric acid).
4. After 20-25 min, when the colour of supernatent containing IAA turned red, the colour absorbance was read using a spectrophotometer at 535 nm. Each test was replicated three times.
5. Pure IAA (dissolved in acetone, Stock 5mg/ml) was used for preparing the standards of 0,10,20,30,40,50,60,70,80,90,100 mg/L.

3.4.1 Standard curve of IAA

Standard curve for IAA		
S.No.	Conc (µg/ml)	OD at 535nm
1	0	0
2	10	0.015
3	20	0.025
4	30	0.03
5	40	0.034
6	50	0.042
7	60	0.05
8	70	0.055
9	80	0.065
10	90	0.075
11	100	0.085



3.5 Determination of antifungal assay in different bacteria (Kumar *et al*., 2002)

Requirements

1) SDA Medium.

Procedure

1) Make wells (5mm) on SDA plates in three different areas.
2) Put Bacterial exract (50µl) on wells.
3) Placed fungus (3mm) in the centre of the SDA plates.
4) Sealed the plates with parafilm and incubated at 28^{0}c for 3 days.
5) The antifungal activity was evaluated by measuring the growth inhibition zone against test fungi.

6) Nystatin was taken as positive control whereas autoclave distilled water was taken as negative control in the above experiment.

Calculations:

$$\% \text{ Inhibition} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

In the present study three different rhizobias Chickpea *rhizobacteria*, Pigeon pea *rhizobacteria* and Groundnut *rhizobacteria* which procured from ICRISAT. *Azotobacter*CBD15 and *Pseudomonas striata* were procured from IARI (New Delhi) and bacterial isolates *Paenibacillus*,*Bacillus subtilis* (T8), *Bacillus licheniformis* (T9), *Bacillus licheniformis* (N15), *Bacillus lichenibacillus* (N14), *Lysinibacillus sphaericus* (DGA) were investigated for different plant growth promoting (PGP) activity such as indole acetic acid(IAA) production, phosphate solubilisation, ammonia production, HCN production and antifungal activity.

4.1 Determination of phosphate solubilisation index in different bacteria (Sesdhari *et al.*, 2000)

Seven cultures were screened for phosphate solubilization at different interval of time i.e. after 5, 7 and 10 days and maximum phosphate solubilisation activity was found in Chickpea*rhizobacteria*after 7 and 10 days as compared to*Azotobacter*CBD 15, *Pseudomonas striata*, *Paenibacillus*,*Bacillus subtilis* T8, Pigeon pea *rhizobacteria* and Groundnut rhizobacteria. Although, phosphate solubilisation activity of groundnut rhizobia was nil after 5 days and pigeon pea and chickpea showed same solubilisation extent after 10 days (Table 1 and Fig 1).

S.No.	Culture	PSI		
		After 5 days	After 7 days	After 10 days
1	Chickpea *rhizobacteria*	1.77± 0.06	2.08±0.1	2.03±0.08
2	Pigeon Pea *rhizobacteria*	1.7± 0.2	1.45±0.1	2.03±0.4
3	Ground nut *rhizobacteria*	1±0.0	1.05±0.05	1.4±0.4
4	*Azotobacter* CBD 15	1.96±0.31	1.67±0.13	1.56±0.03
5	*Pseudomonas straita*	1.45±0.22	1.41±0.08	1.23±0.0 8
6	*Paenibacillus*	1±0.22	1.2±0.08	1.43±0.08
7	*Bacillus subtilis* (T8)	1±0	1±0	1±0

*Values are means of three replicates.

Table 1: Determination of phosphate solubilisation index(PSI) in different bacteria.

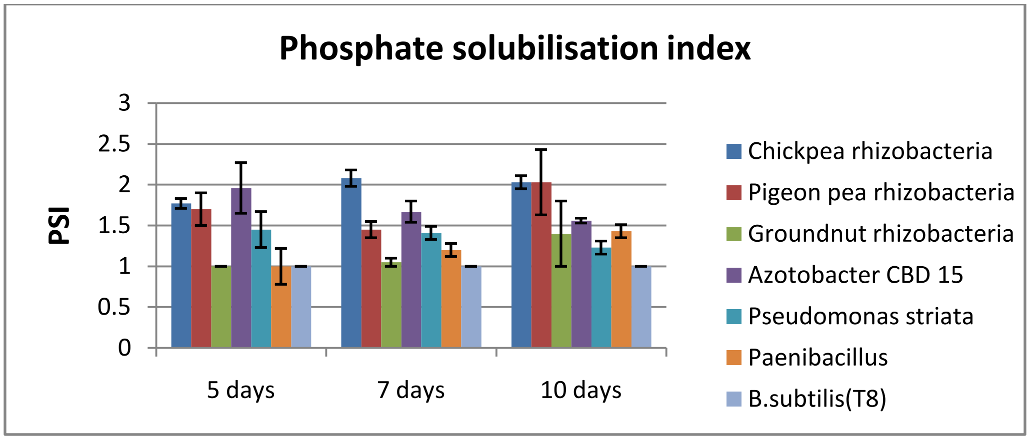


Fig 1: Determination of phosphate solubilisation index of different bacteria

4.2 Determination of ammonia production in different bacteria (Cappuccino and Sherman, 1992).

Cultures were screened for ammonia determination and production of brown colour indicates ammonia production whichwas found maximum in Pigeon pea *rhizobacteria*,*Paenibacillus*, *Bacillus subtilis* (T8) as compared to *Azotobacter*CBD 15, *Pseudomonas striata*, chickpea *rhizobacteria* and groundnut *rhizobacteria*(Table 2).

	Culture	R1	R2	R3
1	Chickpea *rhizobacteria*	Brown coloration, *++	Brown coloration, ++	Brown coloration, ++
2	Pigeon pea *rhizobacteria*	Brown coloration, +++	Brown coloration, +++	Brown coloration, +++
3	Groundnut *rhizobacteria*	Brown coloration, ++	Brown coloration, ++	Brown coloration, ++
4	*Azotobacter* CBD 15	Brown coloration, +	Brown coloration, +	Brown coloration, +
5	*Pseudomonas striata*	Brown coloration, +	Brown coloration, +	Brown coloration, +
6	*Paenibacillus*	Brown coloration, +++	Brown coloration, +++	Brown coloration, +++
7	*Bacillus subtilis* (T8)	Brown coloration, +++	Brown coloration, +++	Brown coloration, +++

* Less, ++ Moderate , +++ Strongly present

Table 2: Determination of ammonia production in different bacteria

4.3 Determination of HCN production in different cultures (Lorck, 1948)

Bacteria were screened for hydrogen cyanideproduction and development of brown colourindicates HCN in King's B medium and red colour in Nutrient medium, respective media and Bussnell haas medium indicates HCN production. Maximum HCN production was shown by *Paenibacillus*, *Bacillus subtilis* (T8) as compared *to Azotobacter* CBD 15,*Pseudomona*s straita, pigeonpea*rhizobacteria*, chickpea *rhizobacteria*, and Groundnut*rhizobacteria* on King's B agar plates.In nutrient agar plates production of hydrogen cyanide was shown by chickpea rhizobacteria, *Azotobacter, Paenibacillus*, *Bacillus subtilis* (T8),*Bacillus licheniformis*(T9). No HCN production was shown by Chickpea *rhizobacteria,* pigeon pea *rhizobacteria* and Groundnut *rhizobacteria* and *Pseudomonas striata.* In nutrient agar plates less cultures (42%) showed HCN production as compared to method in which King's B agar plates were used. In respective media agar plates HCN production was shown by chickpea *rhizobacteria,Azotobacter* CBD 15, *Paenibacillus*, *Bacillus subtilis*(T8),*Bacillus licheniformis*(T9). No HCN Production was shown by *Pseudomonas striata,* pigeon pea*rhizobacteria* and Groundnut *rhizobacteria*. Cultures with their respective media shows better HCN production as compared to other methods in which King's B agar plates and Nutreint agar plates were used. In Bussnell haas agar plates (Minimal media) only *B.licheniformis* N14, *B.licheniformis* N15 cultures showed HCN Production and HCN production was shown by remaining isolates. This meant that this was the most reliable method of HCN Production as compared to other methods which were got clearindication of HCN production on different media. Since, minimal mediacontain only the minimum nutrients necessary for the growth of bacteria (Table 3).

S.No	Bacterial isolate	Test condition	King’s B	NA	YEMA	JM	PKV	NA	BH
1	Chickpea *rhizobacteria*	+B	_	_	No colour	_	_	_	No colour
		+G+PA-B	_	_	No colour	_	_	_	No colour
		+G+PA+B	Brown colour	No colour	Red colour	_	_	_	No colour
		-G+PA+B	_		No colour	_	_	_	No colour
2	Pigeon pea *rhizobacteria*	+B	_	_	No colour	_	_	_	No colour
		+G+PA+B	_	_	No colour	_	_	_	No colour
		+G+PA-B	Light colour	No colour	No colour	_	_	_	No colour
		-G+PA+B			No colour	_	_	_	No colour
3	Groundnut *rhizobacteria*	+B	_	No colour	_	_	_	_	No colour
		+G+PA+B	_	No colour	_	_	_	_	No colour
		+G+PA+B	Light brown colour	No colour	_	_	_	_	No colour
		-G+PA+B	_	No colour	_	_	_	_	No colour
4	*Paenibacillus*	+B	_	_	_	_	_	No colour	No colour
		+G+PA+B	_	_	_	_	_	No colour	No colour
		+G+PA+B	Dark brown colour	Light red colour	_	_	_	Red colour	No colour
		-G+PA+B	_	_	_	_	_	No colour	No colour
5	*Bacillus subtilis*(T8)	+B	_	_	_	_	_	No colour	No colour
		+G+PA-B	_	_	_	_	_	No colour	No colour
		+G+PA+B	Dark	Dark	_	_	_	Dark	No

			brown colour	red colour				red colour	colour
		-G+PA+B	_	_	_	_	_	Red colour	No colour
6	*Bacilluslicheniformis(*T9)	+B	_	_	_	_	_	No colour	No colour
		+G+PA-B	_	_	_	_	_	No colour	No colour
		+G+PA+B	_	_	_	_	_	Dark red colour	No colour
		-G+PA+B	_	_	_	_	_	Red colour	No colour
7	*Azotobacter* CBD 15	+B	_	_	_	No colour	_	_	No colour
		+G+PA-B	_	_	_	No colour	_	_	No colour
		+G+PA+B	Brown colour	Dark red colour	_	Red colour	_	_	No colour
		-G+PA+B	_	_	_	No colour	_	_	No colour
8	*Pseudomonas striata*	+B	_	_	_	_	No colour	_	No colour
		+G+PA-B	_	_	_	_	No colour	_	No colour
		+G+PA+B	Light brown colour	No colour	_	_	No colour	_	No colour
		-G+PA+B	_	_	_	_	No colour	_	No colour
9	*Bacillus licheniformis*(N14)	+B	_	_	_	_	_	_	No colour

		+G+PA-B	_	_	_	_	_	_	No colour
		+G+PA+B	_	_	_	_	_	_	Dark red colour
		-G+PA+B	_	_	_	_	_	_	Red colour
10	*Bacillus licheniformis*(N15)	+B	_	_	_	_	_	_	No colour
		+G+PA-B	_	_	_	_	_	_	No colour
		+G+PA+B	_	_	_	_	_	_	Dark red colour
		-G+PA+B	_	_	_	_	_	_	Red colour
11	*Lysinibacillus sphaericus* (DGA)	+B	_	_	_	_	_	_	No colour
		+G+PA-B	_	_	_	_	_	_	No colour
		+G+PA+B	_	_	_	_	_	_	No colour
		-G+PA+B	_	_	_	_	_	_	No colour

Picric acid(PA) was loaded on filter paper sticking to the lid of plate.

-: Not done; M: media; G: Glycine; PA: Picric acid; B: Bacteria.

NA: nutrient agar; YEMA: yeast extract mannitol agar medium; JM: Jensen medium; PKV: Pikovskaya medium; BH: Bussnell has medium.

Table 3: Determination of HCN production in different bacteria

4.4 Determination of IAA production in different bacteria.

Seven bacteria were screened for IAA production with or without L-tryptophan and IAA production was observed with or without supplementation of L-tryptophan. Maximum IAA production was observed in the presence of L-tryptophan. Since L-tryptophan served as a precursor for IAA production, the production of IAA in presence of L-tryptophan was highest by *Pseudomonas striata*, *Azotobacter*CBD 15, Chickpea *rhizobacteria* as compared to*Paenibacillus*, *Bacillus subtilis* (T8), Pigeon pea *rhizobacteria* and Groundnut *rhizobacteria* (Table 5).

Determination of IAA production (µg/ml) in different bacteria						
S.No	Culture	Tryptophan (0.5%)	IAA(mg/ml)			Mean±SE
			R2	R2	R3	
1	Chickpea *rhizobacteria*	_	0.25	0.28	0.24	0.25±0.01
2	Groundnut *rhizobacteria*	_	0.14	0.15	0.16	0.15±0
3	Pigeon pea*rhizobacteria*	_	0.15	0.19	0.14	0.16±0.01
4	*Pseudomonas* striata	_	0.11	0.16	0.15	0.14±0.01
5	*Azotobacter* CBD 15	_	0.09	0.09	0.09	0.09±0
6	*Paenibacillus*	_	0.41	0.45	0.47	0.44±0.01
7	*Bacillus subtilis*(T8)	_	0.26	0.28	0.29	0.27±0
8	Chickpea *rhizobacteria*	+	1.35	1.34	1.35	1.34±0
9	Groundnut *rhizobacteria*	+	0.48	0.49	0.45	0.47±0.01
10	Pigeon pea *rhizobacteria*	+	0.57	0.58	0.59	0.58±0
11	*Pseudomonas striata*	+	1.1	1.5	1.2	1.2±0.12
12	*Azotobacter*CBD 15	+	0.88	0.98	0.12	0.66±0.27
13	*Paenibacillus*	+	0.71	0.75	0.74	0.73±0.01
14	*Bacillus subtilis* (T8)	+	0.56	0.53	0.55	0.54±0

*Values are means of three replicates.

Table 4: Determination of IAA (µg/ml) production in different bacteria

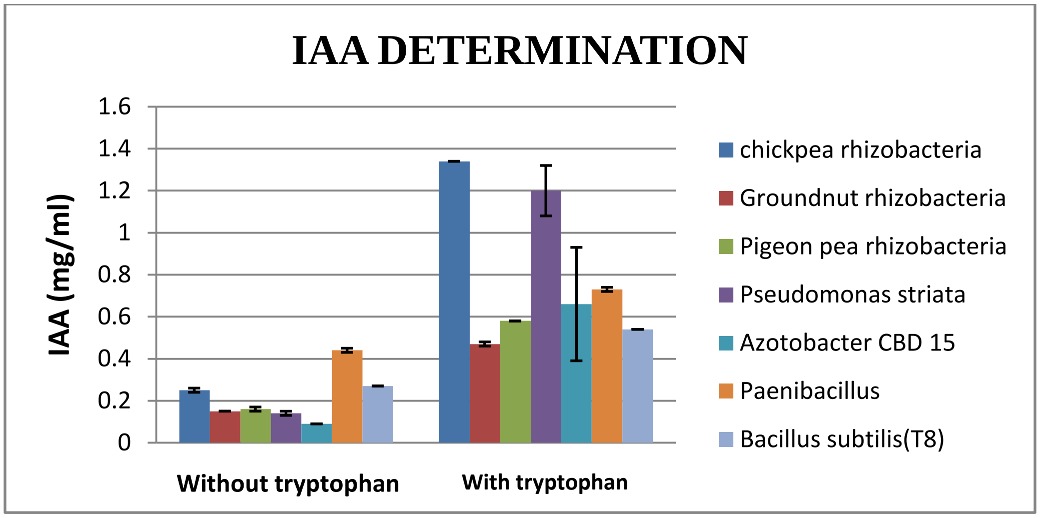


Fig 2: IAA Determination of IAA in different samples with and without L-tryptophan

4.5.1Screening of bacteria for antifungal activity against*Rhizoctonia solani*(Kumar *et al*., 2002).

Antifungal activity of 11 bacteria was checked against *Rhizoctonia solani*, *Fusariumoxysporum* using SDA medium. Groundnut *rhizobacteria*, Pigeonpea *rhizobacteria*, *Bacillus licheniformis* (N15), *Bacillus licheniformis* (N14) induced larger inhibition zones as compared to other cultures against *Rhizoctoniasolani*(Table 6).

Screening of bacteria for antifungal activity against *Rhizoctonia solani* (MTCC 4634)				
Culture	Mean dia (mm)	Control (-ve)	Control (+ve)	% Inhibition
Pseudomonas striata	85	85	55	0
*Azotobacter*CBD 15	85	85	55	0
Chickpea *rhizobacteria*	30	85	55	64.71
Groundnut *rhizobacteria*	23.3	85	55	72.55
Pigeonpea *rhizobacteria*	25.6	85	55	69.88
Paenibacillus	60	85	55	29.41
B.subtilis (T8)	53.3	85	55	37.25
B.licheniformis (T9)	55	85	55	35.29
B.licheniformis (N15)	17	85	55	80.4
Lysinibacillus sphaericus (DGA)	85	85	55	0
B.licheniformis (N14)	12	22.5	18.5	85.88

Table 5: Bacterial isolates showed antifungal activity against *Rhizoctonia solani*

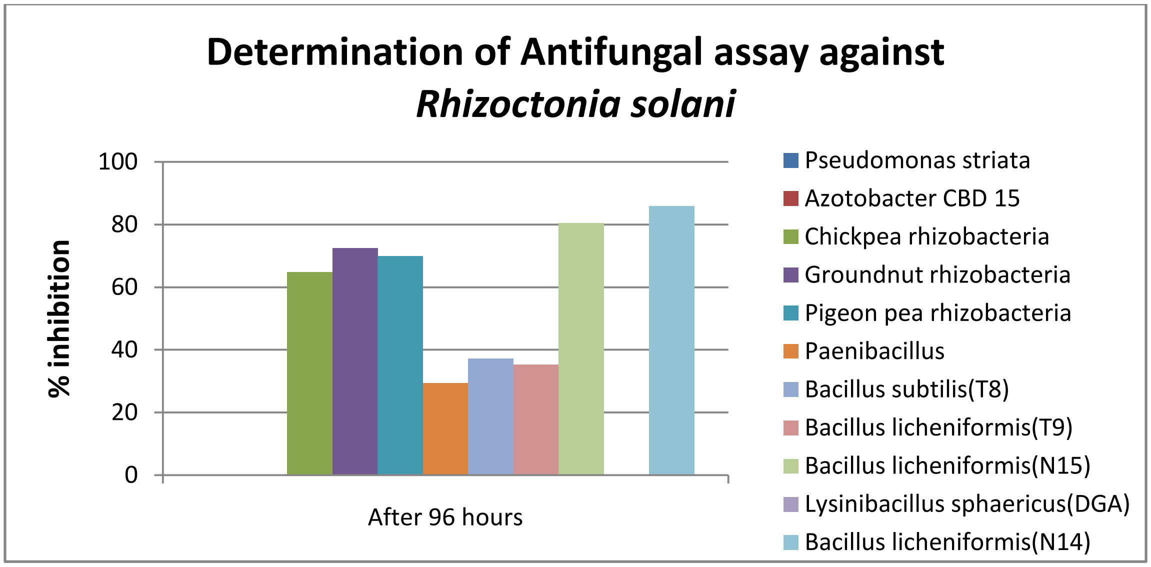


Fig 3: Determination of antifungal assay in different bacteria

4.5.2Screening of bacterial isolates for antifungal activity against *Fusarium oxysporum* .

Antifungal activity of Groundnut *rhizobacteria*, Chickpea *rhizobacteria*, Pigeon pea *rhizobacteria*,*Bacillus licheniformis* (N15)showed good antifungal activity against *Fusarium oxysporum* as compared to other bacteria (Table 7).

Screening of bacterial isolates for antifungal activity against *Fusarium oxysporum* (TBBALM)				
cultures	Mean dia(mm)	Control (-ve)	Control (+ve)	% inhibition
Pseudomonas striata	23.5	22.5	18.5	4.444
Azotobacter CBD 15	19	22.5	18.5	15.55
Chickpea *rhizobacteria*	17.5	22.5	18.5	22.22
Groundnut *rhizobacteria*	17	22.5	18.5	24.44
Pigeonpea *rhizobacteria*	20	22.5	18.5	17.11
Paenibacillus	18	22.5	18.5	20
Bacillus subtilis (T8)	19.5	22.5	18.5	13.33
B.subtilis (T9)	19.5	22.5	18.5	13.33
B.licheniformis (N15)	18	18	18.5	20
Lysini bacillus sphaericus (DGA)	19	22.5	18.5	15.55
B.licheniformis (N14)	19.5	22.5	18.5	13.33

Table 6: Bacterial isolates showed antifungal activity against *Fusarium oxysporum*

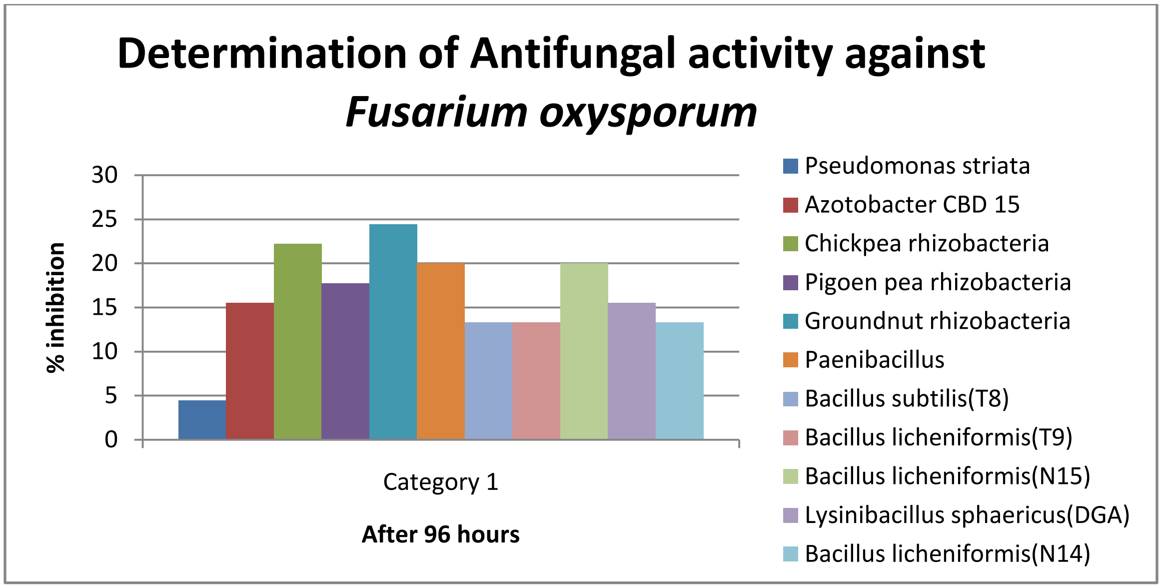


Fig 4: Determination of Antifungal assay in different bacteria

5.1 Phosphate solubilisation index in different cultures.

In the present investigation different isolates were screened for PGP activities. Phosphate solubilisation was most frequently encountered by Chickpea *rhizobacteria* as compared to pigeon pea and groundnut *rhizobacteria, Azotobacter* CBD 15, *Pseudomonas striata*, *Paenibacillus, Bacillus subtilis* (T8). Our finding of phosphate solubilisation in chickpea rhizobacteria culture was in agreement with other workers. The chickpea *rhizobacteria* was showed more solubilisation index 2.08 on pikovskya agar plates containing bromophenol blue at 7 days incubation. According to De Freitas *et al.*, 1997, good phosphate solubilizers were able to produce halos of more than 15mm diameters around their colonies. The phosphate solubilisation activity by different microorganisms was due to the production of different organic acids, in which hydroxyl and carboxyl groups of acids chelate cation (Al, Fe, Ca) and decrease the pH of the medium (Stevenson, 2005).

5.2 Ammonia production

It has been assumed that inoculation with bacteria like *Rhizobium* (Chickpea *rhizobacteria*, Pigeon pea *rhizobacteria*, and Groundnut *rhizobacteria)*, *Azotobacter* CBD 15, *Pseudomonas striata*, *Paenibacillus*, *Bacillus subtilis* (T8) enhanced the plant growth as a result of their ability to fix nitrogen. However, despite of extensive research efforts only *rhizobia* have been shown to increase yields from dinitrogen fixation. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Glick, 1995). Maximum ammonia production was found to be in *Paenibacillus,Bacillus subtilis* (T8), Pigeon pea *rhizobacteria* as compared to *Pseudomonas striata, Azotobacter* CBD 15, Chickpea *rhizobacteria* and Groundnut *rhizobacteria.* Our results support the fact that most nitrogen fixing bacteria showed were able for the production of ammonia (Arshad and Frankenberger, 1993).

5.3 HCN production

Different cultures were determined for HCN production. However, cultures chickpea *rhizobacteria*, pigeon pea *rhizobacteria* and groundnut *rhizobacteria,Pseudomonas striata,Azotobacter*CBD 15, *Paenibacillus*, *Bacillus subtilis* (T8) were showed positive response for HCN production on King's B Medium. Cultures chickpea *rhizobacteria*, pigeon pea *rhizobacteria* and groundnut *rhizobacteria* did not show positive for HCN production on nutrient agar plates as compared to other cultures. In their respective media agar plates, chickpea *rhizobacteria,Azotobacter*CBD 15, *Paenibacillus*, *Bacillus subtilis* (T8), *Bacillus licheniformis* (T9) were detected positive for HCN production. In Bushnell haas medium (Minimal media), *Bacillus licheniformis* (N14), *Bacillus licheniformis* (N15) were detected positive for HCN production. Colour intensity was high in plates of *Bacillus licheniformis* (N15) and *Bacillus licheniformis*(N14) supplemented with glycine as compared to plates without glycine (Castric, 1997). Minimal media contain only the minimum nutrients necessary for the growth of a particular type of cells. Minimal media usually did not contain amino acids and sometimes used to grow so-called "wild type microorganisms or those microorganisms that did not have acquired resistance to a certain agent being tested (such as an antibiotic). Minimal media usually contain a carbon source like glucose, various salts that provide nitrogen, magnesium, sulphur, and phosphorous that allows bacteria to create protein and nucleic acid and water (Allred *et al*., 1963).

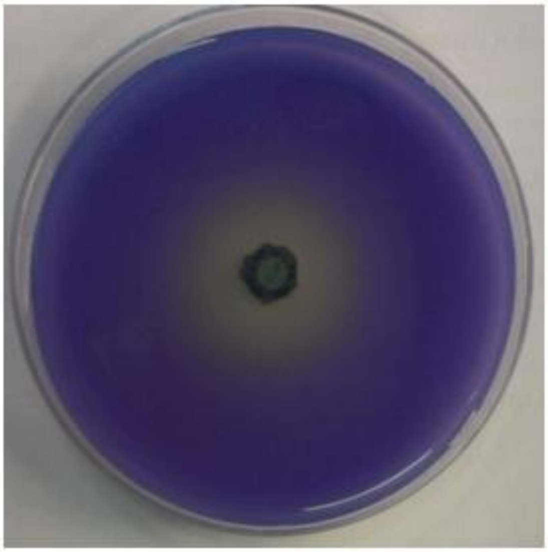
5.4 IAA production

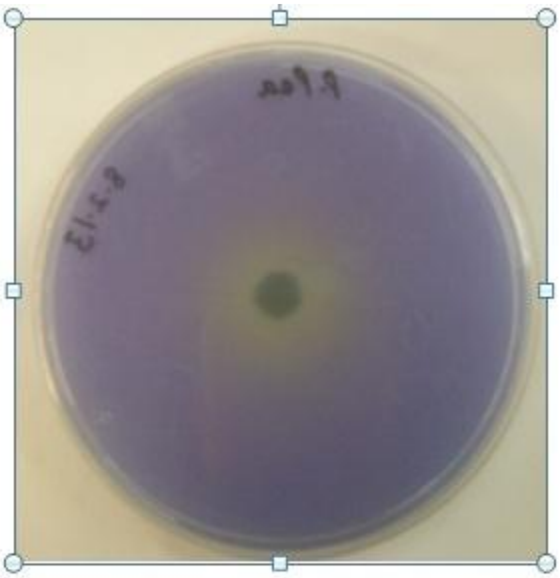
IAA production was detected in chickpea *rhizobacteria*, pigeonpea *rhizobacteria* and groundnut *rhizobacteria*, *Paenibacillus*, *Pseudomonas striata,Azotobacter*CBD 15, *Bacillus subtilis* (T8) with or without L-TRP. There was an increase in the level of IAA production with the addition of 0.5% L- Tryptophan. Similar trend of IAA Production with the increasing concentration of tryptophan was also reported by Barazani and Friedman, 2000. Similar high level of IAA production was recorded by other workers (Xie *et al*., 1996). The production of IAA was found dependent upon type of bacterial cultures and concentration of tryptophan. Such findings may have direct practical application, although intrinsic ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant

(Arshad and Frankenberger, 1993, Gonzalez-Lopez *et al*., 1986; Jagnow, 1987; Nieto and Frankenberger, 1989).

5.5 Antifungal assay

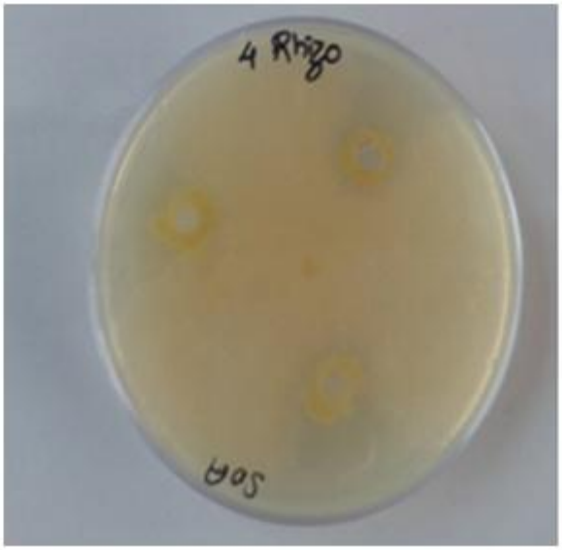
Antifungal activity was shown by 80.4% of *B.licheniformis* (N15)culture, followed by *Bacillus licheniformis* (N14), Groundnut *rhizobacteria*, chickpea *rhizobacteria* and pigeon pea *rhizobacteria* against *Rhizoctonia solani*. Antifungal activity was shown by 24.44% by groundnut *rhizobacteria*, followed by chickpea *rhizobacteria*, *Bacillus licheniformis*(N15) and *Paenibacillus* against *Fusarium oxysporum*(O'Sullivan and O'Gara, 1992; Ahmad *et al*., 2008). PGP activities among PGPR have been reported by some other workers while such findings were commonly explored. The antifungal activity of the test isolates indicated a close relationship between production of HCN. The antifungal activity of test cultures might be due to the production of HCN or synergistic interaction of HCN or other metabolites. Further studies on the performance of these cultures and their mutants on the growth of plant will uncover the mechanism and potential of these PGPR exhibiting multiple PGP activities. (Nagrajkumar *et al.*, 2004).

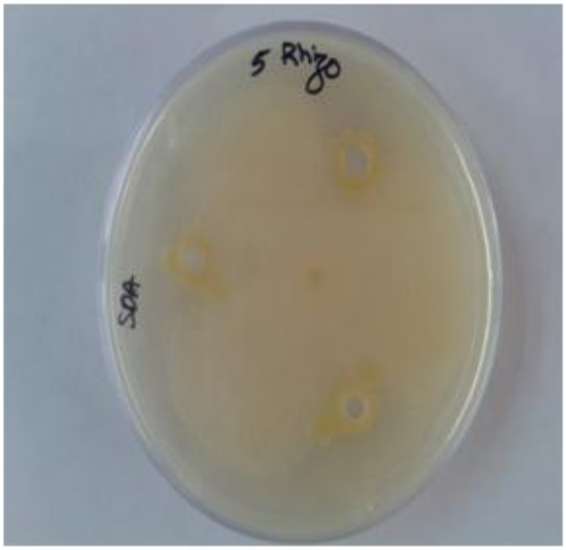




A: Phosphate solubilization of Chickpea *rhizobacteria*after 7 days

B: Phosphate solubilization of Pigeon Pea *rhizobacteria* after 10 days





C: Antifungal activity of Groundnut *rhizobacteria* against *Rhizoctonia solani*

D: Antifungal activity of Pigeon pea *rhizobacteria* against *Rhizoctonia solani*

CONCLUSION

1. IAA production was studied with or without L-TRP and IAA activity was positive in Chickpea *rhizobacteria* (1.35mg/ml) followed by *Pseudomonas striata* (1.1mg/ml) and *Azotobacter* CBD15 (0.88mg/ml) with L-TRP.

2.In present investigation, phosphate solubilisation index was found maximum in Chickpea *rhizobacteria* (2.08 and 2.03 after 7 and 10 days respectively). Chickpea *rhizobacteria* was found efficient plant growth promoting *rhizobacteria* for phosphate solubilisation.

3. Cultures were screened for ammonia production. Maximum ammonia production was found by *Paenibacillu*s, *Bacillus subtilis* (T8) and Pigeon pea *rhizobacteria* after 48-72 h incubation.

4. HCN activity in different cultures after four days of incubation showed HCN production by *Azotobacter*CBD 15,*Pseudomonas striata*, *Paenibacillus*, *Bacillus subtilis* (T8), B*acillus licheniformis* (T9), Chickpea *rhizobacteria*, Pigeon pea rhizobacteria, Groundnut *rhizobacteria*, *Bacillus licheniformis* N14 and *Bacillus licheniformis* N15.

5. Antifungal activity was determined and maximum antifungal activity was shown by *Bacillus licheniformis* (N14),*Bacillus licheniformis* (N15)against *Rhizoctonia solani*.

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APPENDICES

List of media used in the study.

1. Pikovskaya medium

Composition of Pikovskaya Media		
S.NO.	Components	Quantity (g/l)
1	Glucose	10
2	Tricalcium phosphate	5
3	Ammonium sulphate	0.5
4	Sodium chloride	0.2
5	Magnesium sulphate	0.1
6	Potassium chloride	0.2
7	Yeast extracts	0.5
8	Manganese sulphate	Trace
9	Ferrous sulphate	Trace
10	Agar	15
11	Bromophenol blue	0.05%
pH	7.0-7.2	

2. King's B medium

King's B medium agar		
S.No.	Components	Quantity (g/l)
1)	Biopeptone	20
2)	K_2HPO_4	1.5
3)	$MgSO_4.7H_2O$	1.5
4)	Glycerol	10
5)	Agar	15
pH 7.0		

3. Jensen medium

Jensen medium		
S.NO	INGREDIENTS	gm/l
1	Sucrose	20
2	K_2HPO_4	1
3	$MgSO_4$	0.5
4	$NaMoO_4$	0.01
5	$FeSO_4$	0.01
6	$CaCO_3$	2.0
	pH	7.0-7.4

4. Yeast extract mannitol agar

Yeast extract mannitol agar		
S.NO	Ingredients	Gm/l
1	Mannitol	10
2	K_2HPO_4	0.5
3	$MgSO_4..7H_2O$	0.2
4	Nacl	0.1
5	$CaCO_3$	3
6	Yeast extract	0.5
7	Agar	15
8	Distilled water	1000ml
	pH	7

5. Composition of Bussnell haas medium

Bussnell hass Medium		
S.NO	COMPONENTS	gm/l
1	Magnesium sulphate	0.2
2	Calcium chloride	0.02
3	Monopotassium phosphate	1.0
4	Dipotassium phosphate	1.0
5	Ammonium Nitrate	1.0
6	Ferric chloride	0.05
pH 7.0		

6. Compostion of SDA(Sabouraud dextrose agar) medium

Composition of SDA Medium		
S.NO	Ingredients	gm/L
1	Glucose	40g/l
2	Peptone	10gm/l
3	Agar	15gm/l
pH 5.6±0.2		