The Bioscan 9(1): 323-328, 2014 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ISOLATION OF FLUORESCENT *PSEUDOMONAS* STRAIN FROM TEMPERATE ZONE OF HIMACHAL PRADESH AND THEIR EVALUATION AS PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

SHWETA SHARMA[1]*, MOHINDER KAUR[1] AND DURGA PRASHAD[2]
[1]Department of Basic Science (Microbiology Section),
Dr. Y S Parmar University of Horticulture and Forestry Nauni, Solan - 173 230 (H.P.), INDIA
[2]Department of Plant Pathology,
Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan - 173 230 (H.P), INDIA
e-mail: shweta_85sharma@rediffmail.com

KEYWORDS
Pseudomonas
PGP, PGPR
P-solubilization
Siderophore
Antifungal activity

Received on :
01.01.2014

Accepted on :
24.03.2014

***Corresponding author**

ABSTRACT
A bacterial collection of 30 indigenous strains of *Pseudomonas* species were isolated from the normal and replant rhizospheric soil of apple and pear orchard of Kullu District temperate zone of Himachal Pradesh. Twenty three isolates from apple rhizospheric soil and seven isolates from pear rhizospheric soil were characterized on the basis of their morphological, physiological, biochemical and plant growth promoting activities. The strains were further studied on their ability to produce plant growth promoting activities such as plant growth hormone production *viz*., auxins, gibberellins and cytokinins, phosphate solubilizing activity, siderophore activity, protease enzyme activity, ammonia production, HCN production and antifungal activity. Auxins, gibberellins and cytokinins produced by isolates were in the range of 1.83-21μg/ml, 116.1-485.8 μg/ml and 63.6-90.0 μg/ml respectively. *Pseudomonas* isolates showed production of phosphate solubilizing activity in the range of 199.5 to 413.4 μg/ml available inorganic phosphate (Pi) and siderophore production in the range of 20-21 mm in plate assay and 67.27 %SU in liquid assay. Therefore, these results suggested that out of 30 isolates, two isolates An-1-kul and An-13-kul possessed multiple PGP (Plant growth promoting) traits thus can be further explored for its efficacy as effective PGPR (Plant growth promoting rhizobacteria).

INTRODUCTION

Microorganisms that inhabit the rhizosphere may influence plant growth by contributing to a host plant's endogenous pool of bioactive compounds such as phytohormones, antibiotics, siderophores (Patten and Glick, 2002; Mubarikf *et al*., 2010). Those kind of bacterial group are well-known as Plant Growth Promoting Rhizobacteria (PGPR). PGPR can exhibit a variety of characteristics responsible for influencing plant growth (Ahmad *et al*., 2005). PGPR are considered to promote plant growth directly or indirectly. Indirect effects are related to production of metabolites, such as antibiotics, siderophores, or HCN, that decrease the growth of phytopathogens and other deleterious microorganisms. Direct effects are dependent on production of plant growth regulators or improvements in plant nutrients uptake (Bai *et al*., 2003. In the last decades research on PGPR has been increasing at an ever increasing rate since the term was first used by Kloepper and coworkers in the late 1970s (Vessey, 2003). This scientific interest is related to the PGPR potentiality in agriculture which is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Recent progress in our understanding on the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action should facilitate their application as a reliable component in the management of sustainable agricultural system (Bhattacharyya and Jha, 2012). Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms. Some genera of bacteria have been determined as PGPR including *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Bradyrhizobium,* and *Rhizobium*. An effective PGPR should have at least three characters of promoting plant activities which are root colonization competency, phytostimulator and biocontrol agent against plant pathogens (Bloemberg and Lugtenberg, 2001). *Pseudomonas* sp. is one of the most important members of PGPRs showing all the three major group of PGPRs. *Pseudomonas* sp has been reviewed for the biofertilizer, phytostimulator and phytopathogen biocontrol activities. Direct plant growth activities of *Pseudomonas* sp include the production of Indole Acetic Acid (IAA) (Vasanthakumar and McManus, 2004) and siderophore (Dey *et al*., 2004), phosphate solubilization (Wu *et al*., 2005), ACC deaminase, root elongation, degradation of toxic compound (Bano and Musarrat, 2003) and as biological control agent for phytopathogen such as *Aspergillus niger* and *A. flavus* (Dey *et al*., 2004). Of the various rhizospheric bacteria, *Pseudomonas* sp. are aggressive colonizers of the rhizosphere of various crop plants (Schroth and Hancock, 1982) and have

a broad spectrum of antagonistic activity against plant pathogens. The antibiotic produced by *Pseudomonas fluorescens* was found to control damping-off of cotton seedlings caused by *R. solani*. Among *Pseudomonas* species, *Pseudomonas aeruginosa*, a plant growth promoting rhizobacterium has been found to be an effective biocontrol agent of root pathogens. For many pseudomonads, production of metabolites such as antibiotics, siderophores and hydrogen cyanide (HCN) is the primary mechanism of biocontrol. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents. 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 environmental stresses. In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soil borne pathogens (Weller *et al.,* 2002). According to the remarkable PGPR characters of *Pseudomonas* sp, therefore in this study we isolated *Pseudomonas* sp. from the rhizosphere of apple and pear. Thus the present study is undertaken to isolate and identification of indigenous strains of fluorescent *Pseudomonas* sp. from both normal and replant area of apple and pear orchards in Kullu District of Himachal Pradesh. Further these isolates are evaluated for their potential to produce plant growth promoting activities viz., production plant growth regulators, phosphate solubilisation, siderophores, antimicrobials, HCN, ammonia and protease enzyme. The objectives of this study is to screening of *pseudomonas* isolates on over all plant growth promoting activities to develop them as bioinoculants/biofertilizer to reduce the input of synthetic fertilizers and fungicides.

MATERIALS AND METHODS

Site description of soil sampling

Soil samples from the rhizosphere of apple and pear orchards were collected from both normal and replant area in Kullu District of Himachal Pradesh at altitude of 1,278 m or 4,193 ft) latitude 31.58′ N and longitude 77.06′ E.

Isolation and screening of fluorescent *Pseudomonas* species

Bacteria isolated from the rhizosphere, root samples were shaken vigorously to remove loosely adhering soil and 4.5 ml of sterile physiological water was added to 0.5 g of rhizospheric soil and the mixture was shaken at 120 rpm for 2 min. Serial ten-fold dilutions were prepared from the extract and 0.1 ml of each dilution was seeded onto King B medium (Kings *et al*., 1954), supplemented with 100 ìg/ml of cycloheximide to suppress fungi. After 28 ± 2°C for 48 h incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light.

In-vitro assay of fluorescent *Pseudomonas* isolates for plant growth promoting traits

All thirty isolates were screened out both qualitatively and quantitatively for the presence of PGPR traits viz., production of plant growth regulator, siderophore, ammonia, HCN, Phosphate solubilization and antifungal activity by using their standard methods

Quantitative estimation of IAA production

All the 20 isolates were screened for IAA production. Quantitative estimation of auxins was done by colorimetric method (Gordon and Weber, 1951) with slight modifications. 2 to 3 drops of orthophosphoric acid was added to 2 ml supernatant and 4 ml of salper reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 30 % $HClO_4$: prepared fresh). Appearance of red color indicates IAA production OD (optimum density) was measured at 535 nm using spectrophotometer and shown as ìg/ml.

Quantification of gibberellins

The gibberellins were estimated calorimetrically by the method of (Holbrook *et al*. 1961) 15 ml of supernatant, 2 ml of zinc acetate reagent (21.9 g zinc acetate + 1 ml of glacial acetic acid and volume was made upto 100 ml with distilled water) was added. After 2 minutes, 2 ml of potassium ferrocyanide (10.6% in distilled water) was added and was centrifuged at low speed (2000 rpm) for 15 minutes. To 5 ml of supernatant 5 ml of 30 per cent HCI was added and mixture was incubated at 20°C for 75 min. Absorbance was read at 254 nm concentration of gibberellins was calculated by preparing standard curve by using gibberellic acid (GA_3,Hi-media) as standard (100-1000 μg/ml).

Quantification of cytokinins

Radish cotyledons expansion bioassay test was employed (Letham, 1971) for assay of cytokinins the radish seeds (*Raphanus sativus)* were germinated in total darkness for 48 h. at 28°C. The bioassay response (final weight-initial weight) was expressed as increase in weight. Concentration of cytokinins present in the extract was calculated of by preparing standard curve by using kinetin as standard (100-1000 μg/ml).

In vitro antagonism against phytopathogenic fungi

Antifungal activity was observed by the formation of inhibition zone of mycelial growth, based on agar diffusion of extracellular bacterial metabolite. All *Pseudomonas* isolates were tested for their ability to inhibit the growth of soil-borne phytopathogenic fungi *viz*., *Pythium* sp., *Fusarium* sp., *Alternaria* sp., *Dematophora* sp., *Sclerotium* sp. and *Rhizoctonia* sp.) by standard plate assay method (Fleming *et al*., 1975). Petri plates containing sterile potato-dextrose agar were inoculated in the centre with a 5 mm disc of fungal culture grown for 7 days and in the periphery the bacterial strains were inoculated perpendicularly by single streak. Plates were incubated at 28 ± 2°C for 5 days. A bacterial isolate was considered positive for inhibition of fungi when the growth of pathogen under test was absent.

Detection of phosphate solubilising activity

Bacterial isolates were screened on Pikovskaya's agar plates for phosphate solubilization index with known amount of inert phosphorus ($Ca_3(PO_4)_2$) (Pikovskaya's, 1948). Phosphate

solubilisation was expressed in terms of mm diameter of yellow colored zone produced around well. Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was made as described by Bray and Kartz (1945). The absorbance of the developing blue color was read at 600 nm. The amount of soluble phosphorus was detected from standard curve of Potassium dihydrogen orthophosphate (KH_2PO_4).

Assay for siderophore production

Siderophore production by *Pseudomonas* isolates was assayed qualitatively by observing orange halos production around the bacterial colony on CAS agar plates and quantitatively in liquid medium as described by Schwyn and Neilands (1987). Siderophores content was expressed as percentage siderophore units using the formula:

$$\% \text{ Siderophore units} = Ar - As / Ar \text{ -}100$$

Where; Ar = absorbance of reference at 630 nm (CAS reagent)

As = absorbance of sample at 630 nm.

Ammonia and HCN production

Bacterial Isolates were screened for ammonia production by adapting the method of Lata and Saxena (2003) and hydrogen cyanide (HCN) production was detected by the method given by Bakker and Schippers (1987).

Proteolytic activity

All *Pseudomonas* sp. strains were screened out for proteolytic activity by well plate assay method on 1% skim milk agar plates (Kaur *et al.,* 1989) and proteolysis i.e. clear zone (mm dia.) produced around the well was observed. The proteinase liquid assay was based on casein digestion and employed in five minute assay time period. The casein substrate was 2% solution in 0.05M Tris buffer (tris hydroxyl methylaminomethane), pH 8.0. One milliliter of substrate solution was incubated at 37°C with 1ml of enzyme dilution in same Tris buffer (0.05M) for 5 minutes. The mixture was then precipitated with 3 ml of 5%TCA (trichloroacetic acid). The unit of activity was defined as hydrolysis of one equivalent milligram of protein (casein) per milliliter of enzyme per minute under standard assay conditions.

RESULTS AND DISCUSSION

Isolation and identification of indigenous fluorescent *Pseudomonas* species

All the selected thirty isolates from the rhizosphere of apple and pear were found to be fluorescent, with transparent to translucent colonies (irregular to circular colonies with entire edge and flat, raised elevation on nutrient agar plate), pigmented (greenish, brownish, grayish and yellowish pigmentation on King's B medium plates). All bacterial antagonists were found Gram negative, oxidase- positive, rod shaped, non spore forming and all produced yellowish green pigment on King B medium. They all were found to be positive for gelatin liquification, oxidative metabolism, denitrification tests and showed growth at 25°C, 37°C, 41°C by plate method but none of the isolates showed growth at 4°C. None of the isolates were found positive for Tween 80 hydrolysis and lecithinase tests. All isolates showed negative methyl red Voges –Proskaure, citrate test and indole test (Table 1).

***In vitro* screening of plant growth promoting traits of fluorescent *Pseudomonas* species**

Every isolate tested showed at least one of the growth promotion traits that were investigated. The isolates in this study presented several enviable features for PGPR, and multiple action mechanisms which suggest their potential for growth promotion.

Assay of plant growth regulators

Plant growth regulator i.e. auxins production was recorded in the range of 1.83-21μg/ml. The maximum production of auxins was shown by three *Pseudomonas* isolates An-1-kul, An-8-kul, An-13-kul in the range of 14.72-21.00 μg/ml followed by nine isolates that showed less production of auxins i.e. in the range of 7.27-12.98 μg/ml., rest of the isolates showed much less production in the range of 1.83-6.94 μg/mL. All isolates of *Pseudomonas* sp. showed the production of gibberellins in the range of 116.1-485.8 μg/mL. The maximum gibberellins production was observed *Pseudomonas* isolates An-1-kul (485.8 μg/ml) followed by Pn-1-kul and Pn-3-kul (419.2 μg/mL. Each All isolates of *Pseudomonas* species were found to produce cytokinins in the range of 45.4-295.4 μg/mL The maximum production of cytokinins shown by *Pseudomonas* isolates Pn-1-kul (295.4 μg/mL) followed by Ar-3-kul (227.2 μg/mL) and Pn-3-kul (249.9 μg/mL. Isolates were analysised with CRD and found statistically different from each other (Table 2). Production of IAA by PGPR generally affects the root system, increasing the size and number of adventitious roots and also the root subdivision, enabling a bigger soil amount to be exploited by the roots, thus providing large amounts of nutrients accessible to the plant (Ribeiro and Cardoso, 2011). However, IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Sajjad *et al.,* 2001).

Antagonistic activity of fluorescent *Pseudomonas* sp. against plant pathogenic fungi

All thirty isolates of *Pseudomonas* species showed antifungal activity against one or the other plant pathogenic fungi. Nine isolates i.e. six from apple (An-1-kul, An-4-kul, An-7-kul, An-13-kul, An-16-kul and Ar-3-kul) and three (Pn-1-kul, Pn-3-kul and Pr-1-kul) from pear site showed antifungal activity against all the six fungal plant pathogens and ten isolates showed inhibiting activity against five of pathogens. *Pseudomonas* sp. isolates showed antifungal activity against plant pathogen fungi *Alternaria solani* in the range of 1.9-38.00%, *Dematophora necatrix* in the range of 2.22-28.88, *Fusarium oxysporum* in the range of 1.81-26.19%, *Pythium ultimum* in the range of 2.38-38.46%, *Rhizoctonia* sp. in the range of 7.27-53.84 and *Sclerotium rolfsii* in the range of 7.27-26.15% inhibition. Antifungal activities of *P. fluorescence* against phytopathogenic fungi were screened out by Shalini and Srivastava (2008). Secondary metabolites produced by fluorescent Pseudomonads have been reported antifungal activity inhibiting R. solani (Mina *et al.,* 2013). Kamei et al. (2014) revealed that the mycelial growth of R. solani was inhibited up

to 1.9 (cm) by PTR-3 and were found to exhibit antagonism of over 68.9% which is followed by PCF-3(65.6%). It is evident from the present studies that the fluorescent pseudomonads under investigation are capable of producing plant growth promoting substances and antifungal substances. Hence they are potential candidates for the development of bioinoculants for crop plants.

Assay of phosphate solubilization

All the strains are identified as potential phosphate solubilizer based on their capacity to solubilize tricalcium phosphate [$Ca_3(PO_4)_2$] by the formation of clear halo zone on medium. Maximum phosphate solubilization was expressed by *Pseudomonas* isolates on Pikovskaya agar plate in the range of 30-33mm diameter of yellow colored zone. Phosphate solubilization was also expressed in terms of tricalcium phosphate solubilization in liquid medium i.e. PVK broth at 28 ± 2°C for 72h under shake conditions (90 rpm). It was represented as μg/ml of soluble or liberated orthophosphate (Pi) in supernatant as calibrated from the standard curve of KH_2PO_4 (100-1000 μg/ml). Over all *Pseudomonas* isolates showed production of phosphate solubilizing activity in the range of 199.5 to 413.4 μg/ml of available inorganic phosphate (Pi) (Table 3). Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants. The ability of bacteria to solubilize mineral phosphates has been shown of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth. PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to plant that represent a possible mechanism of plant growth promotion under field conditions (Ashrafuzzaman *et al.,* 2009). Free living P-solubilizing bacteria release phosphate from spare soluble inorganic and organic phosphate compounds in soil and so contribute to increase available phosphate for the plants (Gopalakrishanan *et al.,* 2011).

Siderophore production assay

A total of 30 rhizobacterial isolates were screened for siderophore production. All of these isolates grown on CAS agar and produced siderophores. The color of the CAS agar was changed by rhizobacteria from the blue to orange. The variation in color changes in the CAS agar plate (orange, purple or purplish-red) recommend the production of siderophores of a differing nature by the variety of microorganisms isolated and the color intensity can be consequence of siderophore concentration. These siderophore producing microorganisms suppress some soil borne fungal pathogens through direct role of siderophore-mediated iron competition in the biocontrol ability (Chaiham *et al.,* 2009). Maximum siderophore production was shown by *Pseudomonas* sp. in the of range 20-21 mm of diameter of orange color zone by two isolates i.e. one isolate from apple Ar-3-kul (20 mm) and one from pear i.e. Pn-1-Kul (21mm) site. Maximum percent unit of siderophore (%SU) was produced by one isolate An-14-kul i.e. 67.27 %SU followed by 40.00-45.45 %SU (Table 4).

Production of HCN and ammonia

Microbial production of HCN has been suggested as an important antifungal feature to control root fungi pathogen (Ramette *et al.,* 2003). Out of thirty isolates only twenty seven strains showed HCN production on King's B agar amended with glycine (1.4g/L). HCN production by *Pseudomonas* isolates on King's B agar was expressed in terms of change of color of picric acid paper strips from deep yellow (-) to orange brown (+ + + +) at 28°C by *Pseudomonas* species after 4 days. Five isolates each from apple and pear were shown to produced maximum production of HCN in the range of color intensity from yellow to brown (i.e. + + + to + + + +). Ammonia production in peptone water by *Pseudomonas* isolates was expressed in terms of change of color of culture broth from faint yellow (-) to deep brown (+ + + +) at 28°C after 4 days after addition of Nessler's reagent. Cyanide acts as a general metabolic inhibitor to avoid predation or competition. The host plants are generally not harmfully affected by inoculation with HCN production bacteria and host specific rhizobacteria can operate as biological control agents (Saharan and Nehra, 2011).

Assay of protease enzyme

All *Pseudomonas* isolates were screened out for production of protease by well plate assay method using 1% skim milk agar as well as by quantitative assay. Proteolytic activity was expressed in terms of mm diameter of clear zone produced by 100 μl of supernatant around well on skim milk agar plate at 35 ± 2°C after incubation of 48h. 72h old cell free culture supernatants were used as crude enzyme source for protease activity. The presence of protease production was shown by almost all the isolates of *Pseudomonas* sp. in the range of 13-29 mm diameter of clear zone on skim milk agar. Maximum proteolytic activity was recorded in the range of 20-29 mm diameter of clear zone. In quantitative estimation of proteolytic activity was recorded in the range of 58.82-87.05 Units per ml. One unit of protease was expressed as the solubilization of 1mg of casein substrate hydrolyzed by 1ml of crude enzyme under standard assay conditions. Maximum proteolytic activity was expressed in the range of 81.17-87.05 units (Table 5). Thus, it is obvious from this preliminary investigation that the fluorescent *Pseudomonas* strain are able to produce plant growth promoting. The production of secondary metabolites such as IAA, HCN, Salicylic Acid, chitinase, and siderophore by fluorescent Pseudomonads are important feature in plant disease suppression of root rot and enhancement of plant growth (Gade, 2013).

Thus, the potential candidates of PGPR for the development of biofertilizer and bioinoculants for crop plants. The world over is changing from inorganic conventional farming towards organic ecofriendly farming methods. This not only requires the isolation of bioinoculants with high potential for use as biofertilizers but also several other factors right from appropriate application procedures to correct marketing practices also being economically cheaper. These results proved that plant growth activities produced by *Pseudomonas* species could also play a critical role in plant growth promotion. In conclusion, fluorescent *Pseudomonas* strains An-1-Kul and An-13-Kul isolated from apple rhizosphere have possible potential for the plant growth promotion under field condition due to their multifarious plant growth promoting traits. The acquired knowledge can be used to improve the

consistency and level of effectiveness of PGPR.

ACKNOWLEDGEMENT

The authors are highly thankful to the department of Basic Science (Microbiology) and department of Plant Pathology of Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173230, India to carry out the present work.

REFERENCES

Ahmad, F., Ahmad, I. and Khan, M. S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turkish Journal of Biology*. **29:** 29-34.

Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, M.A. and Islam, M.Z. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*. **8:** 1247-1252.

Bai, Y., X. Zhou and D.L. Smith, 2003. Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. *Crop Science*. **43:** 1774-1781.

Bakker and Schippers 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas spp*. Mediated plant growth stimulation. *Soil Biology and Biochemistry*. **19:** 451-457.

Bano, N. and Musarrat, J. 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Current Microbiology*. **46:** 324-328.

Bhattacharya, P. and Jha, D. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*. **1:** 24.

Bloemberg, G. V. and Lugtenberg, B. J. J. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology*. **4:** 343-350.

Bray, R. H. and Kurtz, L. T. 1945. Determination of total organic available forms of phosphorus in soil. *Soil Science*. **23:** 343-353.

Chaiharn, M., Chunhaleuchanon, S. and Lumyong, S. 2009. Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. *World Journal of Microbiology and Biotechnology*. **25:** 1919-1928.

Dey, R. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogea* L.) by application of plant growth promoting rhizobacteria. *Microbiological research*. **159:** 371-374.

Fleming, H. P., Etchells, J. L. and Costilow, R. H. 1975. Microbial inhibition by an isolates of *Pediococcus* from cucumber brines. *Applied Microbiology*. **30:** 1040-1042.

Gade, R. M. 2013. Biological and chemical management of *phytophthora* root rot /collar rot in citrus nursery. *The Bioscan*. **7(4):** 631-635.

Gopalakrishnan, S., Humayun, P., Kiran, B. K., Kannan, I. G. K. and Vidya, M. S. 2011. Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. *World Journal Microbiol Biotechnology*. **27:** 1313-1321.

Gordon, S. A. and Weber, R. P. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiology*. **26:** 192-195.

Holbrook, A. A., Edge, W. L. W. and Bailey, F. 1961. Spectrophotometric method for determination of gibberellic acid in gibberellins, ACS Washington, D.C. pp. 159-167.

Kamei, A., Dutta, S. and Nandi S. 2014. Role of secondary metabolites on biocontrol potentialities of native rhizobacterial isolates against *Rhizoctonia solani*. *The Bioscan*. **9(1):** 253-257.

Kaur, M., Gupta, M., Tripatathi, K. A. K. and Gupta K G. 1989. Lytic effect of *Pseudomonas aeruginosa* elastase on gram positive and gram negative bacteria*: entrablatt Bakt. Indian Journal Microbiology*. **34:** 855-859.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Chemical Medicine*. **44:** 301-307.

Lata and Saxena, A. K. 2003. Characterization of plant growth promoting rhizobacteria. In: Training manual on Biofertilizer Technology (Eds.) A. K. Saxena. IARI Delhi. pp. 24-25.

Letham, A. 1971. Regulator of cell division in plant tissues XII. A cytokinin bioassay using excised radish cotyledons. *Physiology Plant*. **25:** 391-396.

Mina, D. Koche, Gade, R. M. and Deshmukh, A. G. 2013. Antifungal activity of secondary metabolites produced by *Pseudomonas fluorescens*. *The Bioscan*. **8(2):** 723-726.

Mubarik, N. R., I. Mahagiani, A. Anindyaputri, S. Santoso and Rusmana, I. 2010. Chitinolytic bacteria isolated from chili rhizosphere: Chitinase characterization and its application as biocontrol for whitefly (*Bemisia tabaci* Genn.). *American Journal Agriculture Biology Science*. **5:** 430-435.

Patten, C. and Glick, B. 2002. Role of *Pseudomonas putida* indole acetic acid in development of host plant root system. *Applied and Environmental Microbiology*. **68:** 3795-3801.

Pikovskaya, R. E. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologia*. **17:** 362-370.

Ramette, A., Moënne, Loccoz, Y. and Défago, G. 2003. Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. *FEMS Microbiology Ecology*. **44:** 35-43.

Ribeiro, C. M. and Cardoso, E. J. 2011. Isolation, selection and characterization of root associated growth promoting bacteria in Brazil Pine (*Araucaria angustifolia*). *Microbial Research*. **167:** 69-78.

Saharan, B. S. and Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A critical review. *Life Science and Medical Research*. 2011, LSMR-21.

Sajjad, Mirza, M., Ahmad, W., Latif, F., Haurat, J. and Bally, R. 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. *Plant and Soil Science*. **237:** 47-54.

Schroth, M. N. and Hancock, J. E. 1982. Disease suppressive soil and root-colonizing bacteria. *Science*. **216:** 1376-1381.

Schwyan, B. and Neilands, J. B. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytic Biochemistry*. **28(8):** 751-759.

Shalini and Srivastava, R. 2008. Screening for antifungal activity of *Pseudomonas fluorescens* against phytopathogenic fungi.*The International Journal of Microbiol*ogy. **5:** 2.

Vasanthakumar, A. and Mcmanus Patricia, S. 2004. Indole-3- acetic producing bacteria associated with cranberry stem gall. *Journal Bacteriology*. **94:** 1164-1171.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*. **255:** 571-586.

Weller, D. M., Raaijmakers, J., Mcspadden, Gardener, B. and Thomashow, L. S. 2002. Microbial populations responsible for specific soil suppressive to plant pathogens. *Annual Review of Phytopathology*. **40:** 309-348.

Wu, S. C.,. Cao, Z. H., Z. G. Li, Cheung, K. C. and Wonga, M. H. 2005. Effects of biofertilizer containing N-fixer, P and K-solubilizer and AM fungi on maize growth: A greenhouse trial. *Geoderma*. **125:** 155-166.