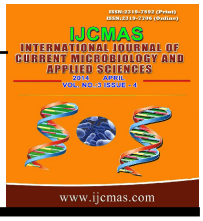
Int.J.Curr.Microbiol.App.Sci (2014) 3(6) 799-809

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 799-809**
http://www.ijcmas.com



Original Research Article

Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna Radita* (L.) R.Wilczek

K.Geetha[1*], E.Venkatesham[2], A.Hindumathi[2] and B.Bhadraiah[3]

Mycology and Plant pathology laboratory, Department of Botany, Osmania University, Hyderabad – 500 007, A. P., India

Corresponding author

K e y w o r d s

PGPR,
Mung bean,
antagonistic activities,
extra cellular enzyme production

A B S T R A C T

Utilization of plant growth promoting rhizobacteria (PGPR) in order to increase the productivity may be a viable alternative to organic fertilizers. The main goal is to reduce the pollution and to preserve the environment in the spirit of an ecological agriculture. Plant growth promoting rhizobacteria (PGPR) influence the plant growth by various direct or indirect mechanisms. . Keeping in this view, the present investigations were undertaken to screen the PGPR isolates from its natural growing zones of plant rhizosphere. A total of 140 bacteria were isolated from plant rhizosphere soils during 2010-2012. Among them 30 potential bacterial strains showing antagonistic and PGP activities were selected for characterization. Out of which 6 strains was selected for further studies based on various morphological, biochemical and physiological screening methods These ten isolates of bacteria, designated as KG-50, WG-57, MG-58, TG-60, MG-64 and BG-72. Of the total, 2 isolates belonged to Gram positive and 4 isolates belonged to Gram negative. The plant growth promoting properties, all isolates exhibited production of indole acetic acid whereas 2 isolates produced HCN and solubilized inorganic phosphate. Subsequently, to investigate *In vitro* test for all the 6 isolates the antagonistic activity against, *Fusarium oxysporum*, *Colletotrichum capsici*, *Rhizoctonia solani*, *Macrophomina Phaseolena* spp. The Selected isolates showed significant plant growth promotion with respect to increase in root and shoot length and number of secondary roots as compared to control. The present study, therefore, suggested that these strains of PGPR potential and biocontrol ability which can be used as biofertilizers as well as biocontrol agents. Hence, these isolates can be further formulated and used for field applications.

Introduction

Green gram [*Vigna radiata* (L.) Wilczek], is an important pulse crop of India. It is also commonly known as mung bean, which is an ancient and well known leguminous crop of Asia. It is quite versatile crop grown for seeds, green

manure and forage and it is also considered as "Golden Bean" because of its nutritional values and suitability for increasing the fertility of the soil, by the way of addition of nitrogen to the soil. On an average, pulse crops add upto 30 kg nitrogen per hectare per year. Because of its short duration, it fits well in crop rotation and mixed cropping systems. Presently, the per capita share of pulses in nutrition supply in India with respect to energy, protein and fat is 117.4 K cal, 6.9 g and 1.0 g per day respectively. An adult male and female requires 80 and 70 g per capita per day, respectively for balanced diet (Anon, 2004) Green gram crop covers a total world area of 5 m ha with a total production of 3 mt (John, 1991). It is widely cultivated throughout the South Asia including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia and South China. India is an important pulse growing country contributing 28 per cent to the global pulse basket from an area of about 37 per cent (Masood Ali, et.al, 2000).

Nowadays, microorganisms play an important role in agricultural system, especially the group of bacteria called plant growth promoting rhizobacteria (PGPR). PGPR are widely studied because of their potential for plant production under three characteristics. Firstly, PGPR acting as biofertilizers (Vessey, 2003). Provide nitrogen via nitrogen fixation reaction, which can subsequently be used by the plants. Secondly, phytostimulators (Steenhoudt, et.al,. 2006) can directly promote the growth of plant, usually by the production of plant hormones. Finally, biological control agents (Costa et al., 2007) are able to protect plant via root system from phyto pathogenic organisms. The application of PGPR in agricultural system as inoculants is being very attractive since it would substantially reduce the use of chemical fertilizers and pesticides as well as a growing number of PGPR is markets in the developed countries as EU and USA. With the use of PGPR gaining acceptance, numerous bacterial species have been isolated and their capacity to promote plant growth has been investigated. In the search for efficient PGPR strains with multiple attributes, various genera of bacteria show promising results. Thus, bacteria genera including Azotobacter, fluorescent *Pseudomonas* species, *Rhizobium* and *Bacillus* are widely used (Teaumroong et al., 2010).

Materials and Methods

Collection of soil samples from green gram rhizosphere

The present investigation was under taken with an objective to select a promising native PGPR strains against pathogen of Green gram. Systematic surveys of Green gram growing areas of Southern and Northern Agro-climatic zones of Telangana region, Andhra Pradesh, India was under taken periodically to assess the prevalence of the disease during Rabhi and Kharif cropping seasons. The samples were placed in plastic bags and stored at 4°C in the Laboratory, Department of Botany, Osmania University for further analysis.

Isolation and maintenance of bacteria

Bacteria was 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 140 rpm for 2 min. Serial ten-fold dilutions were

prepared from the extract and 0.1 ml of each dilution was added onto King's B medium, supplemented with 100 µg/ml of cycloheximide to suppress fungi. The spread-plate cultures were incubated for 48 h at 25 ± 1°C. Ten to fifteen representative colonies, with different morphological appearances, were selected from the countable plates and re-streaked on a new plate containing the same media to obtain pure colonies. A total of 140 candidate isolates obtained in this manner were maintained on nutrient agar slants at 4°C

Microscopic observation and morphological characterization

The selected bacterial isolates were examined for their morphological features. The morphological characteristics were examined on their respective agar plates. The pure cultures from the slants were placed on the agar plates. After the growth of colonies morphological characters of the colonies like the colour, shape, size, surface and gram staining etc. were recorded. All the 80 bacterial isolates were screened for their growth promoting activities like indole acetic acid (IAA) production, ammonia production, phosphate solubilization, HCN production, hydrolytic enzyme production such as catalase, proteases, lipases and amylases and their antifungal activity against the plant pathogen.

Screening of PGPR for multiple plant growth promoting activities

IAA production

Luria Bertani broth medium (25 ml) amended with 50 µ/ml tryptophan was inoculated with the isolated bacteria. They were incubated for 24 h at 28°C on rotary shaker. Cultures were centrifuged at 10,000 g for 15 min. 2 ml of supernatant was taken and 2 to 3 drops of ortho-phosphoric acid was added. 4 ml of Salkowski reagent was added and incubated for 25 min. at room temperature and development of pink colour indicates the IAA production. Absorbance was read at 530 nm. Auxin production was determined by using a standard graph (Gordon, A.S et.al,. 1951).

Production of Ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube separately and incubated for 48-72 h at 28 ± 2°C. Nesseler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappucino et.al,. 1992).

Phosphate solubilizing activity

The plates were prepared with Pikovaskya's medium. The culture of ten isolates were streaked on the plates and incubated in an incubator at 28°C for 7 days. The plates were then examined and data were recorded (Pikovaskya, 1948).

HCN production

Hydrogen cyanide (HCN) production was evaluated by streaking the bacterial isolates on King's B agar medium amended with glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. The plates were then sealed air-tight with Parafilm and incubated at 30°C for 48 h. A colour change of the filter paper from deep

yellow to reddish-brown colour was considered as an indication of HCN production (Baker et.al., 1987).

Extra cellular enzyme activities

Catalase activity

Catalase test was performed by taking a 3-4 drops of hydrogen peroxide (H_2O_2) was added to 48 h old bacterial colony which is grown on trypticase soya agar medium. The effervescence indicated catalase activity (Schaad NW, 1992).

Caseinase (protease) activity

The qualitative assay for protease production was performed on sterile skim milk agar plates (Panc. digest of casein 5.0, Yeast extract 2.5, Glucose 1.0, Agar 15.0, Distilled water 1000 ml, Skim milk 7% was added as inducer). Isolates were spot inoculated and followed by incubation at 30^{o}C and zone of clearance around the colony indicating the enzymatic degradation of protease (Chaiharn 2008).

Lipase activity

Bacteria were grown on nutrient agar amended with egg yolk. After 48 h of incubation the agar medium was flooded with saturated aqueous solution of copper sulphate ($CuSo_4$) and kept for 10-15 min. The excess reagent was poured off. Formation of greenish blue colour zones around the colony indicated the production of lipase (Omidvari M, 2008).

Amylase (starch hydrolysis) activity

The bacterial isolates were spot inoculated on starch agar (Beef extract 3.0, Peptone 5.0, soluble starch 2.0, Agar 15.0, Distilled water 1 lit.) medium plates and incubated at 30°C for 48 h. At the end of incubation period, the plates were flooded with iodine solution, kept for a minute and then poured off. Iodine reacts with starch to form a blue colour compound. This blue colour fades rapidly. Hence the colour less zone surrounding colonies indicates the production of amylase **(**Collins, C. H 1995**).**

Cellulase activity

Cellulase production was determined by using the method (Miller G.L 1959). M9 agar medium with yeast extract plates were inoculated with individual bacterial isolates and incubated for 3-5 d at 28C. Bacterial growth surrounded by clear halos was considered as positive indication of cellulase production.

Pectinase activity

Pectin degrading enzymes were screened by using M9 medium amended with 4g of Pectin per liter. The plates were incubated for 2 days at 28+2°C. The appearance of clear halo around colonies indicates pectinase production (Fogarty WM 1982).

Antagonistic activities against green gram plant pathogenic fungi

The antagonistic effects of all 6 bacterial isolates were tested against fungal pathogens of *Macrophomina phaseolina*, *Fusarium oxysporum*, *Colletotrichum capsici* and *Rhizoctonia solani*) For this the bacterial isolates were streaked at a distance of 3.5 cm from rim of individual Petri plate containing potato dextrose agar (PDA) medium. 6 mm mycelial disc from a 7-day old PDA culture of fungal pathogens were then placed on the other side of the Petri dish and the plates were incubated at 28°C for 4-7 days (Rabindran et.al, 1996). Antifungal activity was estimated from the inhibition of mycelial growth of fungus in the direction of

actively growing bacteria. The level of inhibition was calculated by subtracting the distance (mm) of fungal growth in the direction of an antagonist from the fungal radius. The percent inhibition was calculated using the formula:

$$PI = \frac{(R-r)}{R} \times 100$$

Where ' r ' is radial growth of the fungal colony opposite the bacterial colony and, R is the radial growth of the pathogen in control plate.

Where, PI = Percent inhibition

R = Radial growth of pathogen in control plate

r = Radial growth of the fungal colony opposite the bacterial colony

Results and Discussion

Isolation and characterization of bacterial isolates

A total of 140 bacteria were isolated from various varieties of plant rhizosphere soils from khammam districts during 2011 to 2013(Table1). These isolates were evaluated for their antagonistic and plant growth-promoting traits. Six best potential bacterial strains showing antagonistic and PGP activities were selected for characterization. Among 6 isolates two were Gram positive and four were Gram negative.

Morphological characteristics and Microscopic observations of PGPR isolates

The morphological characteristics of PGPR isolates (KG-50, WG-57, MG-58, TG-60, MG-64 and BG-72) varied widely. All the isolates produced round shaped and raised colonies having rough surface with undulated to erosive margins /smooth shiny surface with smooth margin. No pigmentation was observed on NA media. Microscopic observations were performed to investigate the characteristics of PGPR isolates such as shape, gram reaction and motility. All isolates were rod shaped, motile. In gram reaction two isolates were gram positive reaming four isolates are gram negative reaction (Table2).

Plant growth promoting activities of PGPR isolates

The isolates showed varied levels of PGPR traits such as phosphate solubilization, IAA, ammonia, and HCN production (Table 3).

Phosphate Solubilization

Six of the five strains exerted ability for phosphate solubilization on Pikovskaya medium with different efficacy. Out of six 4 strains showed maximum degree of phosphate solubilization of 52 %. The maximum phosphate solubilization was identified in WG 57 strain. The phosphate-solubilizing activity characterizes the microorganisms with ability to produce and release metabolites such as organic acids that chelate the cations bound to phosphate, converting them into soluble forms.

IAA production

Auxin is the most investigated hormone among plant growth regulators. The most common, best characterized and physiologically most active auxin in plant is indole-3-acetic acid (IAA). IAA is known to stimulate both a rapid response (e.g. increased cell elongation) and a long-term response (e.g. cell division and

differentiation) in plants**.** In our study, 5 bacterial isolates were able to produce indole-3-acetic acid (IAA) growing in medium without addition of tryptophan. Maximum IAA production was recorded in WG-57 strain as compared to other isolates.

HCN production

Ability for hydrogen cyanide synthesis was observed for selected isolates of 6 strains (MG-58, MG-64) Hydrogen cyanide mediated antagonism was observed for isolate R and P6 which is in agreement to earlier reports. The increased production of HCN by the efficient strain of *P. fluorescens* contributed to effective inhibition of mycelial growth of *Rhizoctonia solani in vitro* and appears to be a major factor in control of soil-borne disease by *Pseudomonas fluorescens*

Ammonia production

The production of ammonia observed in all the six isolates. The ammonia is useful for plant as directly or indirectly. Ammonia production by the plant growth promoting bacteria helps influence plant growth indirectly.

Extra cellular enzyme activities

Proteolytic enzyme production

Proteolytic enzyme production was detected as formation of a clear zone around the colony on skim milk agar medium for six strains KG-50, WG-57, MG-58 showed proteolytic activity.

Lipase activity

Bacterial cultures were grown on nutrient agar amended with egg yolk. After 24hr of incubation clear zones around the colony indicates positive for lipase activity. Strain KG-50, TG-60 showed lipase activity.

Cellulase activity

M9 agar medium with yeast extract plates were inoculated with individual bacterial isolates and incubated for 3-5 d at 28C. Bacterial growth surrounded by clear halos was considered as positive indication of cellulase production. KG-50, WG-57, TG-60 and BG-72 showed cellulase activity.

Pectinase activity

Pectin degrading enzymes were screened by using M9 medium amended with 4g of Pectin per liter. The plates were incubated for 2 days at 28+2°C. The appearance of clear halo around colonies indicates pectinase production. All isolates showed Pectinase activity.

Amylase activity

Amylase activity was determined by clear zone on starch agar medium. After 72 t0 96 hr of incubation The plates were flooded with Iodine solution for 1min and pour the excess iodine solution, the appearance of clear zone surrounding the colony indicates positive for starch hydrolysis test WG-57,MG-58, TG-60,MG-64and BG-72 showed Amylase activity.

Catalase activity

Catalase test was performed by adding three to four drops of H_2O_2 on bacterial culture which was grown for 48hr on trypticase soy agar medium. The effervescence indicates Catalase activity KG-50, WG-57, MG-58, TG-60 and BG-72 showed Catalase activity.

The present study revealed the production of mycolytic enzymes viz. cellulase, protease and lipase. Mycolytic enzymes produced by antagonistic microorganisms are very important in biocontrol technology. There are many reports on production of lytic enzymes by microorganisms.

Table.1 Selected Locations for isolation of PGPR

S.NO	Isolates	Location of rhizosphere soil	Variety of the crop
1	WG-57	Wyra	ML-267
2	MG-58	Maddulapally	ML-267
3	TG-60	Thallampadu	Local variety
4	MG-64	Madhira	ML-267
5	KG-50	Kistapuram	ML-267
6	BG-72	Beerolu	Local variety

Table.2 Morphological and Microscopic characters of PGPR isolates

S.No	Isolates	Motility	Shape	Gram stain	Colour	Surface	Margin	Pigmentation
1	KG-50	Motile	Bacilli	+	White	Smooth	Rough	Not showing
2	WG-57	Motile	Bacilli	+	White	Smooth	Rough	Not showing
3	MG-58	Motile	Bacilli	+	White	Smooth	Rough	Not showing
4	TG-60	Motile	Bacilli	-	White	Smooth	Rough	Not showing
5	MG-64	Motile	Bacilli	+	White	Smooth	Rough	Not showing
6	BG-72	Motile	Bacilli	+	White	Smooth	Rough	Not showing

+ =Gram positive, - =Gram negative

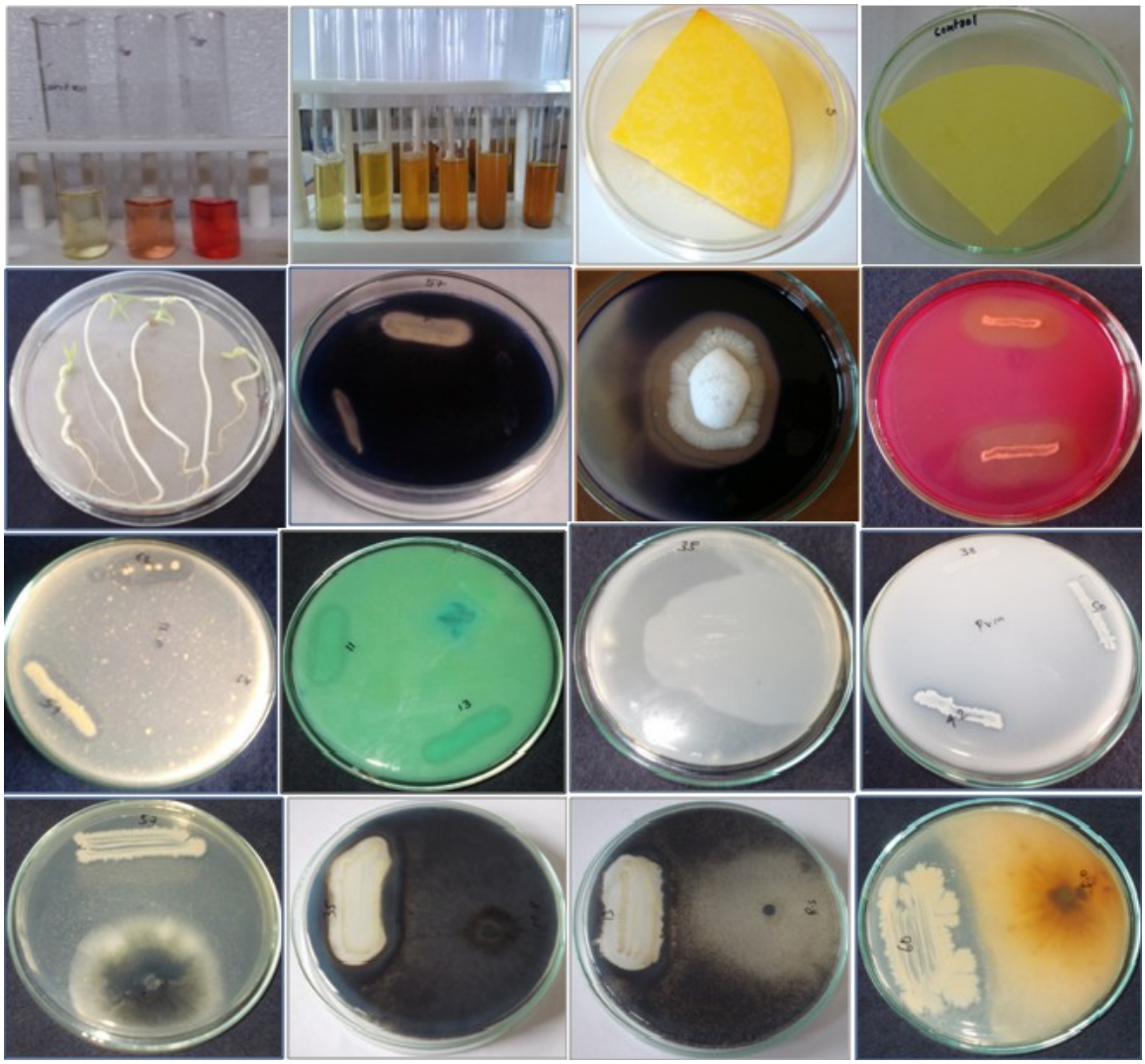
Table.3 Lytic enzymes production and Plant growth promotion traits of selected isolates

S.NO	IAA	Ammonia	HCN	Phosphate solubilization	Enzyme Production					
					Cellulase	Protease	lipase	Pectinase	Amylase	Catalase
KG-50	+	+	-	++	++	++	++	++	-	+
WG-57	+	+	-	++	++	++	-	+	++	+
MG-58	+	+	+	++	-	++	-	+	++	+
TG-60	+	+	-	-	+	-	++	++	+++	+
MG-64	-	+	+	+	-	-	-	++	+++	-
BG-72	+	+	-	-	+	-	-	+	++	+

Table.4 Antagonistic activities against plant pathogenic fungi

Isolates	Antagonistic activity			
	C. capsici	***R. solani***	***M.Phaseolena***	***F.oxysporum***
KG-50	++++	++++	+	+++
WG-57	+	+	+	+
MG-58	++	+	+	++
TG-60	-	+	++	+
MG-64	-	++	+	+
BG-72	++	++	+	++

Figure.1 Antagonism, growth promoting properties and Extracellular enzyme activity of Greengram rhizobacterial isolates:



A. IAA Production 1- Control, 2- High Production, 3- Low Production; **B**. Ammonia Production 1- Control, 2- Low Production, 3 & 4 -High Production, 5 & 6-Moderate Production; **C**. HCN production; **D.** Seed germination; **E**. Amylase activity; **F.** Catalase activity; **G.** Cellulase activity; **H.** Protease activity; **I.** Lipase activity; **J.** Pectinase activity; **K.** Phosphate solubilization; Antifungal activity **L.** *Colletotrichum Capscici* / G57 **M.** *Macrophomina Phaseolena* / G58 **N.** *Rhizoctonia solani* / G50, **O.** *Fusarium oxysporum* / G60.

Plant rhizosphere is known to be preferred ecological niche for various types of soil micro organisms due to rich nutrient availability. It has been assumed that inoculation with diazotrophic bacteria like Rhizobium, Azato bactor, and Azospirillum enhanced the plant growth. However, despite of entensive research efforts, only rhizobia have been shown to increase yields. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (B.R. Glick 1995) & (Blumer C, 2000)

In the present study, Isolation of bacterial cultures from rhizosphere soil samples of green gram. The rhizosphere soil supported a total of 140 PGPR isolates with varied characteristics. Among 140 isolates 35 Bacterial strains were selected out of which 6 Potential strains KG-50, WG-57, MG-58, TG-60, MG-64, BG-72 were selected.

In the present study, from 6 bacterial isolates KG-50, TG-60 showed Gram positive and WG-57, MG-58, MG-64.BG-72 showed Gram negative reaction. All the isolates were screened for their plant growth promoting activities viz., Indole acetic acid production (IAA), Ammonia production, Phosphate solubilization, HCN production, other lytic enzymes like Catalase, Protease, Lipase, Amylase, Cellulose, Pectinase and Antagonistic activies. Among 140 Bacterial isolates 5 isolates showed IAA production , 6 isolates showed Ammonia production, 4 isolates showed Phosphate solubilizing activity, 2 isolates showed HCN production, 6 isolates showed Antagonism against *Rhizoctonia solani, Macrophomina phaseolena and fusarium oxysporum*, 4 isolates showed Antagonism against *Colletotrichum capscici* . The result showed that not all 140 isolates possessed all PGP activity. They differed in the possession of PGP activities. The range of percentage of positive isolates for each PGP activities varied greatly. All most similar results in the assessment of PGP activities of bacterial isolates from the rhizosphere of tomato were reported . This result will lead to developing Bio fertilizers for commercially grown green gram plants.

The most important trait of PGPR is the production of ammonia that indirectly influences the plant growth. All the selected isolates were positive for ammonia production and also produced significant amount of IAA. In our study, from 6 bacterial isolates 4 (KG-50, WG-57, MG-58 and MG-64) were able to solubilize phosphate in the plate-based assay, by showing a clear halo zone around the colony. Among which KG-50, WG-57 showed more production then the other isolates. IAA production promotes plant growth and HCN production has been proposed as a defense regulatory against phyto pathogens was already reported (Dey R, et.al., 2004). Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress , 5 isolates were positive for catalase activity, 4 for cellulose, 3 for protease, 2 for lipase and 5 for amylase. Pectinase showed positive for all 6 isolates. Antagonistic activity of the bacterial isolates were evaluated in terms of inhibition zone diameter as an indicator of the reduction in growth of green gram pathogenic fungi *Rhizoctonia solani, Macrophomina phaseolena, Colletotrichum capscici and fusarium oxysporum*. Bacterial plant growth promotion is a well established and complex phenomenon that is often

achieved by the activities of more than one PGP trait exhibited by plant associated bacteria. In our study 55% of the isolates exhibited more than 2 PGP traits.

Currently our research effort is towards helping the poor farmers in this study we focus on role the of rhizobacteria in plant growth promotion. A pool of promising rhizobacteria was screened through *in-vitro* and their plant growth promoting properties. To evaluate the influence of the most promising bacterial strains on plant growth, bacterized tomato seed were planted in paper towel method. The potential of these has been analyzed,that is to provide data that effect the growth parameters in green gram. Based on the results we suggested that these strains of PGPR potential and biocontrol ability which can be used as biofertilizers as well as biocontrol agents. The differences in plant growth promotion among the isolates are attributed to their individual competencies. Future studies are required to prove the nature of these isolates and to harness their potential as bio-inoculants in agriculture.

Acknowledgment

We are gratefully acknowledging the UGC-Major Research Project New Delhi, for providing financial support for this work. We are thankful to Dr. K.V.B.R Thilak for their kind help in our research work and we are also thankful to Head department of botany, OsmaniaUniversity, Hyderabad for providing necessary laboratory facilities.

References

Anonymous, 2004, Recommended dietary allowance for Indians. *Survey of Indian Agriculture*, Pub. *The Hindu*, pp. 54.

B.R. Glick, (1995) The enhancement of plant growth by free living bacteria Can. J. Microbiol., 41, pp. 109–114.

Bakker, A. W. and Schipperes, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomoas* spp. - mediated plant growth stimulation. *Soil Biol. Biochem.* 19: 451 - 457.

Blumer C and Hass D. (2000) Arch of Microbiol, 173:170-177.

Cappuccino, J.C. and Sherman N, Microbiology(1992): A Laboratory Manual, 3 Edn, Benjamin/cummings Pub. Co. New York. 125–179.

Chaiharn M, Chunhaleuchanon S, Kozo A and Lumyong S, (2008). screening of rhizobacteria for their plant growth promoting activities: M I TL Sci Tech J 8 (1) : 18-23.

Costa, R., Gomes, N. C. M., Kr gerrecklenfort, E., Opelt, K., Berg, G., and Smalla, K. (2007): *Pseudomonas* community structure and antagonistic potential in the rhizosphere: insights gained by combining phylogenetic and functional gene-based analyses. Environ. Microbiol**.** 9, 2260-2273.

Dey R, Pal KK, Bhatt DM & Chauhan SM (2004). Microbiol Res, 159:371-394.

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

John, M. P., 1991, *The Mungbean*, Oxford and IBH Publishing Co. Pvt. Ltd., pp. 375.

Kloepper JW, Ryu CM and Zhang S, (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp: Phytopathology. 94:1259-1266.

Masood Ali and Shivkumar, 2000, Problems and prospects of pulses

research in India. *IndianFarming*, pp. 4-13.

Miller G L, (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar: Anal Chem. 31: 426-428.

Omidvari, M. (2008). Biological control of *Fusarium solani*, the causal agent of damping off, by fluorescent pseudomonads and studying some of their antifungal metabolite productions on it. MS thesis, Tehran University, Iran.

Pikovaskya R.E, (1948). Mobilization of phosphorous in soil in connection with vital activity of some microbial species: Mikrobiologiya. 17: 362-370.

Rabindran R & Vidyasekaran P. (1996).Crop Prot, 15:715-721.

Schaad NW, (1992). Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd Edn, International Book Distributing Co, Lucknow. 44-58.

Steenhoudt, O., and Vanderleyden, J. (2006): *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol. Rev. 24, 487-506.

Sunil a. kulkarni (February 2009), Epidemiology and integrated management of anthracnose of greengram) by department of plant pathology college of agriculture, dharwad , university of agricultural sciences, dharwad – 580 005.

Teaumroong, N., Wanapu, C., Chankum, Y., Arjharn, W., Sang-Arthit, S., Teaimthaisong, K., and Boonkerd, N. (2010): Production and application of bioorganic fertilizers for organic farming systems in Thailand: A Case Study, pp. 293-312.

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