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In vitro evaluation of *Pseudomonas* bacterial isolates from rice phylloplane for biocontrol of *Rhizoctonia solani* and plant growth promoting traits

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Abstract

The ability for biocontrol and plant growth promotion of three *Pseudomonas* bacterial isolates namely *Pseudomonas fluorescens* (UMB20), *Pseudomonas aeruginosa* (KMB25) and *Pseudomonas asplenii* (BMB42) obtained from rice plants was investigated. Fungal growth inhibition by the isolates ranged from 86.85 to 93.15% in volatile and 100% in diffusible metabolites test. Among the isolates, BMB42 showed fungal growth inhibition significantly in the volatile metabolite test. Isolates UMB20 and BMB42 were able to synthesis chitinase with chitinolytic indices of 13.66 and 13.50, respectively. In case of -1,3-glucanase, all the isolates showed activity to produce this enzyme at varied levels and isolate KMB25 showed significantly highest activity (53.53 ppm). Among the three isolates, KMB25 showed positive response to protease production and all of them were negative to pectinase and lipase and positive to the production of siderophore, and HCN, and were able to solubilize tricalcium phosphate. All the three bacterial isolates were capable of forming biofilm at different levels. Above results suggest that phylloplane *Pseudomonas* bacterial isolates have potential for antifungal activities and plant growth promotion.

Key words

Biocontrol, Growth promotion, Hydrolytic enzymes, *Pseudomonas* isolates

Introduction

Bacteria having capacity of exerting the effects on disease suppression and plant growth (Mubarik *et al*., 2010) directly or indirectly are known as plant growth promoting rhizobacteria (PGPR). Effects of these agents on disease suppression are related to exclusion of phytopathogens by aggressive colonization of root environment, or by production of broad spectrum of extracellular lytic enzymes, diverse antibiotics, hydrogen cyanide or by activation of plant defence mechanisms (Jamali *et al*., 2009). Influence of PGPR on plant growth depends on the production of either phytohormones or by enhancing nutrient uptake (Bai *et al*., 2003). Some genera of bacteria such as *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Bradyrhizobium* and *Rhizobium* (Wahyudi *et al*., 2011) have demonstrated PGPR activities in different crops. Among them, *Pseudomonas* is one of the most important group that possesses almost all PGP traits. The beneficial effect of fluorescent pseudomonads have been attributed to production of chitinase, β-1,3-glucanase, hydrogen cyanide (HCN), siderophores, antibiotics, phytohormones,

solubilization of phosphate and induction systemic resistance against a broad diversity of pathogens (Podile and Kishore, 2006). These characteristics make them good candidate as biocontrol agents for suppressing plant pathogens. Particular isolates of *Pseudomonas* like *P. fluorescens*, *P. putida*, *P. aeruginosa* and *P. aureofaciens* have drawn worldwide attention for their potential suppression of soil-borne pathogens through different mechanisms (Karthekeyan *et al*., 2006).

Although *R. solani* is a soil-borne pathogen and for effective control and plant growth promotion biocontrol agents should be obtained from rhizosphere.Several researches have demonstrated the efficacy of phyllosphere bacterial strains on crops from different genera including *Pseudomonas* against different soil-borne pathogens (Sato *et al*., 2014; Kishore *et al*., 2005). Among others, *Pseudomonas* bacteria are common inhabitants of rhizosphere and phyllosphere and are able to cope with wide range of environmental conditions. So far, most of the *Pseudomonas* isolates were obtained from the rhizosphere, but only few from phyllosphere. Plant growth promoting traits of *Pseudomonas* isolates originating from phyllosphere need to be investigated. Hence, the present study was undertaken to determine the biocontrol and plant growth promoting activities of selected phylloplane *Pseudomonas* bacteria under laboratory conditions.

Materials and Methods

Microorganisms and culture conditions : Bacterial isolates *Pseudomonas fluorescens* (UMB20), *Pseudomonas aeruginosa* (KMB25) and *Pseudomonas asplenii* (BMB42) and sheath blight pathogen *Rhizoctonia solani* were collected from Plant Protection Department, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia. Bacterial isolates were identified by Biolog identification system and selected for the study, based on the results of dual culture test (Akter *et al*., 2014). They were preserved in King's B Broth (KBB) broth in 50% glycerol at -20 ºC refrigerator and were revived from frozen condition prior to each experiment.

Determination of metabolites : Inhibitory potential of bacterial isolates against *R. solani* via production of diffusible metabolites was determined following the methodology of Vidaver (1976) with modifications. Double layered petriplates comprising of two layers of different media were used in this test. The bottom layer of potato dextrose agar (PDA) was separated from the upper layer of nutrient agar (NA) by an overlaid sterilized filter paper (Whatman no. 1). Bacterial isolates were streaked on NA surface and incubated at 28±2°C for 2 days. After incubation, filter paper with NA was removed. Subsequently, mycelial plugs of *R. solani* were placed at the center of the plates and incubated at 28±2°C for 5 days. Control plates were maintained by streaking sterilized water on the medium likewise bacterial cultures. The assay was run in a completely randomized design with 5 replications and repeated once. Percent inhibition of diameter growth values were calculated as described by Trivedi *et al*. (2008).

To determine the volatile metabolites, the methodology of Dennis and Webster (1971) was employed with slight modifications. Bacterial isolates were inoculated on bottom lid of petriplate containing KBA medium amended with glycine (4.4 g l^{-1}). The other bottom lid of the petriplate inoculated with fungus was placed face to face and sealed with parafilm to prevent leakage of volatiles produced from the plates. The assay was run in the same manner as diffusible metabolites test.

Lytic enzyme activities : Chitinase production was qualitatively detected following the methodology of Chernin *et al*. (1995) using chitin medium (Kamala and Indra Devi, 2012). Chitinase activity was identified based on the formation of purple coloured zone. Chitinolytic index (CI) was calculated by the formula of Wahyudi *et al.* (2011).

Quantitative determination of chitinase was carried out using liquid medium by following the methodology of Velusamy *et al*. (2011). The activity of b-1, 3-glucanase was assessed by the method of Pan *et al.* (1991). Production of extracellular protease was tested according to Maurhofer *et al.* (1995). Cellulase (Teather and Wood, 1982), pectinase (Gerhardt *et al*., 1994) and lipase were detected (Smibert and Krieg, 1994) as per methodologies.

Determination of plant growth promotion traits : Production of indole 3-acetic acid (IAA) by the test bacterial isolates was detected by adopting the method of Gordon and Weber (1951). Phosphate solubilizing activity was investigated on National Botanical Research Institute's Phosphate (NBRIP) growth medium (Nautiyal, 1999). Presence or absence of visible clear halo zones around bacterial colony on the plates was noted and expressed as phosphate solubilizing index (PSI) using the following formula: [(d clear zone + d colony) ÷ d colony where, d = diameter]. The methodology described by Castric (1975) was used to detect hydrogen cyanide. After incubation for a week at 30°C, the colour change in the filter paper was noted and the HCN production potential of the antagonists was graded as mentioned by Kremer and Souissi (2001).

Siderophore production of bacteria was qualitatively detected using iron deficient succinate agar medium amended with Fe-CAS dye solution at 20% (v/v). Each bacterial isolate was streaked separately on the surface of medium and incubated for 48 hr at 28 ºC. The production of

siderophore was indicated by orange halos around the colonies. Biofilm formation by the bacterial strains was assessed using 96-well microplate by adopting the method of Harvey *et al*. (2007).

Statistical analysis : Quantitative data were subjected to statistically analysis using SAS software version 9.2. The treatment means were compared using the least significant difference (LSD) test at *P*d 0.05.

Results and Discussion

In volatile metabolite test, all the bacterial isolates showed fungal growth inhibition ranging from 86.85 to 93.15% (Table 1). Significantly high percent growth inhibition of *R. solani* was obtained by BMB42 (93.15%) which was followed by UMB20 (88.70%) and KMB25 (86.85%). As compared to the results for controlling of *Phytophthora capsici* reported by Diby *et al*. (2005), results obtained in the present study with *Pseudomonas* are much more promising. In the present study, test isolates exerted more potency in diffusible metabolites activities which was evident by complete inhibition of fungal growth. Getha and Vikineswary (2002) reported antagonistic bacteria as biocontrol agents by producing non-volatile compounds. These results are in accordance with the results obtained by the test bacterial isolates on production of non-volatile metabolites which inhibited the growth of *R. solani.*

Among the test isolates, UMB20 and BMB42 were positive in terms of chitinase production as illustrated by purple zones around the bacterial colonies. Average chitinolytic indices were 13.66 and 13.50 for isolates UMB20 and BMB42, respectively (Table 2). All the test strains were able to produce β-1,3-glucanase at varying level. Isolate KMB25 showed negative test. In terms of protease production, isolates UMB20 and BMB42 were negative, while KMB25 was positive. All the test isolates were able to produce cellulase. None of them produced pectinase or lipase on their respective media (Table 2). *Pseudomonas* bacteria have been reported as the producer of these kinds of enzymes in many studies (Gupta *et al.,* 2006; Saraf *et al.,* 2013). Among the hydrolytic enzymes, chitinase and b-1,3-glucanase are the most important enzyme substances and impart strong antifungal activity against pathogens (Shali *et al.*, 2010; Singh *et al*., 2010). Wide range of bacteria have been reported to synthesis these enzymes. Among these strains, KMB25 did not produce chitinase enzyme, but showed positive response to protease production. Synthesis of protease by *P. fluorescens* has been reported by different researchers (Saikia *et al*., 2005; Ajit *et al*., 2006). Some bacteria are recognized as chitinase non-producers while others cannot produce this enzyme. In this study, cellulase activity was common to all the bacterial strains. In many reports, *Pseudomonas* bacteria were also reported as cellulase producers (Saraf *et al*., 2013). Pectinase and lipase activities were not observed in any of the strains which can be regarded as desirable traits of beneficial bacteria (Cattelan *et al*., 1999).

Among the 3 test isolates, UMB20 and BMB42 showed IAA production activity, (of 51 and 52 ppm), (Table 3). Indole 3-acetic acid (IAA) is one of the most important

Table 1 : Effect of volatile and diffusible metabolites produced by bacterial isolates from phyllosphere on growth of *Rhizoctonia solani*

Bacterial isolate	Volatile metabolites		Diffusible metabolites	
	Fungal growth (mm)	% Growth inhibition	Fungal growth (mm)	% Growth inhibition
P. fluorescens(UMB20)	10.17b	88.70b	0.00	100
P. aeruginosa (KMB25)	11.83b	86.85b	0.00	100
P. asplenii (BMB42)	6.17c	93.15a	0.00	100
Control	90.0a	-	90.0	-

Mean of four replication. Mean values within columns followed by same letter are not significantly different (P d0.05)

Table 2 : Production of hydrolytic enzymes by bacterial isolate from phyllosphere

Bacterialisolate	Production					
	Chitinase (ppm) (CI)	b-1,3- glucanase (ppm)	Protease	Cellulase	Pectinase	Lipase
UMB20	62.66 (13.66)	31.45c±1.42	-	+	-	-
KMB25	-	53.53a±0.20	+	+	-	-
BMB42	65.66 (13.50)	36.87b±0.53	-	+	-	-
Control	-	-	-	-	-	-

Mean values within columns followed by same letter are not significantly different at LSD (P d" 0.05): + = positive response; - = negative response (all the values are the average of 6 replications) and ± represents standard errors

Table 3 : Production of growth related hormone and metabolites by bacterial isolates from phyllosphere

Bacterial isolate	Production and response				
	IAA (ppm)	PS (SI)	HCN	Siderophore	Ammonia
UMB20	+(51)	+(1.94)	+++	+	+
KMB25	-	+(2.51)	+	+	+
BMB42	+(52)	+(1.78)	+++	+	+
Control	-	-	-	-	-

IAA = Indole acetic acid; PS = Phosphate solubilization; SI = Solubilization Index; HCN = Hydrogen cyanide; + = positive response; +++ = strong; - = no response

phytohormone related to plant growth and development. Production of indole 3-acetic acid in *Pseudomonas* bacterial isolates from rice was also observed by Ramezanpour *et al*. (2010). Karnwal (2009) screened fluorescent *Pseudomonas* bacterial isolates based on IAA production. In the present study, both IAA producing bacterial strains produced more than 50 ppm IAA. Recently, Anitha and Kumudini (2014) reported that some *Pseudomonas* bacteria isolated from rhizosphere soil of different crop fields were able to produce IAA. However, the level of IAA produced by them were lower than from the levels obtained by phyllosphere *Pseudomonas* strains in this study.

In phosphate solubilizing test, all the isolates were able to solubilize tri-calcium phosphate in NBRIP medium with phosphate solubilizing index ranging from 1.78 to 2.51. Highest index (2.51) was obtained with isolate KMB25. While the lowest (1.78) was recorded with BMB42. The ability of bacterial strains to solubilize tricalcium phosphate *in vitro* suggested their applicability in crop fields. Many species of rhizobacteria have shown their ability to solubilize inorganic phosphate in agar plate assay (Saranya Devi and Sowndaram, 2014). According to Sarkar*et al.* (2014) *Pseudomonas* spp. having potential to solubilize phosphate *in vitro* significantly enhanced seedling growth and nutrient content in wheat plants.

Response of bacterial isolates for HCN production showed changing colour of filter paper from yellow to orange. Isolate UMB20 and BMB42 changed the colour of paper to completely orange, while KMB25 changed it to brick colour. *Pseudomonas* spp. is known to produce HCN and fungal activity of HCN was observed by Paramageetham and Prasada Babu (2012) to be produced by *Pseudomonas* bacteria. According to Kumar *et al*. (2012) production of HCN is a primary mechanism for biocontrol in many *Pseudomonas* bacteria. In this study, production of HCN by the strains might contribute to effective inhibition of mycelial growth of *R. solani* which was evident in volatile metabolite test.

In siderophore test, all isolates were positive showing orange halo around their respective colonies. Siderophore production was determined by the colour change of Fe-CAS dye in agar medium. It has been reported that *Pseudomonas fluorescens* can secrete fluorescent, yellow-green water soluble siderophores under iron-limiting conditions (O'Sullivan and O'Gara, 1992). Belimov *et al*. (2005) reported that three *Pseudomonas* strains AY197010, AY197006 and AY197009 had the ability to produce siderophores. Rasouli *et al*. (2005) isolated 201 of *Pseudomonas* spp. from Iranian soils which were siderophore producers in CAS-agar medium.

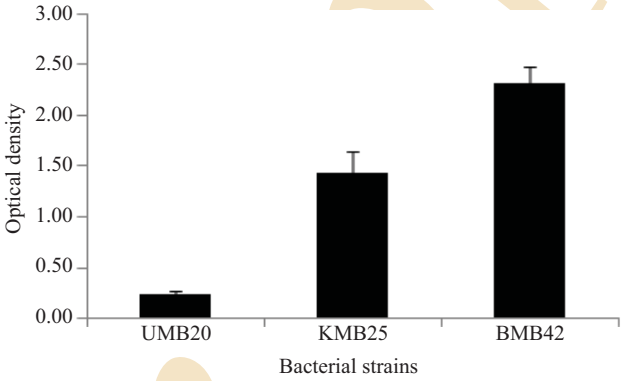

Fig. 1 : Abilities of bacterial strains from phyllosphere to form biofilm in 1% crystal violet after 24 hours of incubation in Luria-Bertany broth (vertical bars represent the standard error)

Another important trait of beneficial bacteria is production of ammonia that influences plant growth indirectly. In the present study, all the 3 test strains showed positive test for ammonia production. Synthesis of ammonia has also been observed by Anitha and Kumudini (2014) in fluorescent pseudomonads. Yadav *et al.* (2010) also observed ammonia production by plant growth promoting bacteria in *in vitro* study in chick pea plants

All the 3 bacterial strains were capable of forming biofilm and showed different levels of biofilm formation (Fig. 1). Strain BMB20, UMB25 and BMB42 showed optical density of 0.23, 1.44 and 2.32, respectively. Highest optical density (2.32) was observed with strain BMB42 followed by

1.44 which was obtained by KMB25. Lowest optical density (0.23) was noted in strain UMB20. Auto aggregation and biofilm formation help to survive and colonize and also increase host plant resistance against antimicrobial compounds (Bogino *et al*., 2013). Biofilms cause beneficial effect on plant growth promotion (Seneviratne *et al*., 2009). *Pseudomonas* sp. form biofilm and was primary model of biofilms research (Parsek and Fuqua, 2003).

The present study was an attempt to demonstrate the multifunctional property of *Pseudomonas fluorescens* (UMB20), *Pseudomonas aeruginosa* (KMB25) and *Pseudomonas asplenii* (BMB42) isolated from rice phylloplane. The broad antagonistic activity of these isolates helped them to establish and to confront against phytopathogens which occupy the similar microbial niche. Their ability to produce siderophore and release antimicrobial compounds reflect their rhizospheric competitiveness that can be beneficially combined with plant protection and their PGPR traits help the enhanced plant growth.

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References

Ajit, N.S., R. Verma and V. Shanmugan: Extracellular chitinase of fluorescent pseudomonads antifungal to *Fusariumoxy sporumf.* sp. *dianthi* causing carnation wilt. *Curr. Microbiol.,* **52**, 310-316 (2006).

Akter, S., J. Kadir, A.S. Juraimi, H.M. Saud and S. Elmahdi: Isolation and identification of antagonistic bacteria from phylloplane of rice as biocontrol agents for sheath blight. *J. Environ. Biol.,* **35**,1095-1100 (2014).

Anitha, G. and B.S. Kumudini: Isolation and characterization of fluorescent pseudomonads and their effect on plant growth promotion. *J. Environ. Biol.,* **35**, 627-634 (2014).

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

Belimov, A., A. Hontzeas, N. Safronova, V.I. Demchinskaya, S.V. Piluzza, G. Bulitta and B.R. Glick: Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L.) (Czern). *Soil Biol. Biochem.,* **37**, 241-250 (2005).

Bogino, P.C., M.D.L. MercedeOliva, F.G. Sorroche, and W. Giordano: The role of bacterial biofilms and surface components in plant-bacteria associations. *Intl. J. Mol. Sci.,* **14**, 15838-15859 (2013).

Castric, P.A.: Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa. Can. J. Microbiol.,* **21**, 613-618 (1975).

Cattelan, A.J., P.G. Hartel and J.J. Fuhrmann: Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. American J.,* **63**, 1670-1680 (1999).

Chernin, L., Z. Ismailov, S. Haran and I. Chet: Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. *Appl. Environ. Microbiol.,* **61**, 1720-1726 (1995).

Dennis, C. and J. Webster: Antagonistic properties of species groups of *Trichoderma.* II. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.,* **57**, 41-48 (1971).

Diby, P., Y.R. Sarma, V. Srinivasan and M. Anandaraj: *Pseudomonas fluorescens*mediated vigour in black papper (*Piper nigram* L.) under greenhouse cultivation. *Ann. Microbiol.,* **55**, 171-174 (2005).

Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Kreig: Methods for General and Molecular Bacteriology. ASM, Washington, DC (1994).

Getha, K. and S. Vikineswary: Antagonistic effects of *Streptomyces violaceus niger* strain G10 on *Fusariumoxy sporum*f.sp. *cubense* race 4: indirect evidence for the role of antibiosis in the antagonistic process. *Indonesian J. Microbiol.*, **28**, 303-310 (2002).

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

Harvey, J., K.P. Keenan and A. Gilmour: Assessing biofilm formation by *Listeria monocytogenes* strains. *Food Microbiol.,* **24**, 380-392 (2007).

Jamali, F., A. Sharifi-Tehrani, M.I. Lutz and M. Maurhofer: Influence of host plant genotype, presence of a pathogen, and co inoculation with *Pseudomonas fluorescens* strains on the rhizosphere expression of hydrogen cyanide and 2,4-diacetylphloroglucinol biosynthetic genes in *P. fluorescens* biocontrol strain CHA0. *Microbial. Ecol.,* **57**, 267-275 (2009).

Kamala, T. and S.I. Devi: Biocontrol properties of indigenous *Trichoderma* isolates from North-East India against *Fusarium oxysoprum* and *Rhizoctonia solani*. *African J. Biotechnol.,* **11**, 8491-8499 (2012).

Karnwal, A.: Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. *J. Plant Pathol.,* **91,** 61-63 (2009).

Karthikeyan, M., K. Radhika, S. Mathiyazhagan, R. Bhaskaran, R. Samiyappan and R. Velazhahan: Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera* L.) root treated with biological agents. *Brazil. J. Plant Physiol.,* **18,** 367-377 (2006).

Kremer, R.J. and T. Souissi: Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. *Curr. Microbiol.,* **43**, 182-186 (2001).

Kumar, A.A., S. Kumar, S. Devi, C. Patil, C. Payal and S.S. Negi: Isolation, screening and characterization of bacteria from rhzospheric soils for different plant growth promotion (PGP) activities: an *in vitro* study. *Rec. Res. Sci. Technol.,* **4**, 1-5 (2012).

Maurhofer, M., C. Keel, D. Haas and G. Defago: Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathol.,* **44**, 40-50 (1995).

Mubarik, N.R., I. Mahagiani, A. Anindyaputri, S. Santoso and I. Rusmana: Chitinolytic bacteria isolated from chili rhizosphere: Chitinase characterization and its application as biocontrol for whitefly (*Bemisia tabaci* Genn.). *Amer. J. Agric. Biol. Sci.,* **5**, 430-435 (2010).

Nautiyal, S.: An efficient microbiological growth medium for screening

phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.,* **170**, 265-270 (1999).

O'Sullivan, D.J. and F. O'Gara: Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol. Rev.,* **56**, 662-676 (1992).

Pan, S.Q., X.S. Ye and J. Kuc: Association of -1,3-glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. *Physiol. Mol. Plant Pathol.,* **39**, 25-39 (1991).

Paramageetham, C. and G. Prasada Babu: Antagonistic activity of fluorescent pseudomonads against a polyphagous soil born plant pathogen-*Sclerotium rolfsii*., *Open Access Scientific Reports*, **1,** 1-9 (2012).

Parsek, M.R. and C. Fuqua: Biofilms, emerging themes and challenges in studies of surface-associated microbial life. *J. Bacteriol.,* **186**, 4427-4440 (2003).

Podile, A.R and G.K. Kishore: Plant growth promoting rhizobacteria. In: Plant Associated Bacteria (Ed.: S.S. Gnanamanickam). Springer, The Netherland, pp. 195-230 (2006).

Ramezanpur, M.R., Y. Popov, K. Khavazi and H.A. Rahmani: Genetic diversity and efficiency of indole acetic acid production by the isolates of fluorescent *Pseudomonas* from rhizosphere of Rice (*Oryza sativa* L). *American-Eurasian. J. Agril. Environ. Sci.,***7**, 103-109 (2010).

Rasouli, M.H., K. Khavazi, H. Rahimian, M.J. Malakouti and H. Asadi-Rahmani: An evaluation of the potential of indigenous fluorescent Pseudomonds of wheat rhizosphere for producing siderophore. *Iranian J. Soil Water Sci.,* **20**, 133-143 (2006).

Saikia, R., B. Singh, R. Kumar and D. Arora: Detection of pathogenesis-related proteins-chitinase and β-1,3-glucanase in induced chickpea. *Curr. Sci.,* **89**, 659-663 (2005).

Saraf, M., A. Thakkar, U. Pandya, M. Joshi and J. Parikh: Potential of plant growth promoting microorganisms as biofertilizers and biopesticides and it's exploitation in sustainable agriculture. *J. Microbiol. Biotechnol. Res.,* **3**, 54-62 (2013).

Saranya Devi, K. and S. Sowndaram: Biological control of rhizobacteria against rice diseases caused by *Rhizoctonia solani* (sheath blight) and *Sarocladium oryzae* (sheath rot). *Int. J. Adv. Res.,* **2**, 818-823 (2014).

Sarkar, A., T. Islam, G.C. Biswsh, S. Alam, M. Hossain and N.M. Talukdar: Screening for phosphate solubilizing bacteria inhabiting the rhizoplane of rice grown in acidic soil in Bangladesh. *Acta Microbiol. Immunol. Hungarica.,* **59**, 199-213 (2012).

Seneviratne, G., R.M.M.S. Thilakaratne, A.P.D.A. Jayasekaran, K.A.C.N. Seneviratne, K.R.E. Padmathilake and M.S.D.L. De Silva: Developing beneficial microbial biofilms on roots of non-legumes: a novel biofertilizing technique. In: Microbial strategy for crop improvement (Eds. M.S. Khan, A. Zaidi and J. Musarrat). Berlin, Heidelberg: Springer, pp. 51-61 (2009).

Shali, A., S. Ghasemi, G. Ahmadian, G. Ranjbar, A. Dehestani, N. Khalesi, E. Motallebi and M. Vahed: *Bacillus pumilus* SG2 chitinases induced and regulated by chitin, show inhibitory activity against *Fusarium graminearum* and *Bipolaris sorokiniana*. *Phytoparasitica,* **38**, 141-147 (2010).

Singh, N., S. Kumar, V.K. Bajpai, R.C. Dubey, D.K. Maheshwari and S.C. Kang: Biological control of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chir-pine. *Crop Protect.,* **29**, 1142-1147 (2010).

Smibert, R.M. and N.R. Krieg: Phenotypic characterization. In: Methods for General and Molecular Biology (Eds.: P.R.G. Gerhardt, E., Murray, W.A.Wood and N.R.Krieg) Washington, DC: American Society for Microbiology pp. 607-654 (1994).

Teather, R.M. and P.J. Wood: Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic from the bovine rumen. *Appl. Environ. Microbiol.,* **43,** 777-780 (1982).

Trivedi, P., A. Pandey and L.M.S. Palni: *In vitro* evaluation of antagonistic properties of *Pseudomonas corrugate*. *Microbiol. Res.,* **163**, 329-336 (2008).

Wahyudi, A.T., R.I. Astuti and Giyanto: Screening of *Pseudomonas* sp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. *Amer. J. Agric. Biol. Sci.,* **6,** 134-141 (2011).

Velusamy, P., J.E. Immanuel and S.S. Gnanamanickam: Rhizosphere bacteria for biocontrol of bacterial blight and growth promotion of rice. *Rice Sci.,* **20**, 356-362 (2013).

Vidaver, A.K.: Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. *Ann. Rev. Phytopathol.,* **14**, 451-465 (1976).

Yadav, J., J.P. Verma and K.N. Tiwari: Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinu* L.) under *in vitro* conditions. *Biol. Forum,* **2**, 15-18 (2010).