Journal of Applied Horticulture, 16(2): 131-135, 2014

Antifungal potential of native strain isolated from rhizosphere soil of *Valeriana jatamansi* from temperate regions of Himachal Pradesh

Anjali Chauhan[1]*, Praveen Pal Balgir[2] and Chand Karan Shirkot[1]

*[1]Department of Basic Sciences, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230 (HP), [2]Department of Biotechnology, Punjabi University, Patiala (Punjab). *E-mail: anjali_chauhan22@yahoo.co.in*

Abstract

Plant growth promoting rhizobacteria (PGPR) are commonly used as inoculants for improving the growth and yield of agricultural crops. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides and supplements. Thus the present study focuses on the phenotypic and genotypic characterization of potent PGPR isolates with multiple plant growth promoting (PGP) traits and antifungal potential against different phytopathogenic fungi. Biochemical, molecular and phylogenetic characterization of four effective PGPR isolates (CKMV1, CKMV2, CKMV3 and CKMV4) of *Valeriana jatamansi* demonstrated that three strains belonged to genus *Bacillus* spp. and one belonged to *Aneurinibacillus* spp. The strain CKMV1 identified as (*Aneurinibacillus aneurinilyticus*) on the basis of 16S rDNA homology showed a considerable antifungal potential against different phytopathogens along with multiple PGP traits like phosphate solubilization, IAA production, HCN production, siderophore production. Significant growth inhibition of phytopathogenic fungi by CKMV1 was obtained in the order *Sclerotium rolfsii* > *Rhizoctonia solani* > *Phytopthora cactorum* > *Alternaria* spp. > *Fusarium oxysporum*. Thus, the secondary metabolite producing *A. aneurinilyticus* strain CKMV1 exhibited innate potential of plant growth promotion and biocontrol activities *in vitro* which can further be used as biofertilizer as well as biocontrol agent.

Key words: *Bacillus*, *Aneurinibacillus*, PGPR, antifungal, antibiotic resistance, *Valeriana jatamansi*

Introduction

Valeriana jatamansi commonly known as Indian Valerian has considerable medicinal value and is found in the temperate regions of Himachal Pradesh. Himachal Pradesh has been a natural habitat for large variety of aromatic and medicinal plants, some of which are on verge of extinction due to unsystematic and unscientific exploitation. Farmers have started cultivation of Indian valerian on large scale in agricultural fields due to which there is a loss of naturally occurring PGPR and might also be exposed to various root rot causing plant fungal pathogens. PGPR are the heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with the roots, which can improve the extent or quality of plant growth directly or indirectly. Also, the use of chemical fertilizers and fungicides has adverse effect on the medicinal value of the plant. Therefore, there is an urgent need to develop effective and more consistent active PGPR inocula as biofertilizer or biocontrol agent in order to increase survival and growth of medicinal plants under field conditions.

Microbial inoculants or biofertilizers are used to hasten biological activity to improve availability of plant nutrients by fixing atmospheric nitrogen, making insoluble phosphate soluble and decomposing farm wastes, which result in the release of plant nutrients and antagonize various pathogenic fungi by producing siderophore, HCN, chitinase, β-1,3 glucanase and different antibiotics.

Indeed, the biological control of plant diseases with bacterial antagonism is a potential alternative to expensive chemical control, preventing the accumulation of hazardous compounds toxic to soil biotes (Bano and Musarrat, 2003). Strains of the genus *Bacillus* are among the most commonly reported PGPR (Compant *et al.,* 2005; Vessey, 2003). The secondary metabolites produced by *Bacillus* spp. are known to have antifungal potential against different phytopathogenic fungus (Asaka and Shoda, 1996; Compant *et al.,* 2005). *Bacillus* spp. has the advantage of being able to form endospores which confers them high stability as biofungicide or biofertilizer (Schisler *et al.,* 2004).

Therefore, in the present study, attempts were directed towards the selection of PGPR isolates exhibiting the maximum number of PGP traits along with antifungal potential under *in vitro* conditions.

Materials and methods

Isolation of plant growth promoting bacteria: For isolation of bacteria twenty *V. jatamansi* plants were randomly selected and rhizospheric soil samples (5 g from each plant), along with roots (1 g from each plant) were collected from moist temperate forest located around Chamba and Palampur, Himachal Pradesh. Modified replica plating technique (Shirkot and Vohra, 2007) was used to isolate PGPR with multiple PGP activities. Briefly, the technique involved, spreading of 100 µL of serially diluted suspension of 1 g soil/roots on enriched soil extract medium. The isolated colonies that developed on the enriched medium (master plate) were replica plated onto the selective media: nitrogen free medium (Jensen, 1942) for N_2-fixing ability, Pikovskaya's medium (Pikovskaya, 1948) for phosphate-solubilizing ability

and chrome azurol S medium (Schwyn and Neilands, 1987) for siderophore production. At the end of incubation period, the colonies that appeared on selective media plate were compared to that on master plate and characterized morphologically.

Phytopathogens: Fungal pathogens *viz. S. rolfsii*, *R. solani*, *P. cactorum*, *Alternaria* spp, *F. oxysporum* were obtained from IARI, Pusa, New Delhi.

***In vitro* screening of bacterial isolates for plant growth promoting activities:** All isolates were screened for phosphate solubilization (Rao and Sinha, 1963), IAA production (Gordon and Paleg, 1957), nitrogen fixing ability (growth on nitrogen free medium), HCN production (Baker and Schippers, 1987), siderophore production (Schwyn and Neilands, 1987) and antifungal activity (Vincent, 1947) against five fungi (*S. rolfsii*, *R. solani*, *P. cactorum*, *Alternaria* spp and *F. oxysporum*). Estimation of different PGP traits like phosphate solubilization, siderophore production, Indole acetic acid (IAA) production, production of antifungal antibiotic activity and HCN production was performed as mentioned by Mehta *et al*. (2010). Chitinase and protease activities were tested as per Robert and Selitrennikoff (1988) and Fleming *et al.* (1975).

All the experiments were conducted in triplicates along with equal number of appropriate controls. Data were statistically analysed by analysis of variance technique (one way classification) using SPSS 16.0 software.

Biochemical characterization of selected bacterial isolates: Characterization of the selected bacterial isolates was done based on their colony morphology, microscopic observations, and biochemical tests (Holt *et al.,* 1994). Colony morphology and cell morphology was observed on nutrient agar medium and nutrient broth, respectively. The biochemical characterization of the isolates was done by using commercial kits (KB009 Hi carbohydrate™ kit and KB013 Hi *Bacillus*™ Identification kit).

Molecular characterization of selected bacterial isolates (DNA isolation, PCR amplification, and rRNA homology): The bacterial isolate CKMV1, CKMV2, CKMV3 and CKMV4 were grown in nutrient broth at 37 ºC. Cells were harvested after 72 h of incubation and were processed immediately for DNA isolation by Real genomic DNA isolation kit. The concentration and purity of DNA were determined by measuring optical density at 260 nm with UV-Vis spectrophotometer. The PCR amplification was carried out in a final volume of 20 µL. Briefly, the amplification reaction containing 50 ng DNA template, 20 pmole each of universal primers (forward primer 5´-GCAAGTCGAGCGGACAGATGGGAGC-3´ and reverse primer 5´-AACTCTCGTGGTGTGACGGGCGGTG-3´), 0.2 mM dNTPs and 1 U Taq polymerase in 1x PCR buffer. Reaction were cycled 35 times as 94º C for 30 s, 58º C for 30 s, 72º C for 1 min 30 s followed by final extension at 72º C for 10 min. Amplified PCR products was separated by gel electrophoresis on 1.2% (w/v) agarose gel. The band of almost 1375 bp was excised from the gels and purified using gel extraction kit (Real genomics). The purified fragments were sequenced from commercial sequencing facility (Xcleris lab). The comparison of sequence was performed via the internet at National Center for Biotechnology Information (NCBI) database by employing BLAST algorithm. The Phylogenetic tree was constructed with the help of ClustalW from the website http://www2.ebi.ac.uk/clustalw/ (Higgins *et al.,* 1994). Tree was viewed with the help of TreeView from the website http://taxonomy.zoology.gla.ac.uk/rod/treeview.html (Page, 1996).

Antagonistic activity testing *in vitro*: Antagonistic activity was tested using the dual culture technique (Mehta *et al.,* 2010). Briefly a loopful of 48 h old culture of each isolate was streaked a little below the center of prepared MEA petriplates. Mycelial discs of the 4 day old culture of the test pathogen *Alternaria* spp. *F. oxysporum, P. cactorum*, *R. solani* and *S. rolfsii* was placed simultaneously. On one side of streak, control test pathogen was kept. The plates were incubated at 24° C and % growth inhibition was calculated according to Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

I = % growth inhibition. *C* = Growth of fungus in control. *T* = Growth of fungus in treated

Antibiotic resistance pattern: For determination of intrinsic antibiotic resistant markers, antibiotic disc amoxycillin, ampicillin, bacitracin, chloramphenicol, gentamycin, lincomycin, matronidazol, streptomycin, novobiocin, neomycin, naldixic acid, penicillin, oxacillin, rifampicin, tetracyclin, kannamycin, polymixin and erythromycin (Hi-media) having varying concentration from 0.5µg to 50 µg of antibiotics, per disc were used (Barry *et al.,* 1970; Ericsson and Sherris, 1971).

Fungicide tolerance: Fungicide tolerance of the bacterial antagonist was determined by spotting the culture on the nutrient agar medium supplemented with different concentrations of fungicides. The plates were incubated at 30º C for 48 h and examined for growth.

Results and discussion

Thirty bacterial isolates in total; twenty three from rhizosphere; and seven from endo rhizosphere of *V. jatamansi* were screened for multiple plant growth promoting (PGP) traits.

All the thirty bacterial isolates showed variation in their ability for different plant growth promoting traits and thus, were ranked accordingly. Most of the isolates exhibited two or more than two common traits. However, bacterial isolates CKMV1, CKMV2, CKMV3 and CKMV4 exhibited higher levels of multifarious plant growth promoting traits [evident from their high score, >60 % (data not shown)] in comparison to other isolates and hence, were selected for further studies. Of these four isolates, CKMV2 and CKMV3 were endophytic whereas, CKMV1 and CKMV4 were rhizospheric in origin. Strains CKMV2, CKMV3 and CKMV4 were positive for chitinase. When tested for protease, all the four strains were positive which supports for the possible antifungal mechanism of the isolates.

CKMV1, CKMV2, CKMV3 and CKMV4 were further characterized up to genus level based on colony morphology, gram staining and biochemical characterization. Characterization data (Table 1) revealed that all the four isolates belong to genus *Bacillus*. All the four isolates were further subjected to molecular characterization based on 16S rDNA sequence homology. Sequencing data revealed that CKMV1 (GQ980020) belongs to genus *Aneurinibacillus* and species *aneurinilytics*, CKMV2

(KC329525) and CKMV3 (KC329526) belongs to *Bacillus subtillis*, however CKMV3(KC329527) belongs to *Bacillus* spp. (Fig. 1).

Antagonistic activity: In order to know the mechanisms of the fungal inhibition the strains, CKMV1, CKMV2, CKMV3 and CKMV4 were tested for their ability to inhibit all tested phytopathogenic fungi for *in vitro* broad spectrum antibiosis. The inhibitory effect of bacterial isolates on growth of different fungi was of different degree after 72h of incubation period (Fig. 2). Maximum growth inhibition was observed by CKMV1 against *S. rolfsii* (93.58 %) and minimum was against *Fusarium* spp. (64.30 %). The difference in the percent growth inhibition amongst *Alternaria* spp. (71.08 %) and *P. cactorum* (71.37 %) was statistically non significant. These results are in agreement with earlier reports on *Bacillus* spp. isolated from many agricultural crops (Singh *et al.,* 2002), horticultural crops (Mandhare and Suryawanshi, 2003; Sood, 2003) exhibiting broad spectrum antifungal activity. We propose that this effect may be caused by the different antifungal metabolites including siderophores, organic acids, IAA and antifungal antibiotics in the culture filtrate (Swinburne, 1978; Utkhede and Smith, 1992). Many workers had also reported the antifungal activity against different phytopathogens due to the production of some specific siderophores, antibiotics, secondary metabolites or hydrolytic enzymes. Therefore, antagonistic bacteria with phosphate solubilization and IAA production could be further exploited both as effective biocontrol agent as well as a biofertilizer. Based on the antifungal potential of all the four isolates, the best isolate CKMV1 was chosen for further studies.

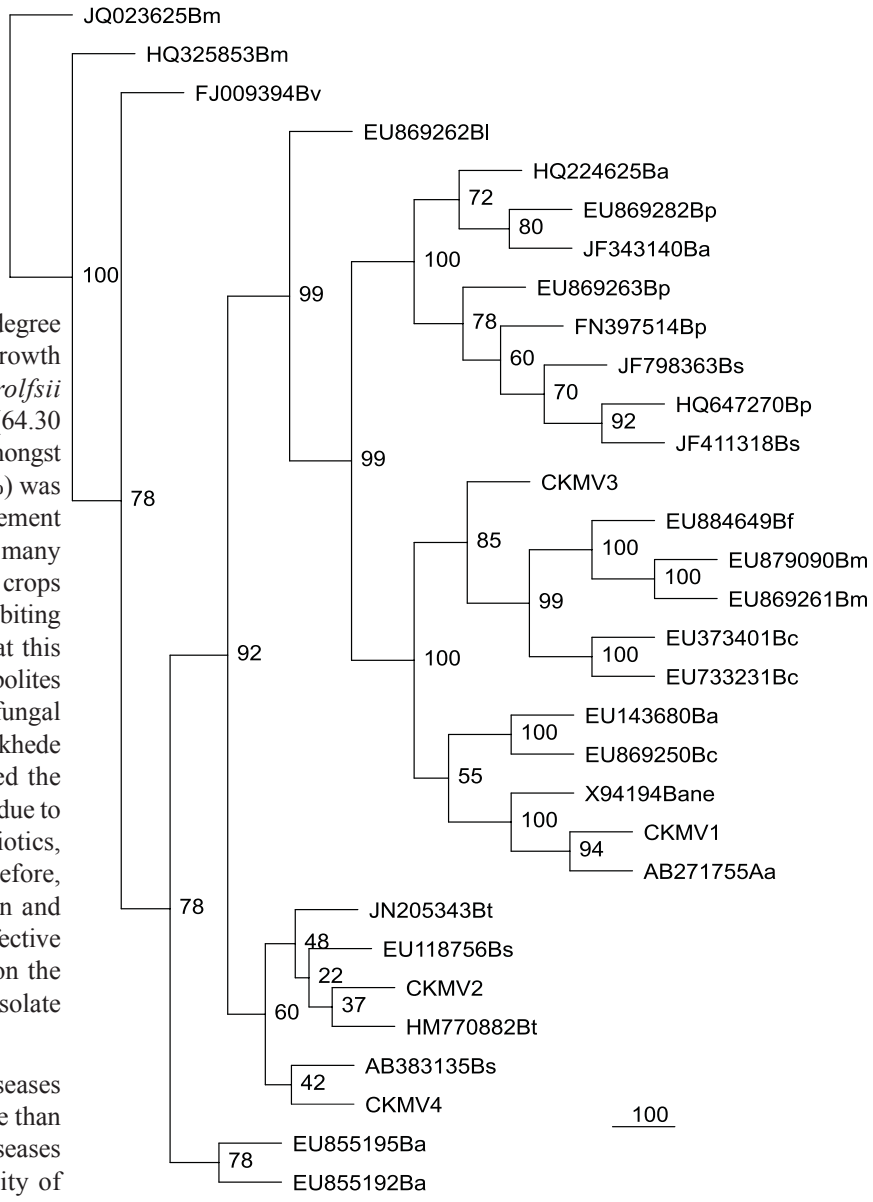

Fig. 1. Neighbor-joining tree based on 16S rRNA sequences showing the phylogenetic relationship of strain CKMV1, CKMV2, CKMV3 and CKMV4. The numbers at the nodes indicate the levels of bootstrap support based on data for 1000 replicates. The scale bar indicates 100 substitutions per nucleotide position.

S. rolfsii is a destructive plant pathogen causing diseases of many monocotyledons plants encompassing more than 500 host species (Punja, 1988). Reports on fungal diseases of *V. jatamansi* are not available and the possibility of plant being infected by common plant pathogens cannot be ruled out. Therefore, CKMV1 of different concentration was thoroughly mixed in medium which was subsequently inoculated with *S. rolfsii* bit in order to check antagonism. It was observed that on average the growth inhibition of *S. rolfsii* increased with the increase in bacterial cell density and in the cell density of O.D. 2.0 the growth of fungus was completely inhibited. Similar trend was found in results where the zone of inhibition was measured. Biological agents including bacteria and fungi are reported to protect crops from *S. rolfsii* infection (Singh *et al.,* 2003).

The results of interaction study revealed that on an average, the percent growth inhibition of *S. rolfsii* increased with the increase in the bacterial density which indicated marked *in vitro* antagonism against plant infecting fungi (data not shown). In such cases of antagonism, a clear zone of inhibition is visible that demarcates the test fungus and the bacterium. The bacterial isolate secrets antifungal substance that diffuse in the medium and inhibit the fungal growth.

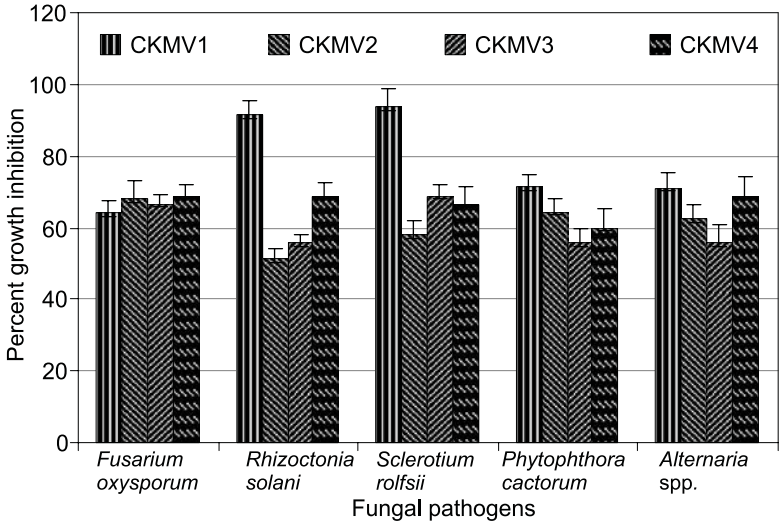

Fig.. 2. Antifungal antibiotic activity of CKMV1, CKMV2, CKMV3 and CKMV4 against some fungal pathogens using dual culture method.

The intrinsic antibiotic resistance (IAR) has been used as quick and simple method for differentiating strains and

Table 1. Metabolic fingerprinting of CKMV1, CKMV2, CKMV3, CKMV4 PGPR isolates

	CKMV1	CKMV2	CKMV3	CKMV4		CKMV1	CKMV2	CKMV3	CKMV4
Gram staining	+	+	+	+	Melibose	-	-	-	-
Spore formation	+	+	+	+	Sucrose	-	+	+	+
Catalase	+	+	+	+	Mannose	+	+	+	+
Malonate	-	+	+	-	Inulin	+	-	-	-
Voges Proskauer's	-	+	+	+	Sodium glucanate	-	-	-	-
Citrate utilization	-	+	+	+	Glycerol	+	-	-	-
ONPG	+	+	+	+	Salicin	+	-	-	-
Nitrate reduction	+	+	+	+	Dulcitol	-	-	-	-
Arginine dihydrolase	-	-	-	-	Inositol	+	+	+	+
Hydrogen sulphide production	+	-	-	-	Sorbitol	+	-	-	-
Gelatin hydrolysis	-	-	-	-	Adonitol	-	-	-	-
Starch hydrolysis	-	+	+	+	Arabitol	-	-	-	-
Indole production	-	-	-	-	Erythritol	-	-	-	-
Esculin hydrolysis	+	-	-	_	α-Methyl-D-glucoside	-	-	-	-
Tyrosine utilization	+	-	-	_	Rhamnose	-	-	-	-
Lactose	-	-	-	-	Cellobiose	-	-	-	-
Xylose	-	+-	-+	+-	Melezilose	-	-	-	-
Fructose	+	+	+	+	α-Methyl-D-mannoside	-	-	-	-
Dextrose	-	+	+	+	Xylitol	-	-	-	-
Galactose	-	-	-	-	D-Arabinose	-	-	-	-
Raffinose	-	-+	-+	-	Sorbose	-	-	-	-
Trehalose	-	-	-	-					

to observe heterogeneity in natural population of rhizobacteria (Beymon and Josey, 1980). Isolate under study CKMV1 was found to be resistant to 50 µg/mL concentration of penicillin and 25 µg/mL of ampicillin. This property may help to study population dynamics of introduced *Aneurinibacillus* strain on roots of *V. jatamansi* seedlings in fields. The rhizobacterial isolate that are resistant to the high concentration of antibiotics, may have the survival and competitive qualities required for a good inoculants strain. The advent of antibiotic resistant marker enabled the first direct proof that some specific strains of introduced bacteria could establish in root zone and sustain bacterial population throughout the growing season (Kloepper *et al.,* 1980).

Pesticides in general and fungicides in particular are essential for disease management and in some cases they may show toxicity towards the introduced rhizobacteria. CKMV1 strain could tolerate as high as 3400 µg/mL of carbendazim under *in vitro* conditions (Table 2). However effect of other fungicides was variable. This in agreement with our published work on characterization of a novel carbendazim tolerant *B. subtilis* strain isolated from tomato rhizosphere that exhibits multiple PGPR activities (Shirkot and Vohra, 2007) suggesting that a single PGPR may have several modes of action.

It was also observed that maximum P-solubilization (250.0 mg/mL) was observed with isolate CKMV1 up to 72 h of incubation. P-solubilization was accompanied by a decrease in pH of the culture filtrate from 7.0 (initially) to 5.09. The decrease in pH indicates the production of organic acids considered responsible for P-solubilization (Daimon *et al.,* 2006). P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant P-nutrition. Maximum growth (43 $\times 10^6$ cfu/mL) coincides with the maximum amount of P solubilization and is in agreement with earlier reports on P solubilization (Vazquez *et al.,* 2000). Production of IAA (7 µg/mL) was observed by CKMV1. The production of IAA increased up to 72 h. IAA production in microbes promotes the root growth by directly stimulating plant cell elongation or cell division (Glick *et al.,* 1995). Another important trait of PGPR, that may indirectly influence the plant growth, is the production of siderophores. They bind to the available form of iron (Fe^{3+}) in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant health. In the present study *A. aneurinilyticus* strain CKMV1 showed maximum siderophore production (53.43 %) at 72 h of incubation period along with high HCN production in which the color of filter paper got changed from yellow to brown.

Recently, there have been numerous reports on the use of PGPR as biocontrol agents on crops like tomato, soyabean, cotton *etc.* However, there has been little information on the occurrence

Table 2. Fungicide tolerance of *A. aneurinilyticus* (CKMV1) using spot plate method

Fungicide	Concentration (µg/mL)	Growth*
Methyl-DL-N(2,6-Dimethyl)-	100	+
alaninate+manganese ethylene	200	+
bisdithiocarbamate+zinc ions	300	+
(Ridomil-MZ)	400	+
	500	-
Manganese ethylene bis-	100	+
dithiocarbamate+zinc ions (mancozeb)	200	+
	300	+
	400	+
	500	-
Methyl-2-banzimidazole carbamate	1000	+
(cabendazim)	1500	+
	2000	+
	2500	+
	3000	+
	3200	+
	3400	+
	3600	-

Growth was assessed visually; + Indicate growth equal to the control (Plate without antibiotic);
- No growth indicate that organism is sensitive to the concentration of fungicide

of PGPR inhibiting so many plant pathogens. Our work shows CKMV1 as effective PGPR with antifungal potential. Further work is required to identify the mechanism of biofertilizer and biocontrol potential of CKMV1 (*A. aneurinilytics*) under field condition.

References

Asaka, O. and M. Shoda, 1996. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtillis* RB14. *Appl. Environ. Microb.,* 62: 4081-4085.

Baker, P.D. and Schippers, 1987. Microbial cyanide production in the rhizosphere in relation to potato yield production and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biol. Biochem.,* 9: 451-457.

Bano, N. and J. Musarrat, 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrolagent. *Curr. Microbiol*., 46: 324-328.

Barry, A.L., F. Garcia and L.D. Trupp, 1970. An improved single disc method for testing the antibiotic susceptibility of rapid growing pathogens. *Am. J. Clin. Pathol*., 53: 149-158.

Beymon, J.L. and D.P. Josey, 1980. Demonstration of heterogeneity in a natural population of *Rhizobium phaseoli* using variation in intrinsic antibiotic resistance. *J. Gen. Microbiol.,* 118: 437-442.

Compant, S., B. Duffy, J. Nowak, C. Clément and E.A. Barka, 2005. Use of plant growth promoting bacteria for biocontrol of plant diseases: Principle, mechanism of action, and future prospects. *Appl. Environ. Microb.,* 71(9): 4951-4959.

Daimon, H., K. Nobuta, M. Ohe, J. Harada and Y. Nakayama, 2006. Tricalcium phosphate solubilizing by root nodule bacteria of *Sesbania cannabina* and *Crotalaria juncea*. *Plant Prod. Sci.,* 9: 388-389.

Ericsson, H.M. and J.C. Sherris, 1971. Antibiotic sensitivity testing report of an international collaborate study. *Acta Pathol. Microbiol. Scand.* Sect B.Suppl. 217: 3-12.

Fleming, H.P, J.L. Etchells and R.H. Costilus, 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Appl. Microbiol.,* 30: 1040-1042.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.,* 41: 109-117.

Gordon, S.A. and L.G. Paleg, 1957. Observations on the quantitative determination of indoleacetic acid. *Physiol. Plantarum*, 10: 39-47.

Higgins, D., J. Thompson, T. Gibson, J.D. Thompson, D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* 22: 4673-4680.

Holt, J.G., N.R. Krieg, P.H.A. Sneathm, J.T. Staley and S.T. Williams, 1994. *Bergey's Manual of Determinative Bacteriology.* 9th edn. Baltimore, MD: Williams and Williams.

Jensen, H.L. 1942. Nitrogen fixation in leguminous plants. *Proc. Int. Soc. NSW*, 66: 68-108.

Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth, 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature*, 286: 885-886.

Mandhare, V.K. and A.V. Suryawanshi, 2003. Antagonistic effect of *Bacillus thermophillus* on some pathogens. *J. Maharashtra Agric. Univ*., 28(3): 279-277.

Mehta, P., A. Chauhan, R. Mahajan, P.K. Mahajan, and C.K. Shirkot, 2010. Strain of *Bacillus circulans*isolated from apple rhizosphere showing plant growth promoting potential. *Curr. Sci.,* 98: 538-542.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.,* 12: 357-358.

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

Punja, Z.K. 1988. *Sclerotium* (Athelia) *rolfsii*, pathogen of many plant species. In: *Genetics of Plant Pathogenic Fungi*. G.S. Sidhu (ed.) Academic Press, London. p. 523-533.

Robert, W.K. and C.P. Selitrennikoff, 1988. Plant and bacterial chitinase differ in antifungal activity. *J. Gen. Microbiol.,* 134: 169-176.

Schisler, D.A., P.J. Slininger, R.W. Behle and M.A. Jackson, 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology*, 94: 1267-1271.

Schwyn, B. and J.B. Neilands, 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.,* 160: 47-56.

Shirkot, C.K. and I. Vohra, 2007. Characterization of novel carbendazim tolerant *Bacillus subtilis* with multiple plant growth promoting activities. Proceedings of XVI International plant protection congress, BCPC, SECC, Glasgow, Scotland, UK, vol. 1 pp. 272-277.

Singh, A., S. Mehta, H.B. Singh and G.S. Nautiyal, 2003. Biocontrol of collar rot disease of betelvine (*Piper betle* L.) caused by *Sclerotium rolfsii* by using rhizosphere-competent *Pseudomonas fluorescens* NBRI-N6 and *P. fluroescens* NBRI-N. *Curr. Microbiol.,* 47: 153-158.

Singh, S.K., R.B. Singh, R. Chand and D. Singh, 2002. Influence of biofertilizer, organic manure and inorganic fertilizers on major foliar diseases of rice (*Oryza sativa* L.) in rainfed conditions. *Indian J. Plant Pathol.*, 20: 48-51.

Sood, S. 2003. Chemotactic response of plant growth promoting bacteria towards roots of vesicular arbuscular mycorrhizae tomato plants. *FEMS Microbiol. Ecol*., 45: 219-227.

Sundara Rao, W.V.B. and M.K. Sinha, 1963. Phosphate dissolving organisms in soil and the rhizosphere. *Indian J. Agric. Sci.,* 33: 272-278.

Swinburne, T.R. 1978. The potential value of bacterial antagonists for the control of apple canker. *Ann. Appl. Biol.*, 89: 94-96.

Utkhede, R.S. and E.M. Smith, 1992. Promotion of apple tree growth and fruit production by the EBW-4 strain of *Bacillus subtilis* in apple replant disease soil. *Can. J. Microbiol.,* 38: 1270-1273.

Vazquez, P., G. Holguin, M. Puente, A.E. Cortes and Y. Bashan, 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. *Biol. Fert. Soils*, 30: 460-468.

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

Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature,* 150: 850.

Received: December, 2013; Revised: February, 2014; Accepted: March, 2014