DISCUSSION

Plant rhizosphere is known to be the preferred ecological niche for various types of soil microorganisms due to rich nutrient availability. Rhizobacteria have received attention for their potential use in sustainable agriculture as biofertilizers and biocontrol agents. However, the effectiveness of PGPR in increasing crop productivity has been limited by variability and inconsistency in their field performance. The selection of stress-tolerant and rhizosphere-competent PGPR with multiple plant growth-promoting attributes and broad-spectrum plant growth-promoting activity has been advocated. Fluorescent *Pseudomonas* commonly found in different types of soil are of enormous importance as the plant growth-promoting and biological control agents in improving agricultural productivity. There has been an ample interest in identifying the species comprising fluorescent *Pseudomonas* group and analyzing their diversity in the soil due to their common association with plant rhizosphere and relative ease in their culturing. A large number of phylotypes have been distinguished based on their 16S rRNA gene sequencing. Certain phenotypic traits such as siderophore types appear to be associated with different genotypes leading to the classification of strains based on siderophore-typing. However, it is not known to what extent other phenotypic traits like phosphate solubilization, auxin production, ACC-deaminase activity, antibiotic production, and stress tolerance can be associated with particular species or phylogenetic clusters in relation to plant growth promotion.

The results of present studies on selection, characterization and evaluation of fluorescent *Pseudomonas* are discussed in the light of available information under the major activities undertaken in developing the microbial inoculants for field evaluation.

5.1 Isolation, Characterization and Diversity Analysis of Phosphate-solubilizing Fluorescent *Pseudomonas*

The development of discrete and conspicuous phosphate solubilization zones by the bacterial colonies indicated the occurrence of efficient phosphate-solubilizing fluorescent *Pseudomonas* in the rhizosphere soil samples from the cold desert of the Indian trans-Himalayas (Fig. 4.1 and 4.2). Nineteen phosphate-solubilizing fluorescent *Pseudomonas* producing greater than 10 mm TCP solubilization zones were characterized based on phenotypic features, carbon-source utilization pattern, FAME analysis and 16S rRNA gene sequencing as the polyphasic approach has been considered most reliable for characterization and identification of bacteria (Chung et al., 2005; Han et al., 2005; Park et al., 2005; Guedes et al., 2008; Tindall et al., 2010). The sharp 23 kb bands resolved in
agarose gels indicated the extraction of DNA with adequate quality from various isolates (Fig. 4.3). The fluorescence of bacterial colonies under UV light and amplification of 900 bp product of 16S rRNA gene region with the genus specific primers confirmed the inclusion of fluorescent *Pseudomonas* in the present studies (Fig. 4.1 and 4.4). The strains belonging to fluorescent *Pseudomonas* are among the common inhabitants of rhizosphere involved in multiple interactions with plants (Rainey, 1999; Botelho and Mendonça-Hagler, 2006; Jha *et al*., 2009; Park *et al*., 2009).

Carbon source utilization pattern indicated variation at strain level as the isolates showing the highest similarity with *Pseudomonas fluorescens* in BIOLOG database were found positioned into different groups and independent branches (Table 4.3; Fig. 4.6). The BIOLOG has been reported as the best test due to high resolution of the closely related isolates for analyzing the diversity (Johnsen *et al*., 1996; Ross *et al*., 2000). The present studies also generated variation among the isolates into different groups and within the groups based on their carbon-source utilization pattern (Fig. 4.6). It has also been reported that different types of microorganisms possess different fatty acid patterns and it is not possible to detect specific species (Barriuso *et al*., 2008). The different groups of fluorescent *Pseudomonas* isolates belonging to the same species acquired by FAME analysis illustrated the existence of strain level variation (Table 4.5; Fig. 4.8). The usefulness of whole-cell fatty acid profile analysis has been reported in strain-differentiation at the intra-species level among *Ralstonia solanacearum* strains isolated from banana, brinjal, chilli, tomato and tobacco (Khakvar *et al*., 2009). However, the groupings based on carbon-source utilization pattern displayed no clear correlation to the groupings based on FAME analysis as the isolate BIHB 736 which grouped with BIHB 730 on carbon-source utilization pattern stood independently outside the major cluster on the basis of FAME analysis (Fig. 4.6 and 4.8). Similarly, the isolates BIHB 747 and BIHB 756 which were included in the major cluster based on carbon-source utilization stood independently outside the major cluster by FAME analysis. Likewise, the isolates BIHB 769 and BIHB 808 grouped based on FAME analysis in the major cluster along with other isolates stood as an independent pair in carbon-source utilization pattern. The results signified specific microbial fingerprint for each isolate for carbon-source utilization and whole-cell fatty acid composition. Earlier also variations in the groupings based on carbon-source utilization pattern and whole-cell fatty acid methyl ester analysis has been reported in *Pseudomonas brassicacearum* and *P. thivervalensis* strains isolated from wheat field soils (Ross *et al*., 2000).
The similarity in 16S rRNA gene PCR-RFLP pattern obtained among the majority of isolates indicated the dominance of a particular species of *Pseudomonas* solubilizing phosphate in the soil samples (Fig. 4.10 and 4.11). Earlier *Pseudomonas* populations belonging to *Pseudomonas pseuodoalkaligenes* and *P. alcaligenes* produced two groups in PCR-RFLP of 16S rRNA gene suggesting predominantly homogenous populations in pearl millet, cotton and paddy rhizospheres (Rangarajan *et al.*, 2001). Similarly, seven 16S rRNA gene PCR-RFLP patterns were obtained for seven ATCC type strains of *Capnocytophaga* spp. with RFLP patterns characterizing particular species (Ciantar *et al.*, 2005).

The phylogenetic tree constructed based on 16S rRNA gene sequences of the test isolates and representative species of *Pseudomonas* formed three clearly distinguishable groups (Fig. 4.16). Group I included fourteen isolates along with *P. trivialis* DSM 14937T, *P. poae* DSM 14936T and *P. antarctica* strain CMS 35T. Group II contained two isolates along with *P. kilonensis* 520-20, *P. syringae* ATCC 19310T and *P. corrugata* ATCC 29736T. Group III included three isolates along with *P. jessenii*, *P. moraviensis* CCM 7280, and *P. pavonaceae*. The 16S rRNA gene sequencing showed identity of ten strains with *P. trivialis* DSM 14937T, three strains with *P. poae* DSM 14936T, one strain each with *P. fluorescens* Pf29A, *Pseudomonas* sp. A-13, *Pseudomonas* sp. F98, *Pseudomonas* sp. H92hy, *Pseudomonas* sp. P 12 and *Pseudomonas* sp. PSB8 (Table 4.6). *Pseudomonas trivialis* DSM 14937T and *P. poae* DSM 14936T have been described as the novel species associated with the phyllosphere of grasses (Behrendt *et al.*, 2003). Recently, *P. poae* has been reported among the siderophore-producing bacteria associated with the tobacco rhizosphere (Tian *et al.*, 2009). Similarly, *P. antarctica* strain CMS 35T has been reported as a novel psychrophillic species from Antarctica (Reddy *et al.*, 2004). Likewise, *P. kilonensis* 520-20 has been described as a novel species from the agricultural soils of Northern Germany (Sikorski *et al.*, 2001). Strains of *P. corrugata* isolated from the subtropical and temperate regions in Sikkim Himalayas have been reported for phosphate solubilization, nitrogenase activity and production of antifungal compounds (Pandey and Palni, 1998). A widespread occurrence has been reported for *P. fluorescens* associated with rhizospheres and non-rhizospheres of several plants, including amaranth, cotton, cucumber, chickpea, ginseng, maize, olive, pea, pepper, pigeonpea, rice, ragi, tomato and wheat (Nautiyal, 1997; Nautiyal *et al.*, 2002; Lucas García *et al.*, 2003; Mercado-Blanco *et al.*, 2004; Negi *et al.*, 2005; Tilak *et al.*, 2006; Raja *et al.*, 2006; Jayaraj *et al.*, 2007; Shaharoona *et al.*, 2008; Park *et al.*, 2009).
The identification of fluorescent *Pseudomonas* based on carbon-source utilization pattern or FAME analysis did not correlate with that based on 16S rRNA gene sequencing which could be due to the limited information in BIOLOG and Sherlock databases pertaining to the strains characterized under the present studies. The results substantiated the earlier reports on the characterization of phenanthrene-degrading fluorescent *Pseudomonas* where the isolates showing identity with *P. corrugata* on the basis of carbon source utilization were identified as *P. fluorescens* strains based on cellular fatty acid profiles and partial 16S rRNA gene sequencing (Johnsen *et al*., 1996). Similarly, the isolates showing the highest similarities with *Enterobacter cancerogenus*, *Kluyvera ascorbata*, *Klebsiella pneumoniae* and *K. planticola* on the basis of FAME analysis were identified as *Pantoea* sp., *P. agglomerans*, *Enterobacter aerogenes* and *E. cloacae* on the basis of 16S rRNA gene sequencing, respectively (Chung *et al*., 2005). Likewise, the isolates showing similarity with *Bacillus sphaericus* and *Pseudomonas putida* based on FAME analysis were identified as *Bacillus fusiformis* and *Pseudomonas fluorescens* on the basis of 16S rRNA gene sequencing (Park *et al*., 2005). A phosphate-solubilizing isolate showing similarity with *Acetobacter liquefaciens* based on FAME analysis was identified as *Gluconoacetobacter* sp. by 16S rRNA gene sequencing (Linu *et al*., 2009).

The high polymorphism obtained in the banding profiles of ERIC and BOX-elements of fluorescent *Pseudomonas* in the present studies indicated genetic variability at inter-specific and intra-specific levels as seventeen strains generated specific and two strains displayed identical ERIC and BOX-PCR fingerprints (Fig. 4.17). The repetitive fingerprint analysis also yielded informative amplicons about the individual strains. rep-PCR displayed variability among strains belonging to the same phylogenetic group based on 16S rRNA gene sequencing exhibiting a poor relationship between phylogenetic groups and rep-PCR clusters (Fig. 4.16 and 4.18). The interspersed repetitive elements found in many bacterial species have been used in genotyping *Aeromonas*, *Escherichia coli*, *Pseudomonas*, *Rhizobium* and *Xanthomonas* (Louws *et al*., 1994; Yang *et al*., 2004; Tacao *et al*., 2005; Tran *et al*., 2008; Menna *et al*., 2009). rep-PCR has also been previously reported for detecting minor differences among strains of the same genus and species and not for establishing phylogenetic relatedness (Versalovic *et al*., 1994; Kaschuk *et al*., 2006; Menna *et al*., 2009). Recently, the genetic diversity of phosphate-solubilizing fluorescent pseudomonads assessed using BOX-PCR fingerprints has also shown a high degree of variability among isolates belonging to the same or different species (Naik *et al*., 2008). A substantial genotypic diversity using BOX-PCR has been reported among the strains
belonging to *Pseudomonas putida* isolated from black pepper rhizosphere (Tran et al., 2008). Likewise, ERIC-PCR and BOX-PCR have been reported to produce highly discriminatory banding patterns among closely related strains of *Leptospirillum ferrphilium* and *Acidithiobacillus ferrooxidans* isolated from the acid-mine drainage sites in China (Xie et al., 2008).

The results of present studies supported the usefulness of polyphasic approach in characterization and identification of bacteria, wherein 16S rRNA gene sequencing was used for identification up to species level while BIOLOG test, cellular fatty acid profiles and rep-PCR fingerprints were used for analyzing variation among the closely related isolates (Johnsen et al., 1996).

5.2 Screening of fluorescent *Pseudomonas* for Multiple Plant Growth-promoting Attributes

5.2.1 Phosphate solubilization

Phosphorus deficiency is a major constraint to crop production due to the rapid binding of applied phosphorus into fixed forms not available to the plants. The role of microorganisms in releasing bound phosphorus has been increasingly recognized for efficient phosphate solubilization (Nautiyal et al., 2000; Fernández et al., 2007; Jha et al., 2009; Selvakumar et al., 2009b). Fluorescent *Pseudomonas* isolated in the present studies appear to be among the most efficient phosphate-solubilizing bacterial strains on comparison with the earlier reports on microbial solubilization of phosphate substrates (Table 4.7). The TCP solubilization by these strains ranged from 319 to 827 µg ml\(^{-1}\) in comparison to 450, 290, 250 and 200 µg ml\(^{-1}\) exhibited by the four most efficient bacterial strains NBRI2601, NBRI3246, NBRI0603 and NBRI4003, respectively, from the alkaline soils of tropical India (Nautiyal et al., 2000), 510 µg ml\(^{-1}\) by the best bacterial strain from the alkaline soils (Johri et al., 1999), 96 to 139 µg ml\(^{-1}\) by 13 best strains clustered under the genera *Enterobacter*, *Pantoea* and *Klebsiella* from the Korean soils (Chung et al., 2005), 900 µg ml\(^{-1}\) by *Pantoea agglomerans* isolated from the acidic soils of Korea (Son et al., 2006), and 248-441 µg ml\(^{-1}\) by *Pseudomonas* strains isolated from the rhizosphere of Chinese cabbage (Poonguzhali et al., 2008). Recently, the phosphate solubilization of 808 µg ml\(^{-1}\) and 515 µg ml\(^{-1}\) have been reported for *Pseudomonas fluorescens* strain isolated from ginseng rhizosphere and *Pseudomonas fragi* strain from the rhizosphere of garlic, respectively (Park et al., 2009; Selvakumar et al., 2009b). The high solubilization of NCRP as compared to MRP and URP by the bacterial strains corroborated the earlier report on solubilization of rock phosphates by *Pseudomonas striata* and *Bacillus polymyxa* (Qureshi and
Narayanasamy, 1999). The solubilization of rock phosphates have been reported to depend on their structural complexity and particle size as well as the nature and quantity of organic acids secreted by the microorganisms (Gaur, 1990). In the present studies P. trivialis appears predominant among the phosphate-solubilizing fluorescent Pseudomonas in the soil samples. Phosphate solubilization have been reported for many Pseudomonas species including P. aeruginosa (Musarrat et al., 2000; Naik et al., 2008; Jha et al., 2009), P. chlororaphis, P. savastanoi, P. pickettii (Cattelan et al., 1999), P. corrugata (Pandey and Palni, 1998), P. fluorescens (Di Simine et al., 1998; Bano and Musarrat, 2004), P. fulva, P. monteilii, P. mosselii and P. plecoglossicida (Naik et al., 2008; Jha et al., 2009), P. jessenii (Valverde et al., 2006), P. lutea (Peix et al., 2004), P. putida (Kumar and Singh, 2001), P. rhizosphaerae (Peix et al., 2003) and P. stutzeri (Vazquez et al., 2000). However, the solubilization of inorganic phosphates by P. trivialis and P. poae has not been previously reported (Table 4.7).

A significant decline in the pH of medium recorded during the solubilization of different phosphate substrates by fluorescent Pseudomonas suggested the secretion of organic acids by the bacterial strains (Table 4.7). Several workers have reported the release of soluble P accompanied by a decrease in pH of culture medium due to organic acid production during phosphate solubilization (Illmer and Schinner, 1995; Chen et al., 2006; Tripura et al., 2007; Park et al., 2009). The release of soluble phosphates is not necessarily correlated with the acidity of culture medium (Asea et al., 1988; Naik et al., 2008; Jha et al., 2009). In the present studies also no relationship could be ascertained between quantity of phosphate solubilization and acidity of medium as decline in pH during solubilization of TCP and NCRP was not statistically different though solubilization was significantly higher for TCP than NCRP. Likewise, the decrease in pH was not always related with the quantity of phosphate solubilized, as the decline in pH was disparate with the quantity of phosphate solubilization by some strains (Table 4.7). Earlier findings on the solubilization of rock phosphates by some efficient microbial strains also indicated that phosphate solubilization varies greatly with the nature of phosphate substrates and the organisms (Bardiya and Gaur, 1974; Vyas et al., 2007; Farhat et al., 2009).

The organic acid production during solubilization of inorganic phosphates by the phosphate-solubilizing strains of Pseudomonas trivialis, P. poae, P. fluorescens and Pseudomonas spp. indicated their involvement in phosphate solubilization (Fig. 4.19; Tables 4.8-4.11). The results corroborated the earlier reports that solubilization of inorganic phosphates is mainly attributed to the production of organic acids and their chelation.
capacity by microorganisms which leads to the lowering of pH in the medium (Goldstein, 1995; Dave and Patel, 1999; Whitelaw et al., 1999; Chen et al., 2006; Trivedi and Sa, 2008; Patel et al., 2008; Park et al., 2009). Gluconic acid was the major organic acid produced as reported during phosphate solubilization by *Azospirillum* spp. (Rodriguez et al., 2004), *Citrobacter* sp. (Patel et al., 2008), *Erwinia herbicola* (Liu et al., 1992), *P. cepacia* (Babu-Khan et al., 1995), *Pseudomonas fluorescens* (Di Simine et al., 1998; Park et al., 2009) and *Pseudomonas* spp. (Illmer and Schiner, 1992; Kaur et al., 2006). The production of 2-ketogluconic, oxalic, malic, lactic, succinic, formic and citric acid in small quantities by *Pseudomonas* strains have also been reported during phosphate solubilization by *Arthrobacter ureafaciens*, *Arthrobacter* sp., *Bacillus coagulans*, *B. megaterium*, *Chryseobacterium* sp., *Citrobacter koseri*, *Delftia* sp., *Enterobacter intermedium*, *Pseudomonas fluorescens*, *Rhodococcus erythropolis* and *Serratia marcescens* (Illmer and Schiner, 1995; Gyaneshwar et al., 1998; Hwangbo et al., 2003; Chen et al., 2006; Trivedi and Sa, 2008). None of *Pseudomonas* strains in the present studies produced propionic acid unlike *Bacillus megaterium* strains and *B. atrophaeus* strain during phosphate solubilization (Vazquez et al., 2000; Chen et al., 2006).

The results indicated that the quantity of organic acids produced differed with the nature of phosphate substrates and *Pseudomonas* strains (Tables 4.8-4.11). The higher solubilization of TCP than URP, MRP and NCRP could possibly be due to the higher gluconic acid production in the presence of TCP. The lower production of gluconic acid and lower TCP solubilization by *Pseudomonas* sp. BIHB 751 than other *Pseudomonas* strains substantiated the involvement of gluconic acid in the solubilization of calcium-bound phosphates. Succinic acid also appeared contributing to TCP solubilization as it was produced by high TCP-solubilizing strains and not by low TCP-solubilizing *Pseudomonas* sp. BIHB 751 strain. The lack of oxalic acid production by phosphate-solubilizing *Pseudomonas* strains signified non involvement of oxalic acid in TCP solubilization though this acid has been implicated besides citric, gluconic, lactic and succinic acids in phosphate solubilization in alkaline vertisols (Gyaneshwar et al., 1998). *Pseudomonas* sp. strain BIHB 751 producing the highest quantity of 2-ketogluconic acid but showing the lowest TCP and URP solubilization also differed from *Enterobacter intermedium* reported for the enhanced phosphate solubilization with increasing 2-ketogluconic acid production (Hwangbo et al., 2003). Likewise, no relationship could be ascertained between the quantity of organic acids produced and the solubilization of rock phosphates by *Pseudomonas* strains as the highest solubilization observed for NCRP among the rock phosphates was coupled to the lowest
production of total organic acids (Tables 4.9-4.11). Previously also the quantities of solubilized phosphorus could not be correlated with the quantities of organic acids produced in the culture medium by *Pseudomonas* sp. and *Penicillium aurantiogriseum* (Illmer and Schinner, 1995). UPR, MRP and NCRP have fluorapatite structure with the highest substitution of phosphate with carbonate in NCRP (Narayanasamy and Biswas, 1998). The higher solubilization and lowered quantities of organic acids detected in the presence of NCRP could be due to the higher reactivity and greater diversion of organic acids in the neutralization of free carbonates in the solubilization of NCRP as compared to MRP and URP (Narayanasamy and Biswas, 1998; Bolland, 2007). Likewise, the higher solubilization and higher production of organic acids in the presence of TCP could be attributed to its amorphous nature with simple structure and absence of any free carbonates as compared to the crystalline lattice structure of rock phosphates (Kumari *et al*., 2008).

Cluster analysis of organic acid profiles generated different groups revealing inter and intra-specific variation in the production of organic acids by *Pseudomonas* strains (Fig. 4.20). The strains clustered together and those standing outside the clusters or sub-clusters belonged to different *Pseudomonas* species. The strains standing outside the clusters differed qualitatively and/or quantitatively from other strains in the production of organic acids (Fig. 4.20; Tables 4.8-4.11). The results implied that *Pseudomonas* strains are independent of the genetic relatedness in their phosphate-solubilizing ability and organic acid production even under similar set of culture conditions. Phosphate solubilization is a complex phenomenon which depends on the nutritional, physiological and growth conditions of the culture (Reyes *et al*., 1999).

Organic phosphate solubilization is another important mechanism in providing available P from the dead organic matter to plants. Phytate the predominant form of organic phosphates in the soil is hydrolyzed to a series of lower phosphate esters of *myo*-inositol and phosphate by bacteria through the production of phytases (Rodríguez *et al*., 2006). In the present studies, the development of phytate-solubilizing zones indicated phytase activity by fluorescent *Pseudomonas* strains (Table 4.20). Phytase production has been reported earlier for species of *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Serratia* and *Yersinia* (Richardson and Hadobas, 1997; Rodríguez and Fraga, 1999; Richardson *et al*., 2001; Idriss *et al*., 2002; Cho *et al*., 2005; Gulati *et al*., 2007; Huang *et al*., 2008). The information regarding phytase-producing *Pseudomonas* strains is limited to *P. fluorescens*, *P. mendocina*, *P. putida*, *P. syringae* and *Pseudomonas* spp. (Richardson and Hadobas, 1997; Cho *et al*., 2005; Hosseinkhani *et al*.,
Discussion

2009). The bacterial strains exhibited phytase activity ranging from 0.27-1.72 U ml\(^{-1}\) as compared to the previous reports of phytase production of 0.37 U ml\(^{-1}\) by *Bacillus amyloliquefaciens* isolated from plant-pathogen infested soil (Idriss *et al.*, 2002), 0.283 U ml\(^{-1}\) by *Bacillus laevo lacticus* isolated from fenugreek rhizosphere (Gulati *et al.*, 2007) and 1.9 U ml\(^{-1}\) by maize endophyte *Staphylococcus lentus* (Hussin *et al.*, 2007). Bacterial phytase activity has also been reported to eliminate chelate-forming phytate known to bind some nutritionally important minerals in the rhizosphere (Reddy *et al.*, 1989). The results showed significant difference in phytase activity by the strains belonging to the same or different species, thereby indicating that the phytase production was not restricted to a particular *Pseudomonas* species.

5.2.2 Production of IAA-like auxins

Plant growth promotion is also influenced by the production of plant growth-promoting hormones by microorganisms in the rhizosphere (Sarwar and Frankenberger 1994). Bacteria inhabiting rhizosphere of various plants are likely to synthesize and release auxins as secondary metabolites because of the rich supplies of tryptophan exuded from the roots (Sarwar and Frankenberger 1994; Kravchenko *et al*. 2004; Kamilova *et al.*, 2006). The present studies also showed the secretion of IAA by fluorescent *Pseudomonas* in tryptophan supplemented medium (Fig. 4.21; Table 4.12). IAA production in the presence of a suitable precursor such as tryptophan has been reported for several PGPR belonging to the genera *Acinetobacter*, *Acetobacter*, *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Xanthomonas* (Patten and Glick, 1996; Pedraza *et al.*, 2004; Kang *et al.*, 2006; Idris *et al.*, 2007; Tsavkelova *et al.*, 2007; Camerini *et al.*, 2008; Gulati *et al.*, 2009; Park *et al.*, 2009; Jha *et al.*, 2009; Abbas-Zadeh *et al.*, 2010). Among the genus *Pseudomonas*, IAA production is widespread in *P. aeruginosa*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, *P. syringae* and *P. stutzeri* (Patten and Glick, 2002; Lucas García *et al.*, 2003; Pedraza *et al.*, 2004; Ayyadurai *et al.*, 2006; Kamilkova *et al.*, 2006; Kang *et al.*, 2006; Khakipour *et al.*, 2008; Karnwal, 2009). The IAA producing bacteria have been reported to promote root growth enabling greater access of nutrients from soil by the plants (Vessey 2003; Fischer *et al.*, 2007; Shahab *et al.*, 2009).

The present studies showed the production of IAA-like auxins of 2.9-26.7 µg ml\(^{-1}\) by the fluorescent *Pseudomonas* strains which compared well with the earlier reports of 10.4-28.3 µg ml\(^{-1}\) by fluorescent *Pseudomonas* (Ahmad *et al.*, 2005), 7.9-8.3 µg ml\(^{-1}\) by *Acinetobacter* sp. strains (Indiragandhi *et al.*, 2008), 10.1-15.0 µg ml\(^{-1}\) by *Azotobacter*
strains (Ahmad et al., 2008), 7.0-19.3 μg ml⁻¹ by Bacillus strains (Wani et al., 2007), 1.5-23.4 μg ml⁻¹ by Pseudomonas strains (Poonguzhali et al., 2008), 0-32 μg ml⁻¹ by Pseudomonas fluorescens strains and 0-24 μg ml⁻¹ by Pseudomonas putida strains (Khakipour et al., 2008), 1.5-3.3 μg ml⁻¹ by Pseudomonas putida strains (Rodríguez et al., 2008), and 0.81-10.17 μg ml⁻¹ by fluorescent Pseudomonas strains (Abbas-Zadeh et al., 2010). Similarly, IAA-like auxin production of 16.8, 6.5, and 0.1 μg ml⁻¹ has been reported for Azospirillum brasilense, A. lipoferum, and Pseudomonas putida, respectively (Mehnaz and Lazarovits, 2006) and 15.6 μg ml⁻¹ by plant growth-promoting Acinetobacter rhizosphaerae strain isolated from the cold desert of the trans-Himalayas (Gulati et al., 2009). The present studies indicated that auxin production was not restricted to any particular Pseudomonas species as different Pseudomonas species and strains within species varied quantitatively in the production indole-derivatives (Table 4.12).

HPLC analysis showed the presence of IAA, IAAld, IAM, IAN, ILA and IPA with quantitative difference in the culture supernatants of fluorescent Pseudomonas strains (Fig. 4.22; Table 4.13). Several indole-derivatives have been reported to be secreted by a particular microorganism into the environment depending on the biosynthetic pathways and genotype (Patten and Glick, 1996; Carreño-López et al., 2000; Ge et al., 2009). The quantity of IAA reportedly the most active natural auxin in the plants varied for the different strains (Table 4.13). The presence of IAA, IAAld, IAM, IAN, ILA and IPA in the culture filtrates suggested the synthesis of IAA through different biosynthetic pathways by all Pseudomonas strains (Table 4.13). The synthesis of IAA through IPA to IAAld to IAA has been reported to be the primary route of auxin biosynthesis by microorganisms (Ivanova et al., 2001; Sergeeva et al., 2002; Woodward and Bartel, 2005; Tsavkelova et al., 2007). The IAA biosynthesis in which tryptophan is converted directly to IAAld bypassing IPA has been demonstrated in Pseudomonas fluorescens (Narumiya et al., 1979; Oberhansli et al., 1991). However, the production of IAA through IAM has also been identified in bacteria including Agrobacterium tumefaciens, Azospirillum brasilense, Bradyrhizobium sp. Erwinia sp., Mycobacterium sp., Pantoea agglomerans, Pseudomonas syringae and Rhizobium (Sekine et al., 1989; Bar and Okon, 1993; Patten and Glick, 2002; Tsavkelova et al., 2007). The synthesis of auxins through IAM and IPA pathways has been reported for different strains of Pseudomonas fluorescens, P. putida and Azospirillum brasilense (Patten and Glick, 1996; Zakharova et al., 1999; Carreño-Lopez et al., 2000; Khakipour et al., 2008; Ge et al., 2009).
The quantity of IAA-like auxins detected for various strains was 1.3-3.2 times higher with HPLC than Salkowski reagent (Tables 4.12 and 4.13). The Salkowski reagent has been reported for its specificity for IAA, IPA, and IAM and non-specificity for ILA and IAAld (Gordon and Weber 1951, Hartmann et al., 1983; Glickmann and Dessaux 1995; Patten and Glick 2002). It has earlier been concluded that Salkowski reagent is sufficient for the rapid screening of isolates for the production of indole-derivatives but not for IAA determination (Sergeeva et al., 2002; Pedraza et al., 2004). HPLC analysis has been employed to more specifically quantify the indole-derivatives (Glickmann and Dessaux 1995; Ivanova et al., 2001; Pedraza et al., 2004; Tsavkelova et al., 2007).

5.2.3 ACC-deaminase activity

The bacteria producing ACC deaminase are known to promote root elongation and plant growth by hydrolyzing ACC from germinating seeds thereby lowering ethylene level and increasing the active rhizosphere zone (Dey et al., 2004; Glick et al., 2007; Rodríguez et al., 2008; Farajzadeh et al., 2010). Several workers have utilized the ability of bacteria to utilize ACC as sole source of nitrogen and enhance seedling root elongation in screening ACC-deaminase producing bacteria (Penrose and Glick, 2003; Dey et al., 2004; Maimaiti et al., 2007; Rodríguez et al., 2008; Husen et al., 2009). Growth of fluorescent Pseudomonas on DF medium with ACC as sole nitrogen source and induction of root elongation in germinated seeds of maize as well as pea over their respective controls indicated ACC-deaminase activity by these bacterial strains (Fig. 4.23; Table 4.14). The fluorescent Pseudomonas strains induced 4.3-121.7% root elongation in maize and 7.5-165% root elongation in pea. An increase in root elongation has been reported by PGPR-26-51% in peanut by Pseudomonas fluorescens and Pseudomonas spp. strains (Dey et al., 2004), 57-108% and 21-92% in spring wheat by Variovorax strains and Burkholderia strains, respectively (Maimaiti et al., 2007), 56-166% in canola by Dyella ginsengisoli, Burkholderia kururiensis, Leifsonia shinshuensis, Pandoraea sputorium and Microbacterium phyllosphaerae (Anandham et al., 2008), 10-53% in Chinese cabbage by Pseudomonas strains (Poonguzhali et al., 2008), and up to 50% in soybean by Pseudomonas strains (Husen et al., 2009).

The strains exhibited significant difference in ACC-deaminase activity among different species and strains within the species revealing that the enzyme activity was not restricted to any particular Pseudomonas species (Table 4.14). The ACC-deaminase activity in the culture filtrates of Pseudomonas strains ranged from 0.23-1389.6 nM α-ketobutyrate h⁻¹ mg protein⁻¹ in comparison to 342 nM α-ketobutyrate h⁻¹ mg protein⁻¹ by Pseudomonas
Discussion

*Fluorescens* strain (Saravanakumar and Samiyappan, 2007), 26.4-3216 nM α-ketobutyrate h⁻¹ mg protein⁻¹ by *Dyella ginsengisoli, Burkholderia kururiensis, Leifsonia shinshuensis, Pandoraea sputorum* and *Microbacterium phyllosphaerae* (Anandham et al., 2008), 2010-7740 nM α-ketobutyrate h⁻¹ mg protein⁻¹ by *Pseudomonas* strains (Poonguzhali et al., 2008), 1100-3470 nM α-ketobutyrate h⁻¹ mg protein⁻¹ by *Pseudomonas putida* strains (Rodríguez et al., 2008) and 793.9 nM α-ketobutyrate h⁻¹ mg protein⁻¹ by *Pseudomonas fluorescens* strain (Farajzadeh et al., 2010). In the present studies, an absence of concomitant increase in root length with respect to ACC-deaminase activity was observed as *Pseudomonas trivialis* BIHB 759 showing significantly higher enzyme activity over other strains did not show the highest root elongation in maize as well as in pea (Table 4.14). Earlier the ACC-deaminase activity did not show any relationship with the root elongation as the *Pseudomonas* strains showing statistically similar root elongation in Chinese cabbage varied considerably in ACC-deaminase activity and also the strain showing the lowest root elongation exhibited relatively high enzyme activity (Poonguzhali et al., 2008). In another study *Pandoraea sputorum* strain showing the highest root elongation in canola exhibited the lowest ACC-deaminase activity (Anandham et al., 2008). However, the fluorescent *Pseudomonas* strains exhibiting enzyme activity up to 15 nM α-ketobutyrate h⁻¹ mg protein⁻¹ showed a non-significant increase in root elongation. The strains exhibiting high enzyme activity of 37-1389 nM α-ketobutyrate h⁻¹ mg protein⁻¹ induced significantly higher root elongation in maize and pea (Table 4.14). The results corroborated the earlier reports that ACC-deaminase activity up to 20 nM α-ketobutyrate h⁻¹ mg protein⁻¹ is sufficient for bacterial strains to promote plant growth and development and the bacterial strains showing high enzyme activity do not necessarily promote root elongation to greater extent than the strains showing low enzyme activity (Penrose and Glick, 2003; Anandham et al., 2008).

5.2.4 Siderophore production, hydrogen cyanide production, ammonia production and *in vitro* antagonism against fungal pathogens

Another important trait of the microorganisms that influences plant growth is the production of siderophores reported to suppress fungal pathogens by rendering iron unavailable in the rhizosphere (Klöepper et al., 1980; Höfte and Bakker, 2007). The type and biological activity of siderophores produced by plant growth-promoting *Pseudomonas* has been considered important for their ability in controlling the plant pathogens (Boopathi and Rao, 1999; Matthijs et al., 2006). In the present studies, ten *Pseudomonas trivialis* strains, three *P. fluorescens* strains, one *P. fluorescens* strain and five *Pseudomonas* spp. strains exhibited siderophore production with 11-24.8 mm wide siderophore zones and
49.7-87.5% siderophore units (Fig. 4.24; Table 4.15). Many *Pseudomonas* species including *P. amygdalae*, *P. asplenii*, *P. aeruginosa*, *P. chlororaphis*, *P. cichorii*, *P. fluorescens*, *P. fucovaginae*, *P. meliae*, *P. poae*, *P. pseudomallei*, *P. putida*, *P. stutzeri*, *P. syringae*, and *Pseudomonas* spp. have been reported to produce siderophores (Sharma and Johri, 2003; Bultreys *et al.*, 2003; Sayyed *et al.*, 2005; Tian *et al.*, 2009). Among the siderophore-producing bacteria such as *Achromobacter xylososioxidans*, *Agrobacterium tumefaciens*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Enterobacter aerogenes*, *E. endosymbiont*, *Pectobacterium carotovorum*, *Pseudomonas aeruginosa*, *P. poae*, *P. putida*, *Pseudomonas* spp., *Serratia marcescens*, *Sphingobacterium* spp. and *Stenotrophomonas maltophilia*, a majority have been reported to produce 20-40% siderophore units while others produced 40-60% siderophore units (Tian *et al.*, 2009). The efficient siderophore producing *Pseudomonas putida* and *P. fluorescens* strains have been reported to produce 83 to 87% siderophore units (Sayyed *et al.*, 2005).

The quantity of hydroxamate-type siderophore ranged from 142-613 µg ml$^{-1}$ for various fluorescent *Pseudomonas* strains (Table 4.15). Hydroxamate-type siderophore has been reported to be 3.8-108.7 µg ml$^{-1}$ for strains of *Bacillus* and *Pseudomonas* (Chaiharn *et al.*, 2009). Resourcing the bacterial iron-siderophore complexes as a means to obtaining iron from the soil has been reported to help plants overcome the stress of iron deficiency (Neilands, 1981; Glick, 1995). The siderophore production by fluorescent *Pseudomonas* strains could also be indirectly augmenting the availability of P as siderophore production has also been reported to release P from iron bound phosphates by chelating iron (Duponois *et al.*, 2006).

A single well defined peak within 360-480 nm with $\lambda_{\text{max}}$ near 405 nm and absence of any other peak in the culture supernatants indicated the production of hydroxamate-type siderophores by the fluorescent *Pseudomonas* strains (Fig. 4.26). The production of hydroxamate-type siderophores has been reported in several *Pseudomonas* species, including *P. aeruginosa*, *P. aureofaciens*, *P. putida*, *P. fluorescens* and *Pseudomonas* spp. (Mahmoud and Abd-Alla, 2001; Gupta *et al.*, 2002; Carrillo-Castañeda *et al.*, 2005; Sayyed *et al.*, 2005; Llamas *et al.*, 2006; Chaiharn *et al.*, 2009). The trihydroxamate nature of siderophores produced by the fluorescent *Pseudomonas* strains was evident by the absorption maxima between 360-480 nm for cell-free supernatants and 420-450 nm for ferric-siderophore complexes, and slight shift in $\lambda_{\text{max}}$ without any colour change over a wide pH range in the reddish-orange ferric-siderophore complexes. The absorption spectra have been employed for determining the nature of siderophores based on functional groups
(Meyer and Abdallah, 1978; Gupta et al., 1999; Storey et al., 2006; da Silva and de Almeida, 2006). Highly discriminating capacity of the spectrophotometric method has been reported to categorize siderophore-producing fluorescent Pseudomonas strains (Carrillo-Castañeda et al., 2005). Fluorescent Pseudomonas have been reported to produce siderophores commonly referred to as pyochelins and pyoverdines (Glick et al., 1999; Llamas et al., 2006). Pyoverdines have been shown to give absorption maxima between 405 to 412 nm as also observed in the present studies which suggested the production of trihydroxamate-type pyoverdines by fluorescent Pseudomonas strains (Stintzi et al., 1996; Bultreys et al., 2003).

The fluorescent Pseudomonas strains exhibited growth inhibition in Fusarium solani f. sp. pisi, F. oxysporum and Alternaria solani on both succinate agar and yeast malt extract medium (Tables 4.16-4.19). The medium-dependent antagonistic activity suggested the involvement of different modes of antagonism. The inhibition of fungal pathogens on iron-deficient medium indicated the involvement of siderophores in controlling these pathogens by the fluorescent Pseudomonas strains (Fig. 4.27; Tables 4.16-4.18). Siderophore-producing fluorescent Pseudomonas have been reported to show antagonism against various fungal pathogens- P. brassicacearum and P. thivervalensis strains against Gaeumannomyces graminis var. tritici (Ross et al., 2000), P. aeruginosa against Aspergillus niger, A. flavus, Fusarium oxysporum and Alternaria alternata (Manwar et al., 2000), fluorescent Pseudomonas strains against Colletotrichum dematium, Rhizoctonia solani and Sclerotium rolfsii (Sharma and Johri, 2003), P. fluorescens against Pyricularia oryzae and Rhizoctonia solani (Prasanna Reddy and Reddy, 2009) and P. aureofaciens against Alternaria sp., Fusarium oxysporum and Pyricularia oryzae (Chaiharn et al., 2009).

The inhibition of growth in fungal pathogens has also been shown by hydroxamate-type siderophore producing bacteria- Azotobacter chroococcum against Fusarium oxysporum (Saikia and Bezbruah, 1995), Pseudomonas sp. against Macrophomina phaseolina (Gupta et al., 2002), and Pseudomonas aureofaciens against Alternaria sp., Fusarium oxysporum and Pyricularia oryzae (Chaiharn et al., 2009).

The decrease in growth inhibition of fungal pathogens with increase in iron concentration confirmed the involvement of siderophores in antagonism by Pseudomonas strains (Fig. 4.27; Tables 4.16-4.18). Decreased antagonistic activity against Aspergillus sp., Colletotrichum dematium, Fusarium oxysporum, Pythium aphanidermatum, Phytophthora parasitica var. nicotianae, Rhizoctonia solani and Sclerotium rolfsii on addition of iron in growth media has also been reported by Proteus sp., Pseudomonas
mediterranea, Pseudomonas sp. and fluorescent Pseudomonas strains (de Boer et al., 1999; Barthakur, 2000; Goel et al., 2000; Sharma and Johri, 2003; Tian et al., 2008). However, the maximum antagonistic activity against various fungal pathogens was not shown by the same Pseudomonas strain (Tables 4.16-4.18). The highest growth inhibition in Fusarium solani f. sp. pisi and Alternaria solani was shown by Pseudomonas trivialis BIHB 728 and in Fusarium oxysporum by P. trivialis BIHB 745. Studies on siderophore-producing bacteria have likewise reported the highest antagonistic activity by Pseudomonas sp. strain against Alternaria sp., Ochrobactrum anthropi strain against Fusarium oxysporum, Bacillus firmus against Pyricularia oryzae and Kocuria rhizophila against Sclerotium sp. (Chaihran et al., 2009). Also the highest antagonistic activity was not shown by the highest siderophore-producing Pseudomonas sp. BIHB 756 (Tables 4.15-4.18). Likewise, the lowest antagonistic activity was not shown by the lowest siderophore-producing Pseudomonas fluorescens strain. The results indicated the operation of other antagonistic mechanisms besides siderophore production in these fluorescent Pseudomonas strains. Fluorescent Pseudomonas have also been reported to suppress fungal pathogens through the production of phenazine-1-carboxylate, 2, 4-diacetylphloroglucinol, HCN, gluconic acid, IAA, chitinases, cellulases and proteases (Castric, 1975; Keel et al., 1992; Compant et al., 2005; Domenech et al., 2006; Kai et al., 2007; Yang et al., 2008; Prasanna Reddy and Reddy, 2009).

The fluorescent Pseudomonas strains exhibited growth inhibition on iron-rich yeast malt extract medium in fungal pathogens ranging from 1.7-24% against Fusarium solani f. sp. pisi, 17-37% against F. oxysporum and 1.4-39% against Alternaria solani (Fig. 4.28; Table 4.19). Pseudomonas fluorescens BIHB 740, Pseudomonas sp. BIHB 751, Pseudomonas trivialis BIHB 763, Pseudomonas sp. BIHB 804 and Pseudomonas sp. BIHB 811 showing moderate to strong HCN production exhibited relatively higher growth inhibition against the fungal pathogens than the non-HCN producing strains, thereby indicating the involvement of HCN in biocontrol by these strains (Tables 4.19 and 4.20). HCN-producing Pseudomonas aeruginosa, P. fluorescens and Pseudomonas spp. strains have been reported to inhibit the growth of Fusarium oxysporum, Helminthosporium sp., Phytophthora capsici and Thielaviopsis basicola (Voisard et al., 1989; Mercado-Blanco et al., 2004; Paul and Sarma, 2006; Hassanein et al., 2009). However, the growth inhibition by non-HCN-producing Pseudomonas strains was statistically not different from HCN-producing strains which suggested the production of metabolites other than siderophores and HCN by these strains. The production of gluconic acid, IAA and ammonia has also
been implicated in suppressing the growth of fungal pathogens (Brown and Hamilton, 1993; Martinez Noël et al., 2001; Bano and Musarrat, 2004; Kaur et al., 2006; Park et al., 2009). Pseudomonas strains under the present studies also produced gluconic acid, IAA and ammonia (Tables 4.8-4.11 and 4.13). However, assigning the mere production of these compounds to antagonistic effects seems not plausible as specific studies would be required to elucidate their involvement in fungal antagonism. The results corroborated the earlier findings that the suppression of fungal pathogens is a complex phenomenon with multiple mechanisms operating singly or concurrently making difficult the assigning of a specific inhibitory compound to the antagonistic effects (Gupta et al., 2002; Arora et al., 2008).

5.2.5 Stress tolerance of fluorescent Pseudomonas strains

The performance of plant growth-promoting microorganisms is often limited by stressful environment which affects their establishment, multiplication and spread through the soil (Latour et al., 1996; Miller and Woods, 1996; Zahran, 1999; Nautiyal et al., 2000; Ruiz-Diez et al., 2009). The present studies showed difference in the stress-tolerance limits of fluorescent Pseudomonas as nine strains belonging to Stress-tolerance Cluster I were highly tolerant to temperature, alkalinity, salinity, Ca salts, and desiccation (Tables 4.21 and 4.22; Fig. 4.31 and 4.32). The high stress-tolerance capacity in the strains belonging to different phylogenetic groups indicated that stress tolerance was not limited to a specific phylogenetic group of fluorescent Pseudomonas in the rigorous environment of cold deserts (Fig. 4.16 and 4.32). However, the results showed that stress-tolerant strains from cold deserts mainly differed in their ability to withstand drought and to some extent temperature, thereby suggesting that screening for desiccation tolerance and temperature should be important attributes in the selection of native strains for developing plant growth-promoting microbial inoculants (Tables 4.21 and 4.22; Fig. 4.31). Drought tolerance is an important factor affecting the performance of microorganisms (Vriezen et al., 2006). The isolation of several stress-tolerant strains from the taxing locale of cold deserts substantiated the earlier reports that stress-tolerant bacteria are more likely to be found in the environments affected by osmotic, pH, temperature and drought stresses (Douka et al., 1978; Hua et al., 1982; Johri et al., 1999). The knowledge regarding the stress-tolerant agriculturally important strains is limited to some microorganisms, including Bacillus sp. tolerant to low pH (Pal, 1998), Rhizobium strains tolerant to high salt, pH, desiccation and temperature (Kulkarni and Nautiyal, 2000; Rehman and Nautiyal, 2002; Arun and Sridhar, 2005; Shamseldin, 2008), Pseudomonas pseudoalcaligenes and P. alcaligenes tolerant to salinity (Rangarajan et al., 2002), Pseudomonas fluorescens tolerant to low temperature (Negi et al., 2005),
Sinorhizobium meliloti USDA 1021 tolerant to desiccation (Vriezen et al., 2006), Pseudomonas sp. tolerant to low temperature (Mishra et al., 2008), Mesorhizobium sp. tolerant to NaCl, pH and temperature (Wei et al., 2008), Xanthomonas campestris tolerant to high salinity, pH and temperature (Sharan et al., 2008), Bradyrhizobium canariense, Mesorhizobium huakuii, Phyllobacterium myrsinacearum and Rhizobium rhizogenes tolerant to salinity, alkalinity and cadmium (Ruiz-Diez et al., 2009), Pseudomonas extremorientalis, P. putida, P. chlororaphis and P. aurantiaca tolerant to salinity (Egamberdieva and Kucharova, 2009), and Exiguobacterium acetylicum, Pantoea dispersa, Pseudomonas fragi and Serratia marcescens tolerant to low temperatures (Selvakumar et al., 2008a, c, 2009a, b). In the present studies, Pseudomonas strains showed tolerance to CaCO$_3$ and CaSO$_4$ but not against high concentration of CaCl$_2$. A reduction in the bacterial populations with CaCl$_2$ has been reported on fresh mushrooms (Chikthimmah et al., 2005). Inhibition in the growth of Selenomonas ruminantium has also been reported as a function of Ca$^{2+}$ concentration (Nakamura et al., 1996). No reports are available on the occurrence of Ca-tolerant microorganisms from the Ca-rich soils of cold deserts.

Phosphate solubilization by Pseudomonas strains at high levels of alkalinity, salinity, calcium salts, and desiccation indicated that these strains have evolved with the ability to solubilize phosphate under stressful milieu (Tables 4.23-4.25). Pseudomonas trivialis BIHB 747 and P. trivialis BIHB 750 exhibiting higher phosphate solubilization at low temperature as compared to other strains could prove more effective as the microbial inoculants in cooler regions. The decline in phosphate solubilization recorded with increasing stress levels was similar to the earlier reports (Johri et al., 1999; Sharan et al., 2008). All nine stress-tolerant strains exhibited reduced phosphate solubilization with increasing alkalinity (Table 4.24). The absence of phosphate solubilization at pH 11 has been reported for two of the four most efficient phosphate-solubilizing bacteria isolated from the alkaline soils of hot, dry, and salt-affected ecosystems (Nautiyal et al., 2000). The decline in phosphate solubilization observed with increasing NaCl concentration was less sharp as reported for Xanthomonas campestris (Sharan et al., 2008). The results varied from the earlier report of increased phosphate solubilization at 2.5% NaCl for the efficient phosphate-solubilizing bacteria from the alkaline soils exposed to high salt stress (Nautiyal et al., 2000). The decline in phosphate solubilization was less marked in the presence of CaSO$_4$ than CaCO$_3$, possibly due to the buffering nature of CaCO$_3$ which provided resistance to decrease in the pH of medium by organic acids produced by the bacterial strains (Table 4.25). Calcium carbonate supplements have been reported to inhibit
phosphate solubilization in the most efficient phosphate-solubilizing bacteria isolated from alkaline soils of the tropics (Nautiyal et al., 2000). The phosphate solubilization in the presence of PEG 6000 by Pseudomonas strains further indicated that these strains could be used as microbial inoculants under regimes of high desiccation in the drought-prone biomes. This is the first study on phosphate solubilization under desiccation by bacteria. The present studies indicated that some fluorescent Pseudomonas strains with the potential to solubilize phosphates under high stress conditions are well adapted to the cold desert environment as the native strains. The results are of practical importance in the context of developing stress-tolerant plant growth-promoting inoculants for application in the rigorous environments.

5.3 Evaluation of fluorescent Pseudomonas for Plant Growth Promotion in Environment Controlled Chambers

The application of PGPR has been reported to enhance plant growth and yield depending upon the plant species/variety, PGPR strains, and growth conditions (Nowak et al., 1998; Mehnaz and Lazarovits, 2006). Enhanced growth and higher N, P and K contents with Pseudomonas treatments in growth promotion experiments conducted on maize in plant growth medium with insoluble phosphate source underlined the advantage of phosphate-solubilizing activity of microorganisms for plant growth promotion (Tables 4.27-4.29). The lowest plant growth-promoting activity observed with the lowest TCP-solubilizing Pseudomonas sp. BIHB 751 further suggested that phosphate solubilization is an important mechanism of plant growth promotion. The increased growth and P uptake have been reported on inoculations with phosphate-solubilizing bacteria- Pseudomonas sp. and Serratia marcescens in maize (Di Simine et al., 1998), Pseudomonas fluorescens in peanut (Dey et al., 2004), Bacillus circulans in mungbean (Singh and Kapoor, 1990), Pseudomonas sp. in wheat (Babana and Antoun, 2006), and Gluconoacetobacter sp. and Burkholderia sp. in cowpea (Linu et al., 2009). The TCP solubilization in soil by fluorescent Pseudomonas strains as supported by in vitro TCP solubilization, increased soil P availability and higher plant P content would be useful in the soils where P deficiency is attributed mainly to the reaction of P with calcium salts forming insoluble di- and tricalcium phosphates. The significantly higher plant growth and N, P, and K contents in plant tissues and soil with some Pseudomonas treatments over NPSSP*K might be due to the immobilization of applied P by native soil microbiota and physico-chemical reactions in the soil. The increased and continuous P availability in the soil also promotes biological nitrogen fixation (Dey et al., 2004).
The decrease in soil pH in *Pseudomonas* treatments indicated the production of organic acids by *Pseudomonas* strains as also reported for phosphate-solubilizing *Aspergillus niger* and *A. tubingensis* (Richa et al., 2007). However, less pH decline in soil during plant growth promotion experiments than phosphate solubilization in culture medium could be due to the buffering nature of soil (Gyaneshwar et al., 1998). The inorganic acids and H⁺ ions of microbial origin and H⁺ ions released from the plant roots during ammonium assimilation have also been reported to influence the soil pH (Flach et al., 1987; Gaur, 1990; Illmer and Schinner, 1995). The studies showed potential for plant growth promotion by *Pseudomonas trivialis* BIHB 745, *P. trivialis* BIHB 747, *Pseudomonas* sp. BIHB 756 and *P. poae* BIHB 808 in the presence of TCP as the phosphate source. The phosphate-solubilizing and stress-tolerant *Pseudomonas* strains are likely to cohabit as effective microbial inoculants with the crops grown in their native habitation.

*Pseudomonas* strains exhibiting high TCP solubilization *in vitro* differed significantly in enhancing plant growth in the soil indicating the interplay of some other growth factors besides phosphate-solubilization (Tables 4.7, 4.27-4.29). In addition to phosphate solubilization, fluorescent *Pseudomonas* strains exhibited plant growth-promoting attributes of auxin production, ACC-deaminase activity, siderophore production, ammonia production, HCN production, and *in vitro* antagonism against fungal pathogens. Rhizobacteria with multiple growth-promoting mechanisms have been considered important to enhancing growth and yield in different crops (Dey et al., 2004; Ahmad et al., 2008; Jha et al., 2009). Bacteria have also been reported to differ in utilizing root exudates in producing biologically active substances and root-colonizing ability known to influence plant growth (Ladha et al., 1986). Plant-microbe interaction is an intricate phenomenon with the interplay of several mechanisms and environmental factors.

The enhanced growth in two varieties each of pea, maize, barley and chickpea in sterilized vermiculite under environmental controlled conditions exhibited broad-spectrum plant growth-promoting activity by fluorescent *Pseudomonas* strains (Tables 4.30 and 4.31). The significant incremental influence on root length, shoot length, and dry matter observed in the test plants indicated potential in these strains as plant growth-promoting microbial inoculants. *Pseudomonas* strains have been reported to enhance growth in pot experiments under controlled environment in several plants, including blackcurrant, maize, okra, olive, pea, peanut, pearl millet, potato, spinach, tomato and wheat (Burr et al., 1978; Dubeikoysky et al., 1993; Dileep Kumar et al., 2001; Pal et al., 2001; Gupta et al., 2002;
Mercado-Blanco et al., 2004; Pandey et al., 2006; Mehnaz and Lazarovits, 2006; Hameeda et al., 2006; Adesemoye et al., 2008; Peyvandi et al., 2010). Significant difference in plant growth-promoting activity of fluorescent Pseudomonas belonging to different species and same species highlighted variation in the plant growth-promoting capability of these strains (Tables 4.30 and 4.31). Pseudomonas trivialis BIHB 745, P. trivialis BIHB 747 and Pseudomonas sp. BIHB 756 showed high plant growth-promoting activity in all the eight test plants exhibiting the attribute of broad-spectrum plant growth promotion. Likewise, Pseudomonas trivialis BIHB 728, P. trivialis BIHB 769 and P. poae BIHB 808 also showed high plant growth-promoting activity in five to six test plants while other strains exhibited high plant growth-promoting activity limited to one to three test plants. The difference in growth-promoting activity of the same strain in different varieties of the crop suggested that plant variety/genotype affects the performance of PGPR strains. Likewise, the difference in growth-promoting activity in the strains of same species suggested that effects of PGPR are also highly specific with respect to plant and bacterial combination. Plant growth-promoting potential of PGPR has been reported to vary with PGPR strains and plant cultivars (Rennie and Larson, 1979; Smith and Goodman, 1999; Arsac et al., 1990; Riggs et al., 2001; Mehnaz and Lazarovits, 2006; Yadegari et al., 2008). The distinction in plant growth response has been related to the specificity of plant-microbe associations and difference in root exudates which support the microbial activity or serve as substrates for the formation of biologically active substances by microbial inoculants (Ladha et al., 1986; Dazzo et al., 2000). The comparative screening of rhizobacteria for multiple plant growth-promoting attributes and growth promotion in multiple crops appears a good strategy in the selection of potential PGPR strains for field evaluation.

5.4 Microplot Evaluation

The strains that work well under controlled environment do not necessarily elicit satisfactory plant response under field conditions necessitating the comparative field evaluation of potential PGPR strains for the selection of most suitable strains in the development of biofertilizers. Pseudomonas strains showed considerable variation in growth-promoting activity in pea in microplots possibly due to the difference in their potential for various plant growth-promoting attributes and ability for stress tolerance (Table 4.32). However, the results on plant growth promotion in microplots were in agreement with the results on plant growth promotion obtained under controlled environment as Pseudomonas trivialis BIHB 745, P. trivialis BIHB 747 and Pseudomonas
sp. BIHB 756 exhibited the highest plant growth-promoting activity under both controlled environment and field conditions (Tables 4.30-4.32).

5.5 Rhizosphere Competence

The success of a microbial inoculant depends on its ability to survive and proliferate in the target habitat. Rhizosphere competence is an important attribute to be considered while screening new strains for developing plant growth-promoting formulations as introduced microorganism might fail to establish itself at the release site (Mazzola and Cook 1991; Glandorf *et al.*, 1994; Hirsch and Spokes 1994; Nautiyal *et al.*, 2002). The rif-resistant mutant strains of *Pseudomonas trivialis* BIHB 745, *P. trivialis* BIHB 747 and *Pseudomonas* sp. BIHB 756 selected on the basis of comparable growth to their respective wild strains statistically showed no difference in their root-colonizing ability compared to their respective wild strains in pea and maize grown in sterilized vermiculite (Fig. 4.33). The wild and mutant strains also exhibited a similar trend in population build up throughout the observation period of eight weeks. The results showed that the wild and their respective mutant strains were ecologically equally competitive as also reported for *Pseudomonas fluorescens* (Nautiyal *et al.*, 2002).

The increase in the population of *Pseudomonas* strains in rhizospheres of maize and pea in non-sterilized soil in the background of native microbial population indicated that the strains were rhizosphere competent (Fig. 4.34). However, the populations of *Pseudomonas* strains in the rhizosphere decreased with time in both maize and pea. The populations of *Pseudomonas* strains were higher in maize rhizosphere as compared to pea rhizosphere possibly due to the difference in root exudates of the two plants (Fig. 4.34). Increased bacterial population during the early phase and decreased population in the late phase of plant growth have been reported due to the changes in root exudation pattern which influence the biological, chemical and physical properties of rhizosphere (Miller *et al.*, 1989; Bais *et al.*, 2006; Nannipieri, *et al.*, 2008; Mishra and Nautiyal, 2009). The significantly lower population recovery of *Pseudomonas* strains from non-rhizosphere in comparison to rhizosphere indicated the dispersion of only small bacterial population of the strains from rhizosphere to non-rhizosphere (Fig. 4.34). The rapid colonization, sustainability of a substantial population in the rhizosphere, and low population dispersion of the rif-resistant mutants of *Pseudomonas trivialis* BIHB 745, *P. trivialis* BIHB 747 and *Pseudomonas* sp. BIHB 756 demonstrated high rhizosphere competence in these strains (Fig. 4.34-4.36). However, the strains differed in their ability to colonize maize rhizosphere and pea rhizosphere with the highest root colonization by *Pseudomonas trivialis* BIHB 745.
followed by *Pseudomonas* sp. BIHB 756 and *P. trivialis* BIHB 747 (Fig. 4.34). Plant growth-promoting *Paenibacillus polymyxa* strains have been reported to differ in their root colonizing-ability in peanut and mouseear cress (*Arabidopsis thaliana*) (Haggag and Timmusk, 2007). Non-significant difference between wild strains and their rif-resistant mutants on growth promotion in maize and pea indicated ecologically fitness of the wild *Pseudomonas* strains (Table 4.26). These strains also appeared ecologically benign as their rif-resistant mutants promoted growth in maize and pea without adversely affecting the resident microbial populations of rhizosphere and non-rhizosphere in both maize and pea (Fig. 4.35 and 4.36; Table 4.26). The rif-resistant mutants of plant growth-promoting *Pseudomonas fluorescens* strain and *Acinetobacter rhizosphaerae* strain have also been reported to enhance plant growth without adversely affecting the native microbial population (Nautiyal *et al*., 2002; Gulati *et al*., 2009).

### 5.6 Field Evaluation

The field evaluation of *Pseudomonas trivialis* BIHB 745 selected on the basis of multiple plant growth-promoting attributes, stress tolerance, rhizosphere competence, and plant growth promotion under controlled environment and microplots was carried out to test the strain in maintaining crop productivity with reduced fertilizer dosage. The results showed enhanced plant growth and yield in pea with the application of *P. trivialis* BIHB 745 with and without an efficient nitrogen-fixing strain of *Rhizobium leguminosarum* BIHB 645 for two consecutive years at two locations (Tables 4.33-4.38). Higher nodulation obtained with *P. trivialis* BIHB 745 over 50% NPK and with the combined application of *P. trivialis* BIHB 745 and *R. leguminosarum* BIHB 645 over *R. leguminosarum* BIHB 645 suggested that the inoculation of *Pseudomonas* strain might have increased the activity of *Rhizobium* strains by providing growth-promoting substances and more active sites to nodulation due to increased root length as reported for peanut by *Pseudomonas fluorescens* strains (Dey *et al*., 2004). The nodulation by *Rhizobium* strains has also been reported to be dependent on phosphorus and iron availability (Cattelan *et al*., 1999; Dey *et al*., 2004; Rosas *et al*., 2006; Dardanelli *et al*., 2008). The test strain has the ability to solubilize inorganic phosphates, and produce auxins and siderophores (Tables 4.7, 4.12 and 4.15). Fluorescent *Pseudomonas* alone or in combination with *Rhizobium or Bradyrhizobium* strains have been reported to enhance nodulation in many leguminous crops, including pea, chickpea, peanut and soybean (Parmar and Dadarwal, 1999; Dey *et al*., 2004; Rosas *et al*., 2006; Egamberdieva *et al*., 2010). The decreased nodule count with the increasing rates of NPK application within the recommended fertilizer dosage suggested that the high fertilizer application
could reduce nodulation by native rhizobial populations. A decline in the efficacy of plant growth-promoting *Pseudomonas fluorescens* strains has also been reported with increasing rates of NPK application in wheat (Shaharoona *et al*., 2008).

Significantly higher plant growth and yield with the combined application of microbial inoculants over the individual applications showed synergism between *Pseudomonas trivialis* BIHB 745 and *Rhizobium leguminosarum* BIHB 645. Synergistic influence of *Pseudomonas* strains and *Rhizobium* strains has been reported in several crops such as chickpea, common beans, lentil, maize, pea, and pigeonpea (Dileep Kumar *et al*., 2001; Martins *et al*., 2004; Tilak *et al*., 2006; Velverde *et al*., 2006; Kumar and Chandra, 2008; Rokhzadi *et al*., 2008; Bano and Fatima, 2009).

Significantly higher total yield with *Pseudomonas trivialis* BIHB 745 over 50% NPK and comparable yield in the combined application of 50% NPK, *P. trivialis* BIHB 745 and *R. leguminosarum* BIHB 645 with 100% NPK signified that the application of *P. trivialis* BIHB 745 alone or in combination with *R. leguminosarum* BIHB 645 would reduce 50% fertilizer application (Tables 4.33-4.38). A saving of 25% NPK has been reported in wheat plants inoculated with PGPR strain (Shaharoona *et al*., 2008). The combined application of phosphate-solubilizing microorganisms and PGPR has also been reported to reduce P application by 50% without any significant reduction of grain yield in maize (Yazdani *et al*., 2009).

Significant difference was observed in plant growth and yield recorded during the two years at both the locations (Tables 4.33-4.38). Significantly higher plant growth and yield in the second year than the first year at the two locations could be due to the residual effect of PGPR strains applied in the first year as the experiments were conducted for the two years in the same fields. The rainfall in the second year in January during the active growth phase of the crop might also have augmented the crop production (Table 3.3). The application of plant growth-promoting fluorescent *Pseudomonas* strains have been reported for significantly higher growth and yield in the third year of field experiments on peanut (Dey *et al*., 2004).

The results demonstrated the potential of *Pseudomonas trivialis* BIHB 745 for improving crop productivity with reduced fertilizer application.
5.7 Optimization of Medium Ingredients for Maximizing Biomass Production of Pseudomonas trivialis BIHB 745

High cell density cultures (HCDC) offer an efficient means to increasing productivity which is the major goal of fermentation in research and industry (Lee, 1996; Reisenberg and Guthke, 1999). Each microorganism has specific requirements for maximum biomass production (Ellobol, 2004). In order to maximize the biomass production of microbial inoculant Pseudomonas trivialis BIHB 745, selection of the suitable growth medium and optimization of the medium ingredients were done to facilitate evolving the commercial production process. The maximum growth was obtained in TSB among TSB, KB, NB, TSBmeth, GYEM, PM2 and PMA (Fig. 4.39; Table 4.39). The results corroborated the relationship between medium composition and biomass production (Preetha et al., 2007; Cho et al., 2009; Manikandan et al., 2009). The information on the optimization of growth conditions for agriculturally important microorganisms is limited to some microorganisms—carbon and nitrogen sources for maximizing biomass of the biocontrol agent Pantoea agglomerans (Costa et al., 2001), aeration, agitation and inoculum size using molasses and urea as the growth medium for postharvest biocontrol agent Candida sake (Abadias et al., 2003), and carbon and nitrogen sources and growth factors for the biomass production and antagonistic compound production by probiotic Pseudomonas strain (Preetha et al., 2007).

In the present studies TSB ingredients were optimized using Box-Behnken Design of RSM for maximizing the biomass production of Pseudomonas trivialis BIHB 745 (Tables 4.40-4.43). The goodness of fit of the model checked by the determination coefficient ($R^2$) revealed that the model used was good fit as indicated by high coefficients of determination in the medium with dextrose or molasses as the carbon source (Tables 4.40-4.43). The values of the determination coefficients of 0.988 with dextrose as carbon source and 0.99 with molasses as carbon source in the medium indicated that only about 1.0-1.2% of the total variations were not explained by the model. The significant effect of independent variables dextrose/molasses, casein enzyme hydrolysate, soya peptone and NaCl as the medium constituents suggested a direct relationship of the selected independent variables with the biomass production (Tables 4.41 and 4.43). The significant interaction between soya peptone and dextrose, soya peptone and molasses, soya peptone and casein enzyme hydrolysate, and NaCl and dextrose further implied that these variables act as the limiting factors and minor variation in their concentration could affect biomass production. The significant effect of carbon and nitrogen sources has been reported for biomass production of a probiotic Pseudomonas strain (Preetha et al., 2007). The changes in NaCl concentration have been reported to affect cell growth and bacteriocin activity of
Discussion

*Lactobacillus curvatus* (Verluyten et al., 2004). RSM designs have been utilized to optimize the medium composition and growth conditions for enhancing the desired activity and biomass production- extracellular protease by *Pseudomonas* sp. (Dutta et al., 2004), poly-hydroxy butyrate by *Ralstonia eutropha* (Khanna and Srivastava, 2005), lipopeptide by *Bacillus subtilis* (Gu et al., 2005), exopolysaccharide by *Rhizobium* sp. (Dutta et al., 2006), cellulose by *Gluconacetobacter hansenii* (Hutchens et al., 2007), biomass and antagonistic compound by *Pseudomonas* sp. (Preetha et al., 2007), lipase by solvent-tolerant *Pseudomonas aeruginosa* (Ruchi et al., 2008), L-cysteine by *Pseudomonas* sp. (Lv et al., 2008), biomass of *Halobacterium salinarum* (Manikandan et al., 2009), and biomass of *Bacillus* spp. (Cho et al., 2009).

The response surface plots generated as the graphical representation of regression equations assist visualizing the interaction of nutrients and optimum concentration of each nutrient for maximum biomass production (Wang and Lu, 2004). Each contour curve represents infinite combinations of two test variables with other variables maintained at their lowest, highest or zero levels. The optimum levels of dextrose, NaCl, casein enzyme hydrolysate and soya peptone construed using Box-Behnken design generated 17.6% higher biomass over the original TSB (Table 4.40; Fig. 4.40). The optimized medium contained 3.2 g dextrose, 3.56 g NaCl, 29.0 g casein enzyme hydrolysate, 5.8 g soya peptone and 2.5 g KH₂PO₄ per litre in comparison to the original TSB with 2.5 g dextrose, 2.5 g NaCl, 17.0 g casein enzyme hydrolysate, 3.0 g soya peptone and 2.5 g KH₂PO₄ per litre. Earlier 19% increase in biomass production of *Pseudomonas* strain has been reported in the optimized medium (Preetha et al., 2007).

The use of low-priced commercial products or by-products of carbon and nitrogen sources from food industries have allowed maximizing biomass production at low costs (Zabriskie et al., 1980; Costa et al., 2001). In the present studies the optimized TSB medium with dextrose as the carbon source showed 4.4% higher biomass over the medium with molasses as the carbon source (Tables 4.40 and 4.42). Moreover, molasses has been reported to show wide variation in nutrient content, flavor, color, viscosity and total sugar content which could limit its application in scaling-up the inoculum production at commercial-scale (Harland, 1995; McGee et al., 1999).

The higher biomass production in comparatively shorter fermentation time makes large-scale inoculum production more economical (Kaur and Satyanarayana, 2005; Lv et al., 2008). A two-fold higher biomass production in the bioreactor with 21 h run period as compared to 30 h incubation in shake flasks could be due to the proper mixing of nutrients,
maintenance of pH and dissolved oxygen in the bioreactor (Fig. 4.43; Table 4.40). The enhanced product formation in reduced time course has been reported in fermenters in comparison to shake-flask experiments: 1.7-fold in higher glucoamylase production by *Thermomucor indicae-seudaticae* in 40 h instead of 48 h in shake flasks (Kumar and Satyanarayana, 2004); 3.11-fold higher biomass generation and 1.38-fold higher poly-β-hydroxybutyric acid production by *Ralstonia eutropha* (Khanna and Srivastava, 2005); extracellular protease production by *Beauveria bassiana* in four-day shorter time (Rao et al., 2006); 1.13-fold higher glucoamylase production in 16 h shorter time by *Thermomucor indicae-seudaticae* (Kumar and Satyanarayana, 2007); 15.6% higher enzyme activity in 6 h shorter time by *Pseudomonas* sp. (Lv et al., 2008); and 64.1-fold higher viable count in *Bacillus licheniformis*, 8.3-fold higher viable count in *B. coagulans* and 3.6-fold higher viable count in *B. subtilis* (Cho et al., 2009). A rapid decrease in dissolved oxygen observed in the present studies during exponential growth phase could be due to the high oxygen demand resulting from rapid multiplication of cells. A decrease in dissolved oxygen during exponential growth phase of *Candida sake* has been attributed to the increased respiration rate of the biocontrol agent (Abadias et al., 2003). During the stationary phase, pO₂ level started increasing probably because of the decrease in the respiration rate of the cells. The increase in pO₂ level attributed to the lack of substrate during stationary phase has been used as a signal to feed the fermenter (Meesters et al., 1996).

5.8 Shelf-life of the Carrier-based Microbial Inoculant *Pseudomonas trivialis* BIHB 745

*Pseudomonas trivialis* BIHB 745 showed the highest survival in activated charcoal followed by talc, peat, vermiculite and lignite (Fig. 4.44). The trend observed for different carriers in supporting the survival of *P. trivialis* BIHB 745 corroborated the earlier study on the shelf life of *Pseudomonas chlororaphis* where the highest viable count was obtained in peat followed by talc, farmyard manure, vermiculite, lignite and gypsum at 28±2 °C (Nakkeeran et al., 2006). Likewise, carrier-based preparations of *Bacillus subtilis* and *Pseudomonas corrugata* developed in five formulations showed the highest viability under storage in alginate beads followed by alginate beads supplemented with skim milk, charcoal, broth and alginate-coated seeds (Trivedi et al., 2005). Similarly, *Pseudomonas fluorescens* has been reported to exhibit the highest survival in peat followed by lignite, lignite and fly ash, and bentonite paste formulations with a shelf-life of 2-3 months in most of the formulations (Jayaraj et al., 2007). Each formulation has its own advantages and drawbacks and the best choice of formulation is dependent on the use and the storage needs of the microbial product (Melin et al., 2007). Charcoal has been used extensively as the
carrier material for microbial inoculants including *Acinetobacter, Azotobacter, Bacillus, Bradyrhizobium, Pseudomonas, Rhizobium* (Bhatia et al., 2002; Kumar et al., 2007; Suneja et al., 2007; Gulati et al., 2009). The higher viable colony count of *P. trivialis* BIHB 745 in charcoal might be due to its neutral pH, higher carbon content, water-holding capacity, surface area and porosity (Steiner et al., 2007).

The decline in viable count in all carriers was higher at ambient temperature as compared to storage at 4 °C (Fig. 4.44). The decline in cell numbers of *Bacillus subtilis* and *Pseudomonas corrugata* has been reported to be greater at room temperature compared to 4 °C in different formulations (Trivedi et al., 2005). Lower temperatures are known to retard the cell division and metabolic activities of bacterial cells resulting in a reduced consumption of nutrients and reduced loss of moisture in the carriers favouring storage of inoculants (van Schreven, 1970). According to Bureau of Indian Standards (BIS) specifications for biofertilizers, *Pseudomonas trivialis* BIHB 745 showed a shelf life of 270 d in activated charcoal, 240 d in talc, 210 in peat, 150 d in vermiculite and 120 d in lignite at 4 °C and 180 d in activated charcoal, 135 d in talc, 120 d in peat, 90 d in vermiculite and 75 d in lignite at ambient temperature. A formulation of *Bacillus brevis* has been shown to have shelf-life of six months in vermiculite as the carrier (Bapat and Shah, 2000). Similarly, peat and talc-based formulations of *Pseudomonas chlororaphis* strain and *Bacillus subtilis* strain have been reported to have a shelf-life of 5 months at 28 °C (Nakkeeran et al., 2006).

The results showed that charcoal was the best carrier in terms of shelf-life for the bacterial inoculant *Pseudomonas trivialis* BIHB 745. It is also a preferred carrier due to easy availability, low cost and neutral pH (Steiner et al., 2007). Charcoal is among the carriers recommended by the Bureau of Indian Standards for field application of phosphate-solubilizing microorganisms.

**5.9 Conclusions**

The present studies revealed that screening plant growth-promoting rhizobacteria based on multiple plant growth-promoting attributes, rhizosphere competence and stress tolerance are important for the selection of potential strains for high field performance. Significant difference in plant growth promotion among the efficient phosphate-solubilizing *Pseudomonas* strains point at the need for selecting the potential strains based on plant growth promotion in the soils supplemented with insoluble phosphates for their targeted application. The study also indicated that the innate ability of *Pseudomonas* strains in exhibiting multiple plant growth-promoting attributes is independent of their genetic
relatedness. The results on field evaluation of *Pseudomonas trivialis* BIHB 745 in conjunction with different levels of the recommended NPK dosage highlighted the usefulness of PGPR in reducing the application of inorganic fertilizers in agriculture. The results also supported the usefulness of polyphasic approach in characterization and identification of bacteria, wherein 16S rRNA gene sequencing was used for identification up to species level, whereas carbon source utilization pattern, whole-cell fatty acid profiles and rep-PCR fingerprints were used for analyzing variation among the closely related isolates.