DISCUSSION

Annipieri et al. (2003) have said that the relation between biodiversity, which can simply be defined as the numerical presence of species in a certain system, relates to the functional dynamics of the soil and therefore, is part of prime concern to be conserved so as to maintain its role in a functional biosphere. In the present study therefore, higher number of countable colonies of bacterial forms in neutral pH and lower number in low pH needs considered attention in potato cultivar soils. pH, as is known, determines the availability of nutrients, thus can have a strong effect on physiological processes, such as root exudations containing signal molecules which consequently, affect the microbial communities in the plant rhizosphere.

Soil pH, aeration, and physicochemical characteristics are jointly responsible for creating specific soil environment, thereby, the rhizosphere microbial communities (Marschner et al., 2001; Gelsomino et al., 1999; Cavigelli et al., 1995). Damodaran et al., (2013) have reported reduced bacterial diversity with increase in soil pH. Role of soil salinity induced bacterial diversity, is suggested as a cause of environmental stress Borneman et al., (1996).
Discussion

The methodology adopted in the present study for PGPR isolates from potato rhizo and non rhizo soils has been employed by other earlier workers also for isolation of PGPR as PSB from rhizo and non rhizo soils of other crop plants (Kumar et al., 2012; Dias et al., 2013; laslo et al., 2012). They also screened the isolates from rhizo and non rhizo soils by observing phosphate solubilisation on Pikovskaya agar medium (PVK) using various pH indicator/s. Bacterial identification by VITEK-2 method as employed in this study has been used by other workers too for bacterial identification (Paim et al., 2014; Chatzigeorgiou et al., 2011; Mory et al., 2009; Ligozzi et al., 2002; Ling et al., 2001). The diverse microbial community was found in three different potato cultivars KS, KC-3 and KL at different time intervals. Ibekwe and Grieve, (2004) and Gomes et al., (2003) have reported that the, rhizospheric bacterial community of varieties 'Monalisa' and 'Asterix' were phylogeneticlly more similar at the early first and second samplings. Thus suggesting that their root signals may be selecting similar bacterial groups. Multiplicity of groups shown by the other cultivars, influence rhizosphere associated microbial communities during early development and then diversifying subsequently as shown by the present three cultivars.

Berg and Smalla, (2009) and Buee et al., (2009) have produced detailed reviews regarding biotic and abiotic factors such as soil type, seasons, plant developmental stage, proximity to root, root architecture, plant species, and cultivars that can affect the structure of microbial communities in the
Discussion

rhizosphere. Various other studies have established that the influence on rhizospheric microbial communities is a synergic effect of both the plant species and the plant genotypes (Weinert et al., 2010, 2009; Andreote et al., 2009; Berg and Smalla, 2009; Van overbeek and Van Elsas, 2008; Smalla et al., 2001). This observation therefore, can also aptly explain the PGPR community structure variation in the present cultivars too.

There are distinct differences in bacterial form between bulk (non rhizosphere) and rhizosphere soil (Burdman et al., 1997; Bertrand et al., 2001; Molla et al., 2001). In the present study species of Bacillus were shown to be abundant in rhizospheric soil than in the bulk soil; this is being followed by Aeromonas and Pseudomonas too. Marques et al., (2014) have reported Bacillus as a dominant form genus in the tuber rhizosphere of sweet potato. Various species of Bacillus constitute major populations in the rhizospheres of Chrysanthemum (Duineveld et al., 2001), barley (Normander and Prosser, 2000), and that of grasses (Felske et al., 1998).

In this study too high number of colonies was found in the rhizospheric compared to non-rhizosphere (bulk) soil and also different species of Bacillus, Pseudomonas and other forms. 70 bacteria species were reported isolated from the rhizosphere of potato cv. Hartapel (Kesaulya et al., 2015). Sati et al., (2013) too have reported 25 morphologically distinct bacterial isolates belonging both to Gram+ve and Gram−ve groups from the Mana potato field. They further reported that in potato these beneficial microbes mainly belonged
to the genera of *Bacillus*, *Pseudomonas*, and *Penicillium*, along with actinomycetes and yeast. This study too recorded that on morphological basis *viz.* shape of colonies, color and elevation and then surface distinctions, the bacterial isolates belonged both to Gram+ve and Gram−ve bacteria. Bacteria belonging to the *Bacillus* and *Pseudomonas* groups are known to be growth promoters (Gupta *et al*., 2005; Picard and Bosco, 2005).

Plant rhizosphere is known to have various types of soil microorganism which are associated with plants and responsible for enhancing plant growth and development. These beneficial bacteria are collectively called plant growth promoting rhizobacteria (PGPR) (Somers *et al*., 2008). In present study higher number of bacterial density was observed in the rhizosphere as compared to the bulk soil. Interesting being that diverse bacterial presence in the bulk than the rhizospheric soil. Higher density of bacteria near roots has been reported in other plants as well (Joshi and Bhatt 2011; Timmusk *et al*., 2011; Nannipieri *et al*., 2007).

Plant growth promoting rhizobacteria (PGPR) are also known as phosphate solubilising bacteria (PSB). They have an inclusive ability to convert insoluble phosphates into available soluble forms for uptake of the host plant (Chung *et al*., 2005; Gulati *et al*., 2010). The aim of the present study was to characterise bacterial isolates from the rhizosphere and bulk soil of potato plant varieties for their phosphate solubilisation/utilisation. These PSB were then further subjected to screening for other PGP (plant growth promoting) traits *viz.*
IAA, HCN, NH₃ and siderophore production and also for their fungal antagonism. All these characteristics of PGPR are already reported to be effective in the plants growth and development directly and indirectly. Direct factors effecting growth promotion due PGPR constitute nitrogen fixation, phosphate solubilisation, IAA, gibberellins and cytokinins production, iron and other heavy metal sequesters-the siderophore production and in some cases lowering of inhibitive ethylene concentration. The indirect factors may include antibiotic production, depletion of iron from the rhizosphere, synthesis of antifungal metabolites, production of fungal cell wall degrading enzymes, competition for target sites on roots and induced systemic resistance. These characteristic features distinguish PGPR from other groups or classes of microorganisms. Synoptically these enhance plant growth by providing additional nutrients and also act as pathogen antagonists (Whipps, 2001).

PGPR effects in plant growth promotion are therefore, a cumulative observation due interaction either by synergism or by inhibition of factors like climate change, weather conditions, soil characteristics and composition cum activity of the other microorganism present in the soil (Naqqash et al., 2016). Akula et al., (2010) reported the benefits of the PGPR colonising the potato root zone under in vitro culturing. They have shown that PGPR inhabiting potato rhizosphere play an important role in tuberisation and also the yield of tubers. Interest in the study of such rhizobacterial associations with cereals and other agricultural crops has brought out their positive and beneficial effects on
the growth and yield under various environmental and ecological conditions (Zhang et al., 2012; Marques et al., 2010; Mehnaz et al., 2010; Ozturk et al., 2003). The observation showed that species belonging to genus Bacillus were found to be numerically dominant in the rhizospheric and non rhizospheric soil of potato. Garbeva et al., (2001) too reported Pseudomonas aureofaciens, P. corrugata, and P. putida, and others viz. Agrobacterium radiobacter, Stenotrophomonas maltophilia, Flavobacterium resinovorans, Bacillus sp., and Sphingomonas paucimobilis from potato growing soil. Various Bacilli forms like Bacillus subtilis, Lysinibacillus, Alicyclobacillus, Brevibacillus, reported by Kumar et al., (2012); Sphingomonas (Panward et al., 2014); Sphingobacterium (Pandove et al., 2016); Pseudomonas, Aeromonas, Klebsiella and Enterobacter (Kundu et al., 2009); Micrococcus (Mbai et al., 2013); Bacillus sp., Neisseria sp., Klebsiella sp., Enterobacter sp., Pseudomonas sp., Proteus sp., (Kadiri et al., 2013); Azotobacter CBD 15, Pseudomonas striata, Paenibacillus, Bacillus subtilis(T8), Bacillus licheniformis(T9), Bacillus licheniformis N14, Bacillus licheniformisN15, Lysinibacillus sphaericus (DGA) (Thakur, 2013); Actinomycetes sp. (Widawati et al., 2008 and Nurkanto, 2007) reported as PSB and/or PGPR.

The presence of a specific bacterial population/s in a certain soil system is due to its survival cum adaptation potential. The factors responsible that effect the presence and growth of bacteria may be due to nutrient uptake, soil pH, moisture content, organic matter and enzyme activities of the organisms.
Discussion

Estimation of PSB populations from potato soil were studied with main focus on their phosphate solubilising capacity on single media with 3 different pH indicators. In present study PSBs grown on Pikovskaya agar medium (PVK) containing different pH indicators showed that the PVK containing methyl red had highest PSB forms growing followed by the presence of bromophenol blue and methyl red orange indicators. Methyl red orange and bromophenol blue are sulphur containing indicators, and sulphur is known to be pH changer and also selectively antagonistic for the growth of some bacteria. The finding therefore, may suggest that the two are selectively inhibitory for certain PGPR.

Parikh and Jha, (2012) using methyl red and Gupta et al., (1994) using bromophenol blue in PVK and bacterial colony showing clear zone around suggested that the zone is due to release of PO$_4$ from tri-calcium phosphate in the media. This they suggested was due to pH change of PVK in the cleared zone. This is what prompted here to use other pH indicators in PVK and ascertain the extent to which other pH indicators can replace methyl red in showing the presence of PSB by developing clear zones due to phosphate release. Perusal of other studies seems to show that employing methyl red, bromophenol blue and methyl red orange to ascertain PSB in PVK is very rare or there hardly being any report. Further, Chung et al., (2005) have reported solubilising activity under liquid medium culturing conditions containing Ca$_3$(PO$_4$)$_2$, AlPO$_4$ and FePO$_4$. According to this study Ca$_3$(PO$_4$)$_2$ is more
phosphate releasing substrate by PSB than AlPO$_4$ and FePO$_4$, whereas, bromophenol blue (BPB) in PVK has been employed to ascertain PSB action in *Pantoea stewartii* by Hu *et al.*, (2010) and for phosphate solubilising fungi by Ejikeme and Uzoma, (2013). The report pertaining the use of methyl red orange (MRO) as PSB indicator in PVK are hardly available.

PSB by utilising various pH indicators in PVK media of 3 different pH, 40 phosphate solubilising bacteria from potato soils from Gwalior are reported. Dawwam *et al.*, (2013) and Malboobi *et al.*, (2009) also reported different strains of PSB from potato soil. However, the PSB here can tolerate extreme climates of Gwalior and hence can be subjected for their exploitation as biofertilisers under arid and semi-arid conditions. It is therefore suggested that whenever, PVK is employed to ascertain PSB, beside MR, MRO, BPB can also be used individually so that the other PSB which could otherwise be missed are also visualised along with change in the pH of the PVK. Soil Microorganisms play a decisive role in maintaining the ecological balance by active participation in carbon, nitrogen, sulphur and phosphorous cycles in nature (Karpagam and Nagalakshmi, 2014).

The present observation is therefore, in agreement with that of Kumar *et al.*, (2012) and Reyes *et al.*, (2006) that PSB are higher in concentration around rhizosphere soils compared to non-rhizospheric soils. The quantitative details of PSB by their workers too are also very close to the values found in this study, as far as P solubilisation of tricalcium phosphate on PVK is concerned.
Phosphorus is one of the major nutrients, second only to nitrogen required by the plants and most of it in soil is present in the form of insoluble phosphates hence unutilised by the plants (Pradhan, and Sukla, 2006). Hanif et al., (2015) have isolated and characterised a PSB bacterium *Bacillus subtilis* KPS-11 from potato rhizosphere having capacity to solubilise and mineralise inorganic phosphate and organic phosphate *in vitro* respectively. Our results show a range of P-solubilisation between 39 to 362.29 µg/ml in both rhizo and non-rhizo focii, which is nearly similar to reported earlier. Kumar et al., (2012) observed that in quantitative estimation, range of tri calcium phosphate solublization was between 362.72 to 562.34 µg/ml. El-Komy, (2005) found that *Pseudomonas fluorescens* and *Bacillus megaterium* strains were having higher solubilisation efficiency up to 350 and 185µg/ml respectively. Park et al., (2010) reported nineteen isolated phosphate solubilising bacteria (PSB) from various soil samples and six of those strains solubilised P from tri-calcium phosphate when amended with National Botanical Research Institute’s Phosphate (NBRIP) medium. There may be more than one reason for fluctuations in bacterial PGPR population density but obviously plant growth stage is the strongest factor that affects the indigenous plant associated communities in field grown potato plants (Van Overbeek and Van Elsas, 2008). Number of plant growth promoting rhizobacteria (PGPR) they say usually are not high enough to compete with other indigenous bacteria commonly present in the rhizosphere.
Certain PGPR have an ability to produce IAA presence and absence of precursor L-tryptophan. IAA can positively influence root elongation and lateral root development, which helps plants to acquire maximum water and essential nutrients. This may ultimately result in a well-established, vigorous and healthy plant. Bacterial strains producing phyto hormones are known to influence the balance of plant phytohormones, eventually inducing different growth stages (Sturz, 1995) as well as, in an overall process, promoting plant growth (Ghyselinck et al., 2013). which do influence root elongation and lateral root development. IAA production is more frequent among rhizosphere inhabiting bacteria than among bulk soil bacteria (Spaepen and Vanderleyden, 2011). Accordingly, in the present study 64% from rhizosphere and 46.6% from non rhizosphere produced IAA.

IAA production by PGPR can vary among different species and strains, and can also be influenced by culture conditions, growth stage and substrate availability (Mirza et al., 2001). In the present study most of the bacterial isolates were positive for IAA production. Of these Bacillus and Pseudomonas sp. were found having higher IAA production as is in consonance with the reports of Kaur and Sharma, (2013) who reported PGPR Pseudomonas diminuta as producing maximum 61.58 μg/ml of IAA. Hanif et al., (2015) too have reported such observations amongst potato rhizosphere for Bacillus and Pseudomonas sp. and that these PGPR isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil.
Discussion

It is also reported that there is however, no relationship between the amounts of IAA and P solubilisation to plant growth promotion due PGPR. Similarly, ammonia is a secondary metabolite product of PSB that indirectly influences the plant growth (Kumar et al., 2012).

In this study some PGPR isolates from both rhizo and non rhizo soils of potato produced ammonia also. Ammonia production by PGPR is also reported by Meena and Saharan, (2013) and Kaur and Sharma, (2013). Kaur and Sharma, (2013) have further reported that PGPR from potato produced HCN. The reason might be the volatility of HCN compounds. This is confirmed by pink coloration and ascribed to HCN reaction with picrate in orthophosphoric acid. This opinion is also being held by Hayat et al., (2010) and is antagonistic for the growth of soil pathogens around the plant rhizosphere.

In the present study PGPR isolates produced both quantitative and qualitative variations in siderophore reactions from both rhizo and non rhizo soils. Shrivastava, (2013); Laslo et al., (2012); Kumari et al., (2009) and Bholay et al., (2012) reported that Pseudomonas sps., Klebsiella, Azotobacter and Agrobacterium were siderophore producers as shown by these on JNFb® broth and CAS agar medium. Siderophore molecules have high affinity for iron ion and are therefore, made unavailable in the natural habitat of phytopathogens. Iron therefore, becomes maximally available for the beneficial PGPR bacteria and/or also for the plant diversity (Haas and Defago, 2005; Beneduzi et al., 2008; Ramesh et al., 2009). Siderophores also react as elicitors
Discussion

for inducing systemic resistance (Höfte and Bakker, 2007; Aarab et al., 2015). Also, the low iron availability in the soil suppresses the growth of pathogenic organisms. Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi (Whipps, 2001).

In the present study PGPR isolates produced quantitative and qualitative siderophore changes from both rhizo and non rhizo potato soils. Bholay et al., (2012) reported *Pseudomonas fluorescens* and *P. aeruginosa* produced siderophore in CAS agar plate assay. Laslo et al., (2012) and Kumari et al., (2009) also reported siderophore production by CAS agar plate assay in PGPR, whereas, Shrivastava, (2013) reported *Pseudomonas, Klebsiella, Azotobacter* and *Agrobacterium* producing siderophore in JNFb’ broth medium.

As shown, both in the rhizospheric and non rhizospheric potato soils, in the present study, some of the *Bacillus* sp like *B. megaterium* produced hydroxamate type of siderophore and some of the *Bacillus* sp like *Bacillus amyloliquefaciens, Lysinibacillus sphaericus* produced catecholate type of siderophore. These results are in agreement with those of Patil et al., (2014) and Radhakrishnan et al., (2014) they too examined and observed that *B. subtilis* as *Bacillus* sp. SD12 are capable of producing hydroxamate type of siderophore. *Bacillus megaterium* and *B.subtilis* in this study produced catecholate type of siderophore which is agrees to reports by Patel et al., (2009). *Basillus cereus* has the ability to produce catecholate-siderophores, and
to utilize transferrin-bound iron as an iron source for growth via the siderophore-mediated iron-uptake system reported by Park et al., (2005). *B. amyloliquefaciens* produce catecholate type siderophore whereas, other sps. of *Bacillus* and also *Paenibacillus, Lysinibacillus* and *Viridibacillus* isolates too produce the catecholate type siderophore (Lyngwi et al., 2016; Clark et al., 2014; Sharma et al., 2013).

In the present observation *Pseudomonas stutzeri* produced both hydroxamate and catecholate type of siderophore in rhizospheric and non rhizospheric potato soils respectively. Bholay et al., (2012) too have reported that PGPR such as *Pseudomonas fluorescens* was found to produce hydroxamate type of siderophore and that *Pseudomonas aeruginosa* produced both the hydroxamate and catecholate type of siderophores.

Siderophores produced by PGPR function as suppressors for the growth of phytopathogenic fungi such as *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*, and are also reported, being inhibitors of various other phytopathogenic fungi such as *Fusarium solani, Aspergillus niger* and *Phythium* sp (Radhakrishnan et al., 2014; Manivannan et al., 2012). The siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins (Nachin et al., 2001; Nudel et al., 2001). The production of siderophores by microorganisms is thus beneficial for plants, since these can inhibit the growth of plant pathogens (Sharma and Johri, 2003).
In this study PGPR isolates from both rhizospheric and non rhizospheric soils of potato produced antagonistic effects in vitro against *Fusarium* and *Pythium* sp. Calvo *et al.*, (2010) too have reported *Bacillus* and *Pseudomonas* sps being inhibitive against *Fusarium* and *Pythium* sp. They have also observed that higher population presence of *Bacillus* being antagonistic towards *Rhizoctonia solani* and *Fusarium solani* in the rhizosphere of potatoes growing under natural habitat in the Andes. Radhakrishnan *et al.*, (2014) and Palumbo *et al.*, (2007) too have reported antagonistic effects of PGPR against *Fusarium solani* and *Pythium* sp.

The present study therefore can create an assumption that isolates PR1 (*Bacillus subtilis*) from rhizospheric soils and PB11 (*Pseudomonas stutzeri*) in non rhizospheric soils of potato comparatively showed maximum PGP traits and could hence be further screened and tested under field and/or green house conditions. The studies though preliminary for the selection of effective PGPR strains for consequent use as bioinoculants can yet be a step further to consort as bioinoculants for sustainable potato crop growth, and also as controls for potato field pathogens.

There are reports related to the advantages and characterisation of PGPR from crop plants particularly wheat, pea, rice, maize, chickpea, *aloevera*, saffron, sugar cane and french bean in rhizosphere soil but there is little information about screening of and using PGPR in both rhizospheric and non-rhizospheric (bulk) soil with potato. Present study which analysed five bacterial
isolates from rhizo and bulk soil of three different cultivars of potato plants using sequencing of their gene 16s rRNA were characterised as *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Lysinibacillus boronitolerans*, *Pseudomonas brassicacearum* and *Bacillus subtilis*. *Bacillus sp.* were present in both rhizo and bulk soil whereas *Pseudomonas sp.* was present only in the bulk soil, an observation in agreement with that of Ambardar and Vakhlu, (2013).

Single sharp band was visualised suggesting 16s rRNA gene can successfully be amplified. All five isolates analysed by 16s rRNA, two forms PR1 and PR3 from rhizo and three forms PB10, PB11 and PB12 from bulk soil showed this characterisation. 16s rRNA gene sequences from PR1 and PR3 strains were found to be 99 percent similar whereas PB10, PB11 and PB12 were found to be 93, 96 and 99 percent similar respectively. Our results seem relevant and in agreement with Ambardar and Vakhlu (2013). Their analysis of 16s ribosomal RNA sequences and subsequent phylogenetic analysis of sixteen different bacterial species, report *Bacillus aryabhattai* presence both in rhizospheric as well as in the bulk soil. *Pseudomonas sp.* reportedly was present in the rhizosphere and *Bacillus* and *Brevibacterium* sp. were present in the bulk soil as the dominant genera respectively.

Present study is in agreement further with Naqqash et al.,(2016) and Verma and Shahi, (2015) who too have reported species of *Azospirillum*, *Agrobacterium*, *Pseudomonas*, *Enterobacter*, *Rhizobium* and *Enterobacter*
Discussion

cloacae strains isolated from the potato rhizosphere respectively. Dawwam et al., (2013) isolated Bacillus cereus and Achromobacter xylosoxidans, from sweet potato rhizosphere. Using 16s rRNA sequencing Aarab et al., (2015) have also reported the isolation of Pseudomonas, Aeromonas, and Enterobacter and have provided their phylogenetic analysis. Calvo et al., (2010) on the basis of 16S rRNA gene phylogeny suggested isolation of novel Bacillus species, however, majority of the strains were ascribed to B. amyloliquefaciens by then; it being a species reported with several PGPR characters.

16s rRNA gene is reported to be a highly conserved within a species and among the species of the same genus. This therefore, is a common target for taxonomic purposes (Olsen and Woese, 1993; Gürtler and Stanisich, 1996). In the present study, Bacillus was found to be more dominant species in both rhizosphere and bulk soil of potato cultivars.

Microorganisms such as bacteria show extreme diversity due diverse characteristics. Therefore, suggestively molecular approach becomes useful in establishing their phylogeny (Agrawal et al., 2011). Certain earlier analysis mainly focussed to isolate novel species using 16s rRNA sequencing technology (Jiménez et al., 2013; Joseph et al., 2012). In this study, however, the 16s rRNA sequencing was employed to assess the molecular identity of rhizospheric and non-rhizospheric bacteria. Earlier, Giongo et al., (2010) used the BLASTn tool to determine the identity of unknown 16s rRNA gene sequence with the repository bacterial sequences in NCBI GeneBank database.
Similarly, here too an online BLASTn search tool was accessed for relative strains and retrieved for further analysis. Representative phylogenetic tree based on BLASTn analysis revealed that most of the isolates were scions of similar firmicutes and few seem to show a relation with proteobacteria particularly Pseudomonads.

Van Noorden et al., (2014) suggested that multiple sequence alignment (MSA) is one of the widely used modelling approach in biology after Thompson et al., (1994) describing ClustalW a most cited tool of all time. Therefore, this study expends the analytical view point for molecular characterisation using MSA. Sequence alignment (SA) of rhizosphereic and bulk soil PGPR showed quite a similar matching of nucleotide sequence with homological strain retrieved from NCBI GeneBank and pairwise and multiple sequence alignment (MSA) using Clustal W.

Based on 16s rRNA gene sequencing, phylogenetic trees of almost all known strains of PGPR have been constructed, using UPGMA. This was to determine the phylogeny of PGPR isolates and each one of these showed genetic relationship with top 5-6 matches in the gene bank. UPGMA phylogeny can be inferred as ancestral and predecessor relationship with top relatives in NCBI GenBank database. Among PGP strains, B. subtilis DRKJUPR3 was closest relative of B. amyloliquefaciens DRKJUPR1. Lysinibacillus boronitolerans DRKJUPB10 and Pseudomonas brassicacearum DRKJUPB11 clustered together. However, Bacillus subtilis DRKJUPB12 was observed as
Discussion

predecessor of all strains. In this study, rhizospheric soil isolates PR1 and PR3 had good similarity on phylogenetic level and similarly bulk soil bacteria PB10 and PB11 had relative similarity on molecular level as an influence based on over all comparative tree construction using pairwise alignment and UPGMA.

The observation obtained thus indicate that the rhizosphere of potato as grown in Gwalior, MP., India is a good source of potential PGPR strains of Bacillus and also others viz. Lysinibacillus boronitolerans and Pseudomonas brassicacearum. These forms are currently being tested by the present lab for their cumulative plant growth promoting effects on potato. Isolated bacteria here seem to be as first report in the non-rhizospheric soil of potato. However, there is an ardent need for their infield studies and also a further consistent validation is recommended.

The study implies that the Arbuscular Mycorrhizal Fungi have a strong association with potato grown in the Gwalior-Chambal region of India. The association it seems is assured in the potato roots despite varied edaphic conditions. The presence of AMF, barring plants belonging to few angiosperm families is ubiquitous in the plant roots (Koul et al., 2012). These are known to synergise the various growth and developmental parameters. The observations here, are therefore, in conformity with other earlier studies where the presence of AMF in the potato plant rhizosphere are already reported (Baradar et al., 2015; Lone et al., 2015; Wu et al., 2013; Lakshmipathy et al., 2012; Das and
Kayang, 2010). These reports also pertain to various geographical regions of the world; hence being reports from diverse environmental conditions.

There are studies where AMF inoculations of the soil have led to the improved growth and development of the plants. In certain cereal crops the use of AMF inoculations are now becoming a common practice for better crop yield. Singh and Adholeya, (2013) reported the AMF diversity of wheat crop soils in India. Priyadharsini et al., (2012) have inventorised the AMF associated with the Allium cepa crop. The dominance of Glomus intraradices amongst the AMF associated with potato was established by Cesaro et al., (2008) and as also reported by others (Bhat et al., 2014; Sheikh et al., 2013; Das and Kayang 2010; Singh et al., 2007). In this study too the genus Glomus and its species dominate both rhizospheric and the bulk soil which therefore, seems one of the adaptive convenience of this genus with potato. G. intraradices here shows highest spore numbers intra Glomus species. This condition is related to various other crops and also potato where G. intraradices is the dominant species compared to all other species of Glomus and plus those of other genera. Some workers explain this as a co-evolution of genus Glomus with plants over varied niches and therefore, applicable to association with potato roots too (Baradar et al., 2015; Lone et al., 2015; Cesaro et al., 2008; Bharadwaj et al., 2007).

Results show that in potato propagules the AMF hypha penetration is steady which becomes subsequently vigorous once the potato plant is
established and shoots emanate from the propagules. However, the differentiation of other AMF structures in the roots *viz.* arbuscules or vesicles seems to be cultivar oriented, for their presence in the roots varies with time after propagule plantation. Since AMF symbiotic association with plant roots is a mutuality of carbon skeleton sharing as photosynthates from the plant to AMF in exchange with the soil water carried nutrient enhancement it therefore, is obvious that immediately after the potato plants are established, irrespective of the cultivars, the AMF hyphal establishment in roots is demonstrated. This function of symbiosis being primary, the secondary structures wait and vary in formation either due to both internal physiological responses of the cultivars or due to external factor like pH, soil conductivity or even mineral and other microbial organisations in the potato root vicinity. Such inferences for other crop plants are already available, for example that of Wu *et al.* (2013) saying that the level of mineral availability specifically phosphorus are inversely related to the AMF colonisation and more recently by Halder *et al.*, (2015).

Despite varietal differences in the extent of root colonisation or soil sporal density, there seems a similarity of trends. All the varieties show two peaks of spore densities commensurate with the beginning of the sharp shoot and root growth after plantation and the other at the time of underground tuber formation. Obvious inference could be that these are two important stages in the life cycle of the potato plant when roots have to organise nutrients and translocate these maximally first for growth and development of the plant
followed by the formation of underground tuber acting as the nutrient and photosynthate sinks. Once, the tuber formation has matured and the shoot root disfunction is organised before harvest, the living roots as remnants remain interestingly bereft of any AMF infestation. This seems an acquired character since all the cultivars follow this trend despite differences in the extent of AMF root colonisation at various stages of growth. Interestingly the presence of AMF hyphae has been reported in the potato peels also which is it seems a phenomenon of a post tuber differentiation (Lone et al., 2014). Rhizo soil AMF, seem play dominant role in the root penetration as the spore density around potato roots almost all through, and is 5 to 10 folds higher than the bulk non-rhizo soils. Bulk soil spore density is almost negligible at the time of planting subsequently remains constant till the time of harvest.

The present study was undertaken to ascertain a preliminary potato plant growth and development vis a vis use of AMF, PGPR individually and in combination in each of the three varieties of potato. Individual inoculum treatments of AMF have been widely available, Berruti et al., (2016); Rozpadek et al., (2016); Lin et al., (2015); Sarikhani and Aliasgharzad, (2012); Gallou et al., (2011) and PGPRs Hanif et al., (2015); Kumar et al., (2014); Kaushal et al., (2013); Sharma et al., (2013); Ibiene et al., (2012) and so are on their effects when supplemented with PGPRs Baradar et al., (2015); Hassani et al., (2014); Otroshy et al., (2013); Lowe et al., (2012); Hemashenpagam and Selvaraj (2011). AMF and PGPR both increased the growth significantly when
compared to their control. This is irrespective of the variety but dual inoculums of AMF and PGPR in combinations were the best.

Saia et al., (2015) have suggested that AMF and PGPR, used alone or in combination is an additional option for agricultural fraternity to improve the sustainability of the agro-ecosystem/s and said further studies are needed for evaluating the benefits of the soil inoculation in wheat with efficient consortia of both AMF and PGPR and characterise the balanced use of applied fertilisers. This implies for potato too. Palacios et al., (2009) have reported and then suggested that the inoculation of native diazotrophic bacteria and AMF in the micropropagated in vitro potato plantlets can increase growth.

These AMF and/or PGPR consortium used as biostimulant, bioprotectent and biofertiliser for growth and antagonist against different soil borne pathogen also increases the growth and yield of potato plant. Otroshy et al., (2013) revealed that potato plants treated with Azotobacter + Bacillus + Glomus had positive and highest yield of potato. AMF and PGPR formulations containing two or more AMF and/or PGPR species have been tried for cultivation in other crops and plants (Yan et al., 2002; Mia et al., 2005; Domenech et al., 2006; Rodriquez Romero et al., 2005; Vestberg et al., 2004). In the present study dual inoculums of AMF and PGPRs invariably increases the overall growth and development of plant viz. plant height, fresh weight of tuber, root and shoot and dry matter content of tuber, root, shoot and also increases the yield of potato in all the three varieties KS, KC-3 and KL.
Baradar et al., (2015) revealed that interaction of chelating factor of iron (EDTA and EDDHA) mycorrhizal colonisation and PGPR strains had positive effects on root colonisation and in consequence lead to increase in fresh and dry weight, other growth factors and chlorophyll in potato tuber. Similarly, Vosatka and Gryndler, (1999) described that dual inoculums of PGPR (Pseudomonas putida) and AMF increase the total weight of potato tubers and enhance the mycorrhization and extraradical mycelium activity of plants. Plant’s ISR/SAR response, serially with the use of plant activators and elicitors of SAR, may offer an effective strategy for controlling both soil-borne and foliar diseases of potato plants. PGPR or AMF combined with foliar spraying with an elicitor can be used to control late blight of potato caused by Phytophthora infestans (O’Herlihy et al., 2003).

The effects of inoculation on crop productivity in field conditions are complex phenomena in both the selection of inoculants and also the field site characteristics which will contribute to the successful persistence and effectiveness of the given microbes after inoculation (Farmer et al., 2007). It is difficult to rule out the contribution of indigenous strains, even those strains are less effective than the introduced ones (Koide et al., 1999; Bernard et al., 2012). As a result, inoculants need to be carefully selected to ensure the best combination among host, microbial, substrate and inoculants type in the process of potato production (Klironomos and Hart, 2002). Mostly, the interaction studies between AMF and other microbes were based on studies in
**Discussion**

pot and greenhouse systems and little has been done in large-scale field trials (Wu *et al.*, 2013). This type of study in future is suggested and that too on an exhaustive scale in Gwalior-Chambal region.

After treatment of AMF and/or PGPR in potato plants hyphae, arbuscules, were found in the roots of KC-3 and KL whereas, hyphae, arbuscules and also vesicles were found in the roots of KS varieties. Highest root colonisation and spore density were found in KS variety followed by KC-3 and KL. These results are more close to the finding of Davies *et al.*, (2005b) who reported that 70 to 80 percent root colonisation and spore density was found in Yungay potato. Similarly, Ngakou *et al.*, (2006), Halder *et al.*, (2015) and Pellegrino and Bedini, (2014) described that after inoculation of AMF root colonisation and spore density increases. After inoculation of AMF potato plant increases the root colonisation, spore density and also further increases the yield of potatoes (Douds *et al.* 2007; Douds *et al.* 2012). Mycorrhizal root colonisation may be increased by soil environmental factors (Shi *et al.* 2014). Lone *et al.*, (2015) reported AMF (*Glomus intraradices* and *G. mosseae*) colonisation improved positively the overall growth and development of *Solanum tuberosum* (TPS, SM/93-237) and potato tubers var. Jyoti both cultivars of potato plant. AMF can increase chlorophyll content growth and development parameters and therefore, increases the production of potato.

In an overall assessment in present study the increments in plant height, root length and number (yield) of tubers, fresh weight and dry weight of root,
Discussion

Shoot and tuber were significant as were recorded in dual inoculums of AMF and PGPR in each three varieties KS, KC-3 and KL. This study shows that these inoculums can increase growth and development and also yield of potato tuber. The present study therefore, is a preliminary investigation to report effects of PGPR and AMF provided to potato plants individually and in combination, under a series of pot experiments. The ultimate aim is to procure a consortium of microbes to be used as a biofertiliser for potato crop, which may lead to lesser use of chemical fertilisers and other synthetic chemicals for pathogen control.

In future studies therefore, more detailed investigations of the relationships in various pathosystems and of the interactions between the microorganisms and the host plant are needed and also for developing a biocontrol mechanism for the related diseases.