REVIEW OF LITERATURE

The varieties of potato plant grow about 60 cm in height. The leaves die back after flowering, fruiting and depending on underground tuber formation. Flowers are red white, blue and/or purple among yellow stamens. In general, the tubers of varieties with white flowers have white skins, while those of varieties with colored flowers tend to have pinkish skins (Winch, 2006). Potato plants produce small green fruits resembling green cherry red tomatoes. Each fruit contains about 300 seeds. Except the tubers all other parts of the plant contain the toxic alkaloid solanine therefore, rendered unsuitable for consumption. New potato varieties are now seed grown, hence also called "true potato seed", "TPS" or "botanical seed". This is to distinguish it from seed tuber propagation. New varieties grown from seed can then be propagated vegetatively by seed tubers, (pieces of tubers cut to include one or two eyes) or cuttings. Plants propagated from tubers are clones of the parent, whereas those propagated from seed produce a range of different varieties (Amador et al., 2001).
2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria. These vigorously colonise the rhizosphere around plant roots, and are now known to bring about growth and yield enhancement of plants when applied additionally to the crops, directly or through seed treatments (Kumar et al., 2014). Most of the PGPR strains are reported to exhibit multiple of these properties. However, there do exist differences in the growth responses of plants to different PGPR strains. This depends upon the strain specific internal differences of both the plant and the PGPR together (Ghyselinck et al., 2013; Qaisrani et al., 2014). PGPR consortia are being recommended as biofertiliser for various field crops. These therefore, serve as an alternative cum reduction in the use of chemical fertilisers and also their behaviour in crop protection through pathogen control (Egamberdiyeva and Hoflich, 2004).

Rhizobacteria are plant root associated bacteria having ability to colonise roots and also their continued presence within or outside the roots of plants. Due to raised nutritional concentrations and root exudates, plant roots thus serve as ‘microbial hot spots’ in the soil (Kuzyakov and Blagodatskaya, 2015). Rhizobacteria can colonise diverse sites of the rhizosphere such as the root surface or inside the root and directly and/or indirectly stimulate the plant growth. When introduced through seeds, roots or the soil, these bacteria, which include plant-growth-promoting rhizobacteria (PGPR) can solubilise insoluble phosphates, produce plant growth hormones, convert atmospheric nitrogen to
ammonia and suppress the growth of the plant phytopathogens (Pérez-Montaño et al., 2014). Studies on PGPR have both experimental approaches like *in vitro* and *in vivo*, and are being carried out for different crops such as potato (Kesaulya et al., 2015; Verma and Sahi, 2015; Naqqash et al., 2016), wheat (Majeed et al., 2015), onion (Reetha et al., 2014), maize (Qaisrani et al., 2014), bean (Pérez-Montaño et al., 2014) chickpea (Kaur and Sharma, 2013), *Aloe vera* (Meena and Saharan, 2013), sweet potato (Dawwam et al., 2013), sunflower (Shahid et al., 2012), pigeon pea (Usha rani et al., 2012), rice (Lucas et al., 2009), soybean (Cassán et al., 2009). Studies have revealed the potential of PGPR to encourage the growth and yield of such crops, with slight inputs of chemicals and minimal damaging consequences for the environment.

The iPGPR are mostly Gram-negative and rod-shaped, with less proportion of Gram-positive rods, cocci and pleomorphic forms. ePGPR are non nodulous, and increase plant growth through other various mechanisms (Gray and Smith, 2005). They include such genera as *Bacillus, Pseudomonas, Erwinia, Caulobacter, Serratia, Arthrobacter, Micrococcus, Flavobacterium, Chromobacterium, Agrobacterium, Hyphomycrobium, Alcaligenes, Azospirillum, Azotobacter, Bradyrhizobium, Burkholderia, Enterobacter, Frankia, Klebsiella, Rhizobium*, and free-living nitrogen-fixing bacteria. These are commonly known PGPR which are growth beneficial for different crops (Tailor and Joshi, 2014; Bhattacharyya and Jha, 2012).
2.1.1 Phosphate solubilisation by PGPR

Phosphorus (P) is the second most important macro-nutrient; next only to nitrogen in plant growth and development (Fernandez et al., 2007; Xiao et al., 2011). Jones and Oburger, (2011) observed that the level of soil phosphorus over all is low, usually as low as one-tenth to one-fourth of nitrogen (N), and as low as one twentieth of potassium (K). Soil P is usually between 400 and 1,200 mg kg\(^{-1}\) within pH range from 5.5 to 7. It presents mainly in two insoluble forms. One being the mineral forms such as apatite, hydroxyapatite, and oxyapatite, and the other organic forms including inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters, and phosphotriesters (Khan et al., 2007). Workers have reported various different bacterial species have capacity to solubilise insoluble inorganic phosphate such as dicalcium phosphate, tricalcium phosphate, rock phosphate and hydroxyapatite mainly through acid production and also through other mechanisms. These are thus termed as phosphate solubilising bacteria (PSB) (Nautiyal et al., 2000; Chen et al., 2006). A large number of bacterial genera like Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Micrococcus, Rhizobium, Serratia, Bacillus megaterium, B.circulans, B.subtilis, B.polymyxa, B.sircalmous, Pseudomonas striata, and Enterobacter and their species have been reported to solubilise phosphate. The list continues increasing (Reena et al., 2013; Kumar et al., 2012; Reyes et al., 2006).
Mahdi et al., (2011) suggested that organic and/or inorganic acids convert tricalcium phosphate to mono and di basic phosphates which are then conveniently available for roots to take up. This is how several forms of phosphate are made increasingly available for bacteria and plants. The P absorbed by the bacteria is in the form of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$. This depends upon soil pH. The type and extent of organic acid produced differ with different organisms. Gluconic acid and 2-ketogluconic acid are reported to be the often used agent of elemental phosphate solubilisation (Song et al., 2008; Park et al., 2011). Ahmed and Shahab, (2011) have listed other organic acids, such as acetic, citric, lactic, propionic, glycolic, oxalic, malonic, succinic, fumaric, and tartaric also having phosphate solubilising action. Acids also enhance their fixation sites for Al and Fe insoluble oxides. On reacting, these stabilise them as ‘chelates’ and the process is also one of an important phosphate solubilisation processes (Whitelaw, 2008). Depending on the particular properties of a soil, phosphate anions are extremely reactive and may be immobilised through precipitation with cations such as $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{Fe}^{3+}$ and $\text{Al}^{3+}$.

(Dicalcium phosphate) \[ \text{CaHPO}_4 + \text{H}^+ \leftrightarrow \text{H}_2\text{PO}_4^- + \text{Ca}^{2+} \]

(Hydroxyapatite) \[ \text{Ca}_5(\text{PO}_4)_3(\text{OH}) + 4\text{H}^+ \leftrightarrow 3\text{HPO}_4^{2-} + 5\text{Ca}^{2+} + \text{H}_2\text{O} \]

(Walter and Vega, 2007)
Chen et al., (2006), showed *Bacillus megaterium* (CC BC 30) and *Rhodococcus erythropolis* (CC BC 17) could solubilise 140.6 μg/ml and 151.2μg/ml phosphorus under broth conditions. Banerjee et al., (2010) reported that *Bacillus sp.* TRSB16 can consistently show high solubilisation rates of Ca$_3$(PO$_4$)$_2$ (144μg/ml), whereas low solubilisation of Ca$_3$(PO$_4$)$_2$ (71μg/ml) was observed by an *Arthrobacter sp* TRSB10. The combinations of either *P. agglomerans* or *M. laevaniformans* strains with *Pseudomonas putida* have shown to produce higher biomass and potato tuber number both under greenhouse and field trials (Malboobi et al.,2009). *Bacillus cereus*, *Achromobacter xylosoxidans* and *Azospirillum* sp. have ever been recommended as biofertilizers for potato plant and *Aeromonas, Pseudomonas* and *Enterobacter* in rice for reducing the dependence on chemical fertilisers. This therefore, provides a way towards sustainable agriculture (Dawwam et al., 2013; Aarab et al., 2015; Naqqash et al., 2016). Hanif et al., (2015) reported
that PGPR from the potato soil include mostly *Bacillus* and *Pseudomonas* sp. and that these have been used for improving uptake of phosphorus.

### 2.1.2 The role of Siderophore production

Iron constitutes an essential growth element for all living organisms. Siderophores meaning in the Greek: "iron carriers" are small, low molecular weight compounds. These are high iron chelating compounds secreted by various bacterial and fungal microorganisms. Iron is an essential element for cellular growth and metabolism. The Fe$^{3+}$ acquisition through siderophore production therefore, plays an essential role in determining the competitive fitness of bacteria to colonise plant roots and also to compete for iron with other microorganisms in the rhizosphere (Miller *et al.*, 2009; Crowley, 2006; Crowley and Gries, 1994). Marschner and Romheld, (1994) suggested that plants utilise siderophores secreted by PGPR for sequestering iron. Plants such as oats, sorghum, cotton, peanut, sunflower and cucumber have the ability to use microbial siderophores as the only source of iron than their own siderophores (phytosiderophores) (Crowley *et al.*, 1988; Wang *et al.*, 2006). By removing Fe$^{3+}$ around the rhizosphere, PGPR are also able to inhibit the proliferation of pathogenic microorganisms (Siddiqui, 2006). Crowley (2006), describe that iron (Fe) reduction in the rhizosphere does not affect the plant, because the low concentrations of iron (Fe) take place at microsites of high microbial activity during establishment of the phytopathogen.
Two physiological mechanisms involved are proposed for iron binding siderophore. One being the usual siderophore iron delivery mechanism in which ferric siderophore is bound to the protein receptor. This causes a conformational change in the protein. The ferric siderophore is then pumped through a receptor into the periplasmic space. The release of the ferric from siderophore takes place and, the receptor protein returns to its original conformation. The other mechanism of delivering is siderophore shuttle iron delivery mechanism. In this the iron free siderophores initially bound to the receptor protein, a second, iron-loaded siderophore binds to the receptor. Iron exchange between the two siderophore take place which induce iron exchange. This therefore, brings about a conformational change and the iron complex of the originally iron-free siderophore enters the cytoplasm where it releases the ferric siderophore. The receptor protein returns to its original conformation now with the originally iron-loaded siderophore bound to the receptor protein Stintzi et al., (2000).

More than 500 different siderophore are reported produced both by Gram-positive and Gram-negative bacteria (Wani et al., 2007; Ahemad and Khan, 2010a,b). *Rhodococcus* genera are the Gram-positive bacteria reported to produce siderophores (Tian et al., 2009). In Gram-negative bacteria, the ferric-siderophore complex must cross the outer membrane and the cytoplasmic membrane before delivering iron within the cytoplasm (Stintzi et al., 2000).
Various workers have isolated siderophore producing rhizospheric bacteria. These belong to the genera *Bradyrhizobium* (Khandelwal *et al.*, 2002), *Pseudomonas* (Boopathi and Rao, 1999), *Rhizobium* (Roy and Chakrabartty, 2000), *Serratia* and *Streptomyces* (Kuffner *et al.*, 2008). The major groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (e.g. derivatives of citric acid). Neilands, (1995) described hydroxamate siderophores. He explained that the siderophore ferrichrome is produced by *Ustilago sphaerogena*, Desferrioxamine B (Deferoxamine) by *Streptomyces pilosus* and *Streptomyces coelicolor*, Desferrioxamine E by *Streptomyces coelicolor*, Fusarinine C by *Fusarium roseum* and Ornibactin by *Burkholderia cepacia*. Wheat rhizosphere in black cotton soils of North Maharashtra region is reported to produce catechol type of siderophores by
*Acinetobacter calcoaceticus* during its exponential growth phase. This was also influenced by the iron content of the medium. There are some other examples of catecholate siderophores like Enterobactin produced by *Escherichia coli*, bacilli bactin by *Bacillus subtilis* and *Bacillus anthracis* and vibriobactin by *Vibrio cholerae*. Siderophores with mixed ligands are azotobactin produced by *Azotobacter vinelandii*, pyoverdine by *Pseudomonas aeruginosa* and yersinia bactin by *Yersinia pestis*.

### 2.1.3 Production of Indole acetic acid (IAA)

The production of phytohormones such as auxin Indol acetic acid (IAA), cytokinins and gibberellins by naturally occurring soil microbial communities is now established. These both include symbiotic and non-symbiotic microbial forms promoting plant growth (Poonguzhali *et al.*, 2008; Ahemad and Khan, 2010c,d). The IAA is established to affect cell division, root initiation, growth rate, phototropism, geotropism and apical dominance. It also induces expansion and differentiation of plant cells and tissues. Precursor for IAA synthesis is amino acid tryptophan. It can also be synthesized via tryptophan-independent pathways, (Tsavkelova *et al.*, 2006; Spaepen *et al.*, 2007). Thus far, six pathways for the biosynthesis of IAA have been identified in rhizobacteria, five of which are tryptophan dependant and one is tryptophan independent. The tryptophan dependent one utilizes the presence of indole-3-glycerolphosphate. However, it is now known that the rhizo bacteria can synthesise IAA through various different pathways.
Spaepen et al., (2007) has described that microbes can synthesise IAA through any one of the following three pathways: (1) IAA formation via indole-3-acetic aldehyde is found in the majority of bacteria viz. *Erwinia, Agrobacterium, Pseudomonas, Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella* and *Enterobacter*, (2) The conversion of tryptophan into indole-3-acetic aldehyde which may also involve an alternative pathway in which tryptamine is formed by *Pseudomonads* and *Azospirilla*, (3) IAA biosynthesis via indole-3-acetamide formation as formed by *Agrobacterium tumefaciens, Pseudomonas syringae, Erwinia herbicola, Pseudomonas putida* and *Pseudomonas fluorescens, Rhizobium sp., Bradyrhizobium sp.* and *Azospirillum sp.* In plants IAA biosynthesis involves tryptophan conversion into indole-3-acetonitrile, which is similar to one by *Alcaligenes faecalis* and *cyanobacterium*, Though, IAA of bulk soil remains in conjugated throughout storage to avoid degradation. Stimulation mechanisms of shoot and root development by IAA, gibberellins and cytokinins still fragmentary (Van Loon, 2007). Cytokinins, have been concerned in nitrogen fixing, cell division and nodule development (Murray et al., 2007; Tirichine et al., 2007). They also promote rapid growth of the primary root and enhance branching (Ortiz-Castro et al., 2009).
Fig. 2.3: Biosynthetic paths of Indole acetic acid (IAA) as adopted by the rhizobacteria, (Ahemad and Khan, 2011)

2.1.4 Production of Hydrogen cyanide (HCN)

HCN is a volatile, secondary metabolite that suppresses the development of microorganisms (Siddiqui et al., 2006). It has severe toxic properties. Although cyanide acts as a general metabolic inhibitor, it is synthesised, excreted and metabolised by hundreds of organisms, including bacteria, algae, fungi, plants, and insects, as a means to avoid predation or competition.

The HCN production by rhizobacteria may suppress plant growth in soil. HCN production by virulent *Pseudomonas* affects plant root growth and also other live processes in the rhizosphere (Rudrappa et al., 2008). Various types of bacterial genera are capable of HCN production, which include species of *Alcaligenes, Aeromonas, Bacillus, Pseudomonas* and *Rhizobium* (Devi et al., 2007; Ahmad et al., 2008). The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and root
nodules. This is therefore, considered a serious environmental pollutant and thereby a biocontrol metabolite (Charest et al., 2005; Ahmad et al., 2008). *Mesorhizobium loti* MP6 produces hydrocyanic acid (HCN) under normal growth conditions and is reported to enhance the growth of mustard (*Brassica campestris*) (Chandra et al., 2007).

### 2.1.5 PGPR as Biocontrol and/or Biofertiliser agents

PGPR also act as biofertiliser and/or biopesticide. Biofertilisations are available through nitrogen fixing biofertilisers eg. *Rhizobium, Bradyrhizobium, Azospirillum and Azotobacter*, phosphorus solubilising biofertilisers (PSB) eg. *Bacillus, Pseudomonas and Aspergillus*, phosphorus mobilising biofertilisers eg. Arbuscular Mycorrhiza Fungus (AMF) and plant growth promoting biofertilisers eg. *Pseudomonas sp.* Alternative to chemical fertilisers for improvement in crop yield is the use of PGPR. This has found a role in developing a sustainable crop production system (Sturz et al., 2000; Shoebitz et al., 2009). Kumar et al., (2012) examined *Acinetobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Micrococcus* sp., and *Pseudomonas* sp., as PGPR for environmental friendly and sustainable approach to the increased production of crops and their health. Samuel and Muthukkaruppan, (2011) reported use of such a mixed portion of *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Azospirillium* sp., *Phosphobacteria* sp., *Glucanacetobacter* sp., for similar purpose. *Aspergillus niger and Penicillium sp.* were reported from various different sources viz. rice field, mangroves and effluent soil. Rhizobacteria
tolerant to multiple heavy metals have been exhibited by a couple of bacteria with PGP activities. Various bacteria which are mostly used as a biological control agents include the genera of *Streptomyces, Bacillus, Burkholderia, Pseudomonas* and *Agrobacterium*.

### 2.1.6 Molecular approach to PGPR

16s rRNA gene is now, an established technique in studying evolutionary changes and phylogenetic relatedness of organisms (McDonald *et al.*, 2012). Using fatty acid methyl ester (FAME) analysis and partial sequencing of 16s ribosomal RNA genes. Agrawal *et al.*, (2011) reported that the most frequently isolated strains more than 5% were characterised as different *Pseudomonas* sp., eg. *P. aureofaciens, P. corrugata, and P. putida, Agrobacterium radiobacter, Stenotrophomonas maltophilia, and Flavobacterium resinovorans*. Other Proteobacteria or Firmicutes were also found mostly in potato stem tissue.

Morphological, physiological and molecular approaches based on fatty acid analysis, mole percentage G+C contents, DNA–DNA hybridisation and 16s rRNA sequencing characteristics help in defining the taxonomy and nomenclature of PGPR (Figueiredo *et al.*, 2010). The molecular fingerprints generated by PCR with primers annealing to repetitive sequences (rep-PCR: REP, ERIC and BOX) and random DNA stretches, or amplified ribosomal DNA restriction fragment analyses (ARDRA) have been carried out on various PGPR by Rademaker and Bruijn, (1997) and Marten *et al.*, (2000).
PGPR have further been characterised by one or more additional methods *viz.* restriction fragment length polymorphism (RFLP), plasmid profiling, ribotyping, amplified ribosomal DNA restriction analysis (ARDRA), pulsed field gel electrophoresis (PFGE), and randomly amplified polymorphic DNA (RAPD) (Oliveira et al., 2000, Von der et al., 2000, Depret and Laguerre 2008 and Monteiro et al., 2009). The 16s rRNA gene sequence is deposited in databases such as Ribosomal Database (http://rdp.cme.msu.edu) and Gene Bank (http://www.ncbi.nlm.nih.gov). On the basis of comparative phylogenetic analysis sequences of related species for can also be retrieved from these databases. Then after, sequence comparing with BLAST and CLUSTAL W software are used for alignment of partial 16s rRNA gene sequences and genetic relatedness between bacterial species examined by the construction of phylogenetic trees or dendrograms.

Altschul et al., (1997) reported sequencing of 16s rRNA gene with BLAST analysis for their identification of the respective strains *Pseudomonas chlororaphis, Paenibacillus polymyxa, Serratia plymuthica, Lysobacter antibioticus* and *Lysobacter gummosus*. Calvo et al., (2010) revealed some bacterial strains, characterised by BOX fingerprinting and 16s rRNA gene phylogeny, which belong to *Bacillus* species but the common of the strains were attributed to *B. amyloliquefaciens*, which reported as PGPR in potato rhizosphere. Akula et al., (2010) described *Bacillus, Variovorax, Proteobacterium, Staphylococcus, Agrobacterium, Chrysobacterium* and
Plantibacter. These were identified as PGPR upto genus level based on the 16s rRNA sequence analysis. PGPR had been identified as being the most common residents in the rhizosphere of potato using cpn60 pyrosequencing or 16s rRNA sequence analysis (Turnbull et al., 2012). Naqqash et al., (2016) examined, bacterial isolate TN10 and presumptively identified it as Azospirillum sp., TN14 as Agrobacterium sp., TN36 as Pseudomonas sp., TN38 as Enterobacter sp., while TN42 was a Rhizobium sp., TN14 and strain TN42 belong to Rhizobium, strain TN10 which clustered in an Azospirillum clade. Strains TN38 and TN36 were affiliated with Enterobacter and Pseudomonas respectively. All these observations were based on 16s rRNA gene sequence analyses.

2.2 Arbuscular Mycorrhizal Fungi (AMF)

The term mycorrhiza comes from the Greek words for “fungus” and “root” and describes many diverse root-fungus associations (Bonfante, 2001). Arbuscular mycorrhizal fungi are soil fungi that form a symbiosis with most of the terrestrial plants (Smith and Read, 2008; Scheublin et al., 2010). AMF are important especially for the phosphate nutrition to the host plant ascribed to their symbiosis in enhanced uptake of immobile mineral nutrients, improved water relations, and increased resistance to pathogens. It also has an ability to protect plants from biotic and abiotic stresses (Cho et al., 2006; Borie et al., 2010). AMF generally stimulates plant growth by increasing the capability of the root system to absorb and translocate nutrients through extensive mycelia including an improvement in soil aggregation by hydrophobic glycoprotein
(glomalin) released from extra-radical hyphae (Giri et al., 2005; Rilling and Mummey, 2006; Cavagnaro et al., 2006 and Cavagnaro, 2008). They also confer resistance to the plants against phytopathogen (Wehner et al., 2011) and enhancement in water relations (Auge, 2004). AMF have a role in impacting soil structure also (Leifheit et al., 2014).

AMF are the most widespread soil fungi, and the human activity that has an impact on soil, such as, agricultural practices has a side effects on these. These practices, alone or in combination, exert an enormous selective pressure on AMF. These also shape their community structure and evolution by modifying their biological features like sporulation strategy, resource allocation and spatial distribution (Verbruggen and Kiers, 2010). Many studies have indicated that AMF diversity, effectiveness, abundance and biodiversity decline in agroecosystems subjected to high input practices (Borriello et al., 2012; Lumini et al., 2010).

AMF are therefore now, considered important for sustainable farming due to their providing efficient and additional nutrient availability to the plants. This they achieve by freeing nutrients bound to soil particles and/or organic matter. Agricultural crops are already known to be benefiting from AMF association, which include maize, potato, sunflower, wheat etc. especially when these crops are sown under conditions where availability of nutrient concentration could be limiting for the plant growth (Halder et al., 2015).
Lakshmipathy et al., (2012) reported that different AMF species *viz.* *Glomus fasciculatum, G. geosporum, G. mosseae* were found in both premonsoon and post monsoon. They also found other variations in sp., as well as higher number of sp. in the post monsoon than premonsoon in a paddy field or in the natural forest. Singh and Adholeya, (2013) described *Glomus* as an abundant genus present in wheat agro system and suggested that *G. albidum* and *G. macrocarpum* spores have a capability of adaptation in wheat host plant in comparison of others AMF species.

![AMF establishment in host plant root cortex cells.](www.biostim.com.au)

**Fig.2.4: AMF establishment in host plant root cortex cells.**

(www.biostim.com.au)

### 2.2.1 Nutrients uptake of Arbuscular mycorrhizal fungi (AMF)

Phosphorus (P) is one of the major nutrients, which is essential for the plant growth and development. In soil, phosphorus occurs in three forms such as, soluble inorganic P, insoluble inorganic P and organic P. Uptake of P symbiotically by arbuscular mycorrhizal fungi (AMF) is in addition to one by
the plant roots. AMF have an ubiquitous presence in most of the soils. They are commonly found in association with agricultural crops (Bagyaraj et al., 2015). Borie et al., (2010) say that AMF stand out since they are extremely important for the phosphate (P) nutrition of plants under acidic soil. Phosphorus is the soil nutrient which has the highest primary role in the AMF symbiosis. Advantages of AMF have been attained through application of suitable AMF inoculum thus augmenting native soil AMF. These activities are employed through manipulating agricultural practices to the favour of these fungi (Bagyaraj et al., 2015). AMF can enhance plant uptake of inorganic phosphorus (Pi) from soil through hyphal scavenging of soil volumes that are in accessible to the roots. These fungi normally exploit the pools of Pi that are naturally available to the roots (Bolan, 1991), however, these bypass the slow process of breakdown and diffusion by translocating soluble Pi from non rhizospheric soil to their host through extraradical hyphae.

2.2.2 AMF as a bio-fertiliser/biocontrol agent

AMF can act as biofertilisers, bioprotectants, and/or biodegraders. These can incorporated as bio-fertilisers to enhance crop productivity and thus reduce need for chemical fertiliser/s (Xavier and Boyetchko, 2002). Bio-fertilisers are the organic substances which make use of microorganisms to increase the fertility of soil and are made from the microbial mixtures. These fertilisers are least harmful to crops or other plants then the chemical fertilisers. Microorganisms are used to increase the level of nutrients in the plants. They
allow the plants grow in a healthy environment. Being environment friendly do not cause pollution. Use of these bio fertilisers adds to the plant health by protecting them from pathogens. The main sources of bio-fertilisers can be either or bacteria, fungi, cyanobacteria. After culturing these are transferred by inoculating seed or soil or both under ideal conditions. To increase the accessibility of plant nutrients, mycorrhiza is an important inoculant used in agriculture fields for the cultivation of various crops (Sadhana, 2014). AMF inoculum production on large-scale is cumbersome especially due to the techniques currently available. The main obstacle being their peculiar symbiotic relation with their host plant for growth. The maintenance of AMF reference collections requires methodologies that are rather different from those used for other microbial collections and then inoculum production. Non-obligate symbionts distant from AMF, production of AMF inoculum require the mutual obligatory control and effective both host and fungal development (Berruti et al., 2014).

### 3.1 PGPR in potato rhizosphere

Verma and Shahi (2015) reported *Enterobacter cloacae* strain AB2, isolated from potato rhizosphere, that PGPR can be used as biofertilisers and may offer an attractive way to replace chemical fertilisers. PGPR were isolated from the rhizosphere of potato cultivar Hartapel for their physiological characters which revealed their potential use as biostimulant, biofertiliser and bioprotectant against soil borne pathogens (Kesaulya et al., 2014). Plant growth-promoting
rhizobacteria (PGPR) colonise potato plant roots and induce an increase in plant growth (Vessey, 2003). *Bacillus and Pseudomonas* are the most commonly reported among the PGPR (Compant *et al*., 2005; Vessey, 2003), whereas *Pseudomonas* and *Azotobacter* are highest phosphate solubilising genera. However, *Bacillus* strains are also phosphate solubilising bacteria (Chatli *et al*., 2008; Vessey, 2003; Nautiyal, 1999). *Pseudomonas* strains (Deshwal *et al*., 2013) and species of *Bacillus and Pseudomonas* (Sati *et al*., 2013) were isolated from potato rhizosphere by these workers. Hanif *et al*., (2015) observed that potato inoculation with *B. subtilis* strain KPS-11 increased the root and shoot length and root and shoot weight of potato as compared to non-inoculated control plants. Naqqash *et al*., (2016) reported that *Azospirillum* sp. TN10 has the greatest potential to increase the growth and nitrogen uptake by potato hence, it is being suggested as a good candidate for the production of potato biofertiliser for integrated nutrient management.

Nookaraju *et al*., (2011) reported *in vitro* and *ex vitro* tuberisation influenced potato growth by lipoxygenase (LOX) associated with plant growth promoting rhizobacteria (PGPR) isolated from potato fields.

Vrany and Fiker, (1984) reported 4-30% increase in plant growth and tuber yield when potato seed tubers were inoculated with PGPR before planting. Non-fluorescent *Pseudomonas* sp. show *in-vitro* effects on growth, enhancement and developmental modifications (Frommel *et al*., 1991) and also yield of tuber (Sturz, 1995) in potato. Akula *et al*., (2010) reported *in vitro*
tuberisation of potato influenced by plant growth promoting rhizobacteria (PGPR) isolated from the potato fields and further Malboobi et al., (2009) observed that combinations of either *P. agglomerans* or *Microbacterium laevaniformans* strains with *Pseudomonas putida* led to higher biomass and potato tuber under both greenhouse and field conditions. Al-Ani et al., (2013) reported *Pseudomonas fluorescens, Rhodotorula* sp can protect potato plants against potato virusY disease, whereas, Rahman et al., (2012) have demonstrated that the identified antagonistic bacterial strain E-65 (*Bacillus sp*) can significantly inhibit the growth of potato soft rot bacteria *in vitro* and in storage. Potato tubers with antagonistic bacteria successfully prevented the initial infection and reduced soft rot disease subsequently the multiplication of rot bacteria.

PGPR mutants resistant to rifampicin (rif) and nalidixic acid (nal) retained plant growth-promoting activity under greenhouse assay. These mutants are thus reported to induce increase in plant weights by five folds and develop larger root system with increased branching due PGPR (Kloepper et al., 1980). *Bacillus subtilis* is found to be more dominant species in the potato rhizosphere. The bacteria isolated also include species of *Bacillus, Variovorax, Proteobacterium, Chrysobacterium, Staphylococcus, Agrobacterium* and *Plantibacter*. These genera represent both Gram-negative (*Chrysobacterium, Agrobacterium, Proteobacterium* and *Variovorax*) and Gram-positive (*Bacillus, Staphylococcus* and *Plantibacter*) bacteria. Some of the strains of the
PGPR belonging to these genera are reported to be beneficial for the potato crop plants (Banik and Dey, 1982; Datta et al., 1982; Cezon et al., 2003). Using direct PCR-DGGE on the DNA extracted and also employing fatty acid methyl ester (FAME) analysis and/or sequencing of their partial 16s ribosomal RNA genes various species and strains of PGPR. Those characterised were as different *Pseudomonas* sp. i.e *P. aureofaciens*, *P. corrugata*, and *P. putida*, and others *Agrobacterium radiobacter*, *Stenotrophomonas maltophilia*, *Flavobacterium resinovorans*, *Bacillus sp.*, and *Sphingomonas paucimobilis* (Garbeva et al., 2001).

Calvo et al., (2010) suggested rhizosphere of native potatoes growing in their natural habitat in the Andes being a rich source of *Bacillus* acting as fungal antagonists, which thus have a potential to be used in the future as PGP inoculants to improve potato crop. Ahmad et al., (2008) evaluated *Azotobacter*, *Pseudomonas fluorescent*, *Mesorhizobium* and *Bacillus* isolates exhibiting multiple plant growth promoting (PGP) traits however, a soil-plant system is needed to uncover their efficacy as effective PGPR. Two *Bacillus* isolates amongst the 13 exerted significant positive influence of isolates in vitro potato tuberisation and were also investigated for their influence on abiotic stress tolerance in potato plants. The putative changes in the antioxidant pathway, gene expression and photosynthetic efficiency conferred by PGPR inoculation have also been described by Upadhyay et al., (2011).
3.2 AMF in Potato rhizosphere

A total of 2648 potato-associated bacteria were screened by dual testing of antagonism to the soilborne pathogens *Verticillium dahliae* and *Rhizoctonia solani* and the most prominent species characterised was *Pseudomonas putida* (Berg *et al.*, 2005). Additionally, 800 species were isolated from rhizospheres and endospheres at the flowering stage of potato plants. One of *Lysobacter* sp. was considered to be maximally antagonistic (Van Overbeek and Van Elsas, 2008). Sixteen AMF morphotypes were identified in potato under field conditions in India, and they proved that dark septate endophytes (DSE) and AMF colonisation progressed synchronously with the dominance of *Glomus tortuosum* (Das and Kayang, 2010). Mycorrhizal roots due to their external hyphae are capable of absorbing and translocating more nutrients, by exploring more soil volume. This increases the supply of slowly diffusing ions, such as phosphate to the potato plant (McArther and Knowles, 1993; Hodge *et al.*, 2010). AMF significantly suppressed disease due to pathogen *Fusarium sambucinum* on potato plants (Ismail and Hijri, 2012). The growth and yield of potato tubers was most affected by phosphorus (P) nutrition since P deficiency developed increased stress during the period of tuberisation and bulking (Nelson *et al.*, 1947; MacKay *et al.*, 1988). Ngakou *et al.*, (2006) suggested that AMF and soil solarisation enhanced growth of potato, to a degree saving N and P fertilisers, which earlier were considered as remedials of agricultural soils from biopollutants. Douds *et al.*, (2007) revealed that inocula of AMF and
also with vermiculite mixtures one can increase the yield of potato tubers by 20%
. AMF inoculation of low P availability prenuclear minitubers of Peruvian potato increased yield by an average of 85% (Davies et al., 2005a).

McArthur and Knowles (1993), observed that the growth responses of potato were different with different AMF however, all enhanced nutrient uptake particularly P (Black and Tinker, 1977). AMF are also reported to increase productivity of potatoes by increasing disease resistance (Graham et al., 1976; Niemira et al., 1995, 1996). Micro-propagated plant material with AMF strains *Glomus* and *Gigaspora* inoculum improved growth both in minituber and potato seedling and tuber production (Cheng et al., 2008). Micropropagated virus-free tuber has been shown to optimize and improve the tuber yield and quality (Donnelly et al., 2003). Duffy and Cassells, (2000) too report that mycorrhizal inoculation can influence the yield quality of potato microplants. AMF can raise or decline yield depending on the host genotype and mycorrhizal isolate. Now a days, the cultivation of potato to AMF are potentially important tools in agriculture that reduce or eliminate chemical input. The benefit of mycorrhiza as biofertilisers, increases the tuber yield, nutrient uptake and phosphorus use efficiency (PUE) of ‘Yungay’ variety (Davies et al., 2005b; Douds et al., 2007).

Mycorrhisation at potato microplant establishment significantly improved microplant growth and yield of saleable potato minitubers. In
protected cropping, mycorrhizal inoculation can increase or decrease yield quality of microplants. This depends on the mycorrhizal isolate and host genotype (Duffy et al., 1999). Sarikhani and Aliasgharzad, (2012) have shown that non mycorrhizal treatments in comparison to mycorrhizal treatments, especially *G. etunicatum*, had higher content of K in shoot. Interestingly they further reported that AMF treatments had higher dry matter in tuber, percent of starch, and specific gravity even when mycorrhizal association with potato plants form very weak root colonisation under field conditions.

Bharadwaj et al., (2007) suggested for crop rotation to enhance efficacy of AMF inocula for potatoes. *G. mosseae* is the most abundant species under monocultures at the field level. Lone et al., (2015) reported AMF (*Glomus intraradices* and *G. mosseae*) colonisation improved positively the overall growth and development of *Solanum tuberosum* var. TPS, SM/93-237 plant and also that of potato tubers var. jyoti, both cultivars of potato plant. AMF could improve chlorophyll content and other plant growth and developmental parameters and subsequently therefore, increase the production of potato tuber.

Gallou et al., (2011) investigated impact of *in vitro* AMF on *Phytophthora infestans* where in leaf infection index decreased in mycorrhiza associated potato plants. Potato is the main staple crop in the highlands of Peru and Bolivia comprising the center of origin and diversity of the cultivated potato (Devaux et al., 1997). For sustainable potato crop production in Peru it
is important that native AMF isolates are to be selected. Because of low soil fertility average potato yields from 5 to 8 t ha\(^{-1}\). While small producers apply organic N as animal or green manure, P is applied via chemical fertilisers which are expensive and are rarely available. A limitation of mycorrhiza utilisation is its commercial availability and the added production cost. The flavonoid, formanonetin has been reported to enhance mycorrhizal effectivity of mycorrhizal plants (Nair et al., 1997; Davies et al., 1999; Koide et al., 1999).

Cesaro et al., (2008) suggested that diversity of AMF species in rhizosphere and in bulk soils from the two studied areas of potato roots were selectively colonised by one AMF species *G. intraradices*. Gene expression analysis at different stages of AMF establishment with the MDP *in vitro* culture system, they observed the induction of PT3 gene at the late stage of potato *G. intraradices* interaction. Two genes PR1 and PR2 were induced prior to root colonisation. They showed a transient expression; being demonstrated further by the initiation of GST1, Lox, MAPK, and PAL genes at different stages of potato *G. intraradices* interaction. PT3 gene is a plant gene marker of AMF root colonisation which confirmed that the potato *G. intraradices* association was successfully established and the exchange between the two partners was mutually effective (Gallou et al., 2010).
3.3 PGPR and AMF in potato rhizosphere

Towards the management of natural microbial soil biota, positively effecting plant development, nutrition and health, Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth Promoting Rhizobacteria (PGPR) are now recognised as major agricultural inputs to reduce dependence on chemical pesticides and fertiliser, thus improve the sustainability of potato growing soils. These environmental friendly, natural bio-fertilisers and bio-protectors prove as cost effective inputs for small farmers and resource poor agricultural systems (Franco et al., 2011). 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 increased fresh and dry weight, endogenous growth factors and chlorophyll contents in potato tuber and plant.

The microbial community in the rhizosphere plays an important role and may have a positive or negative influence on plant growth. Microbes are essential for the mobilisation of plant nutrients and may produce plant growth hormones which are important for plant development (Lynch, 1990). AMF and PGPR formulations containing two or more AMF and/or PGPR species have been tried for cultivation (Yan et al., 2002; Mia et al., 2005; Domenech et al., 2006; Rodriguez Romero et al., 2005; Vestberg et al., 2004). Further reports combinations of other AMF, PGPR and Trichoderma harzianum (Srinath et al., 2003); AMF, PGPR and other bacteria (Bashan, 1998); AMF, PGPR and
nitrogen-fixing rhizobacteria with stimulants of AMF spore germination and plant colonisation, plant stimulants and fertilisers (Handelsman and Stabb, 1996). *Pseudomonas putida* culture can be mixed along with AMF inoculation in potato plants to increase the total weight of tubers and enhancement of mycorrhisation and extraradical mycelium activity of plants (Vosatka and Gryndler, 1999). Palacios et al., (2009) has suggested that the inoculation of native diazotrophic bacteria and AMF in micropropagated *in vitro* potato plantlets can increase growth.

AMF and their bacterial associates are essential living components of the soil microbiota. Bacteria associated with AMF referred as AMB isolates also stimulate mycorrhizal formation. *Paenibacillus sp.* isolated from surface-sterilised *Glomus mosseae* spores stimulates mycorrhizal formation in *Sorghum bicolor* (Budi et al., 1999), while *Bacillus pabuli* isolated from *G. clarum* spores enhances *G. clarum* colonization in pea roots (Xavier and Germida, 2003). Arbuscular Mycorrhizal Bacteria play vital role in the growth of AMF. Those Arbuscular Mycorrhizal Bacteria (AMB) that help in the growth of mycorrhizal symbiosis are also known as mycorrhiza helper bacteria (MHB) (Garbaye, 1994). It has been suggested that AMB can also function as plant growth-promoting bacteria (PGPB) because they improve the nutrient acquisition of plants (Artursson et al., 2006). The use of AMF and/or PGPRs to leading the 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).

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OBJECTIVES

The present study focuses on the following aspects of the potato crop:

- Isolation of Plant Growth-Promoting Rhizobacteria (PGPR) from the rhizosphere and non-rhizospheric soil (bulk) of the three different varieties of potato plant at various stages of its growth.

- To identify genetic relatedness of the associated PGPR species based on the morphological, physiological and molecular characterisation.

- To perform comparative phylogenetic analysis based on 16s ribosomal RNA sequence databases on the associated PGPR.

- Identify the extent of the colonisation and sporal density of associated Arbuscular Mycorrhizal Fungi (AMF).

- To assess the interaction between isolates of AMF and PGPR species from different varieties of potato (*Solanum tuberosum* L.).

- To formulate effects of PGPR and AMF individually or in combination on the growth of commercial crop.