INTRODUCTION

The layer of soil called rhizosphere, the one closely surrounding the plant root is most important for being an active area for root activity and metabolism (Kennedy, 1998). The concept of rhizosphere was first introduced by Hiltner (1904). It says that rhizosphere is the volume of soil surrounding both plant root and an organism where intensive interactions occur between the roots, soil, soil microflora and microfauna. There are different types of substances such as carbohydrates (sugars and oligosaccharides), organic acids, vitamins, nucleotides, flavonoids, enzymes, hormones, and volatile compounds that diffuse from the roots which stimulate the microbial activity, (Prescott et al., 1999). Further on, the rhizoplane is considered an active area for plant root activity and metabolism. The rhizosphere concept was later enlarged to include the root’s ever-present mycorrhizal fungal associates and PGPR (Rawlings, 1958).

Bulk soil is outside the rhizosphere and is not penetrated by plant roots. Natural organic compounds are much lower in bulk soil than in the rhizosphere. Populations of microbes are usually lower in bulk soil than in
rhizosphere. Moreover, bulk soil inhabitants are generally smaller than identical species in the rhizospheric soil (Stotsky 1996; 2000). Soil microbial population can be measured comparing the population density (Colony Forming Unit, CFU) (Atlas and Bartha, 1997).

Bacteria, fungi, protozoa and algae coexist in the rhizosphere; bacteria being the most abundant among these. Microorganisms closest to root epidermis, host plants secrete certain organic signal molecules. These are for heterogeneous microbe activity in the root zone. This is the stage where pathogenicity, association, symbiosis, or neutralistic adaptation of microbes with the plant is decided (Hayat et al., 2010).

In the mycorrhizosphere two different zones are distinguished. First zone being under the joint influence of the root and fungal components of the mycorrhiza, and the second which is affected by the mycelium of the mycorrhizal fungus only, called the hyphosphere (Marschner, 1995). Moreover bacterial species load is heavier in rhizosphere than in the hyphosphere soil. This may therefore, support biotic activities exclusive from those where in soils are under joint root and fungal influence. Microbial activity may thus differ in mycorrhizosphere, hyphosphere, rhizosphere and bulk soils (Andrade et al., 1997).

Ever since studies were initiated on PGPR in the 1950’s, hundreds of PGPR candidate strains have been screened across the world and laboratory
evaluated under both greenhouse and field conditions. PGPR are now commonly used in various countries as inoculants on millions of hectares of land (Zehnder et al., 2001). These influence plant health and productivity by variety of mechanisms involving solubilisation of mineral nutrients, stimulation of root growth, and suppression of root pathogenicity.

Fig.1.1: Schematic representation of (A) Rhizosphere (B) Mycorrhizosphere, (Vega and Walter, 2007)

The study of PGPR therefore, reveals these being functionally multifaceted. Specifically, term rhizobacteria is used for those rhizospheric bacteria which are capable of colonising the root and then promote the plant growth. These beneficial bacteria are then referred as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper et al., 1991, Kloepper, 1994). Their additive characteristics are the mobilisation of nutritional elements, nodulation and nitrogen fixation (Zhang et al., 1996), synthesising phytohormones (Khalid et al., 2004; Spaepen et al., 2007), microbial iron transport agents or developing
siderophores (Kloepfer et al., 1980a), antibiotic production against plant pathogens (Sandra et al., 2001; Morales et al., 2008) and suppressing pathogen, and/or combinations of these processes (Somers et al., 2008).

Certain PGPR are also defined as MHB (Mycorrhization Helper Bacteria). These are bacteria associated with mycorrhizal roots and mycorrhizal fungi helping promote the establishment of mycorrhizal symbioses (Garbaye, 1994). PGPR are also referred as phosphate solubilising bacteria (PSB) for their ability to convert insoluble phosphates into available soluble forms of phosphate for uptake of the host plant. This may be brought about through acidification, chelation, exchange reactions, or sometimes by production of gluconic acid (Chung et al., 2005; Gulati et al., 2010).

**Fig. 1.2:** Various mechanism interplays by rhizobacteria in plant growth promotion (Ahemad and Kibret; 2014)

Three intrinsic characteristics define these PGPRs- (i) able to colonise the root (ii) survive and multiply in habitats associated with the root surface in
competition with other microbiota, (iii) must promote plant growth. These are also considered as highly efficient microbial competitors in the soil-root zone. Generally species belong to the genera *Serratia, Pseudomonas, Burkholderia, Agrobacterium, Erwinia, Xanthomonas, Azospirillum, Bacillus, Enterobacter, Rhizobium, Alcanigenes, Arthrobacter, Acetobacter, Acinetobacter, Achromobacter, Aerobacter, Azotobacter, Clostridium, Klebsiella, Micrococcus, Rhodobacter, Rhodospirillum* and *Flavobacterium, Bradyrhizobium, Frankia, Rhizobium* (Rodriguez and Fraga, 1999; Bloemberg and Lugtenberg, 2001; Esitken et al., 2003; Bhattacharyya and Jha, 2012 and Tailor and Joshi, 2014). PGPR are now used as biofertiliser for different crop plants as an alternative source to chemical fertilisers to improve plant root growth and nutrition uptake (Egamberdiyeva and Hoflich, 2004).

PGPR differentiation is based according to their association with plant root as free living or symbionts. One being an extracellular plant growth promoting rhizobacteria (ePGPR, free living) and the other intracellular plant growth promoting rhizobacteria (iPGPR, symbiotics) (Martinez-Viveros et al., 2010). These may increase plant growth directly or indirectly. When direct these are known to secrete certain plant growth promoting substances, siderophores, or enzymes in rhizosphere, whereas under indirect process PGPR strains secrete antimicrobial substances like HCN, antibiotics, enzymes which inhibit the growth of pathogenic microorganisms (Deshwal et al., 2013).
The 16s rRNA is the most employed gene in understanding microbial ecology and ascertaining bacterial phylogeny (Louws et al., 1999). The small subunit of the gene can represent a valued candidate molecule due to its sequence uniformity and function which are due to regions of highly conserved, variable and hypervariable sequences being therefore important in the identification of species and being limited to 1500 bases. It is therefore, easily sequenceable and meanwhile, sufficient enough for the identification and phylogenetic analysis (Spratt, 2004; Agrawal et al., 2011). Molecular studies regarding assay of microbial diversity include DNA base ratio (mole % G+C) analysis, DNA-DNA hybridisation, DNA microarray, reverse sample genome probing, 16s rDNA sequencing and amplified rDNA restriction analysis (Muyzer et al., 1993). Comparing the differences in the base sequence of this 16s rRNA gene is, therefore, an excellent means of studying evolutionary changes and phylogenetic relatedness of organisms (McDonald et al., 2012).

Based on 16s rRNA gene sequences large number of genotypes can be distinguished. However, detection and diversified selection of nucleotide of 16s rRNA gene sequences are not yet studied in detail (Quast et al., 2013).

Potato (Solanum tuberosum L.) is a herbaceous perennial plant belonging to family Solanaceae. Cytogenetically the plant is a tetraploid and is propagated vegetatively by an underground modified stem. The word "potato" refers both to the plant and the edible underground tuber (Merriam-Webster Dictionary). The English word for potato is derivative of Spanish patata.
The Spanish Royal Academy describes the Spanish word as a compound of the Quechua *papa* (potato) (Real Academia Espanola, 2010). Potato is a staple crop in 130 countries and a common consumed food of north India. It is relatively insensitive to losses induced by soil salinity, drought, and/or low nutrient availability (Van der Linden *et al.*, 2011). It is a source of vitamin C, niacin and vitamin B_6_ and most important source of starch. Potato starch is of a superior quality and also many interesting properties make it attractive. Starch is used in the food industry for making various different products (Christensen and Madsen, 1996; Jansen *et al.*, 2001). Quantity and quality of potato depends on balanced nutrition. Potassium and nitrogen are found in large amounts in potato tuber, followed by Ca and Mg, hence is in high demand for its potassium (Mengel and Kirkby, 2001; Fageria, 2009). The edible potato undergoes five steps of growing sprout development, vegetative growth, tuber initiation, tuber bulking and maturation.

Potato is the fourth major food crop of the world after rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Czajkowski *et al.*, 2011). India is the second largest potato producer in the world after China. Worldwide, under diverse range of altitudes, and climatic conditions, distribution ranges from that of the sea level to more than 4000 m elevation. Crop is unmatched in its production of food energy and also food value per unit area (Sieczka and Thronton, 1993). Potatoes are generally cultivated in the highlands, therefore can constantly damage the environment, due to occurrence
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of soil erosion and thus lowered productivity (Kesaulya et al., 2015). This important crop was domesticated by pre-Columbian civilizations in the Andean highlands of Peru and Bolivia (Davies et al., 2005a).

![Diagram of potato growth stages](image)

**Fig. 1.3: Different growth stages of potato (Johnson, 2008)**

The Food and Agriculture Organization (FAO) of the United Nations reported that the world production of potatoes in 2013 was about 368 million tonnes (FAOSTAT, 2015). Just over two thirds of the global production is consumed directly by humans and the rest being fed to animals or used to produce starch. This means that the present annual diet of an average global citizen in the first decade of the 21st century included about 33 kg (or 73 lb) of potato. However, each locality has importance of potato and is variable and rapidly changing. It remains an essential crop in Europe where per capita production is still the highest in the world, but the most rapid expansion over
the past few decades has occurred in southern and eastern Asia (The Potato, 2008).

In India predominantly 48 varieties of potato are cultivated the listing being available with Potato Research Station Shimla, H.P., India. However, the base characteristic of the three varieties used in the present study are given as follows:

**Kufri Lauvkar:** - Plateau regions of Karnataka, Madhya Pradesh and Maharashtra. It can be grown both in Kharif and Rabi seasons. It can build up yields rapidly under warmer climate. Early (70-90 days) maturity variety. Yield of this variety is 200-250 q/ha. It is suitable for preparation of flakes, flour, chips and dehydrated products. Suitable for low-input eco-system. Easy to cook, texture floury, flavour mild, free from discoloration after cooking.

![Kufri Lauvkar variety](cpri.ernet.in)

**Fig. 1.4: Kufri Lauvkar variety**

(cpri.ernet.in)
**Kufri Sindhuri:** - Late (> 110 days) maturity variety and suitable for cultivation in North Indian plains. This variety has replaced Kufri Red and Kufri Kisan. Yield of this variety is 300-350q/ha. Moderately resistant to early blight but tolerant to leaf roll. Slow rate of degeneration. Suitable for low-input eco-system. Easy to cook, texture waxy, flavour mild, free from discoloration after cooking.

![Fig.1.5: Kufri Sindhuri variety](cpri.ernet.in)

**Kufri Chipsona-3:**- Medium (90-110 days) maturity variety and suitable for cultivation in North Indian plains. Yield of this variety is 300-350q/ha. Resistant to late blight. Suitable for making chips and French fries. Easy to cook, texture floury, flavour mild, free from discoloration after cooking. High dry matter, reducing sugars and low phenols. This variety conforms to the norms of protection of plant varieties and farmers rights (PPV & FR) authority.
Mycorrhiza term was proposed by Frank in 1885 as an association between a soil-borne fungus and plant roots. The term symbiosis was first introduced by De Bary in 1887. It included a mutualistic association in which two partners would benefit from each other. An antagonistic symbiosis an association where only one partner would benefit from the other (De Bary, 1887). These fungi produce characteristic finely branched hyphal structures, termed arbuscules, inside cortical cells of plant roots. 80-90% families of land plants are associated with AMF. AMF and soil microbial underground community shows symbiosis where in several kinds of bacteria are associated with AMF spores (Bonfante, 2001). Arbuscular Mycorrhizal Fungi (AMF) are now established plant root symbionts and have widespread symbiotic association with the roots of plants belonging to angiospermophyta, pteridophyta, bryophyta and coniferophyta. Except for plant species mainly
belonging to the families cruciferae, chenopodiaceae, cyperaceae, caryophyllaceae and juncaceae, which do not show AMF association, almost all other plant species are known to be associated with AMF (Harley and Smith, 1983; Smith and Gianinazzi-Pearson, 1988; Azcon-Aguilar and Bago, 1994).

These fungi come under the phylum Glomeromycota which formerly was Glomales within the Zygomycota (Schüßler et al. 2001). The earlier order of Glomales stood divided into two sub-orders Glomineae and Gigasporineae due to vesicles in the root for the suborder Glomineae or absence of vesicles in the root for the suborder Gigasporineae. The sub-order Glomineae contain four families of Glomaceae, Acaulosporaceae, Paraglomaceae and Archaeosporaceae (Pirozynski and Dalpche, 1989). Further, Glomaceae has form genus Glomus, Acaulosporaceae has two viz. form genera Acaulospora and Entrophospora, Archaeosporaceae is having a form genus Archaeospora and Paraglomaceae with a form genus Paraglomus. Family Gigasporaceae includes genera Gigaspora and Scutellospora. Taxonomy of Glomalean fungi is primarily based on stable and discrete morphological characters of fungal mycelium and spores (Morton and Benny, 1990).

Symbiosis between AMF and the plant involves a mutual sharing of assimilated carbon from the plant to AMF in exchange for soil-derived nutrients from the AMF. Extensive networks of mycelia external to roots in the
soil enables the AMF to take up and subsequently translocate nutrients to inside roots through arbuscules and hyphal mass into the plant. The hyphal networks help increase the fungal surface area thereby increasing availability of soil nutrients to the host plant (Smith and Read 2008). Improved water relations by AMF association can be another functional benefit to the host plant (Auge, 2004). AMF symbiosis with the host plants provide the fungi with their hydrocarbon needs in exchange for nutrients, mainly phosphorous. These symbiotic properties are determined by: (i) the ability of a plant to acquire nutrients through a fungus (mycotrophy), (ii) physiologically obligate symbiont (host plant dependency), (iii) plant and their characteristics degree of dependence on mycorrhiza for its proper development (mycorrhizal dependency of a living plant). These fungi are having external hyphae which absorb and translocate nutrients for symbiotic plant growth by their penetration to more soil volume for such nutrients (Joner and Jakobsen, 1995).

Fig.1.7: AMF Classification (Harrison, 1997; Olsson, 1994).
AMF is not readily identifiable by their morphological characterisation. Colonisation and morphotyping may not therefore, be a suitable criteria for species level studies. Isozyme differences or antibody reaction techniques are difficult to employ when more than one fungus are symbiotic in the roots. Though, specific primers for species identification are used to amplify for genes fungi from root tissue by the PCR amplification (Abbas et al., 1996; Millner et al., 1998; Van Tuinen et al., 1998; Zézé et al., 1994). This advance may be suitable for field studies only when unaccompanied species may colonise the roots. Using SS rRNA gene sequences to understand AMF community structure too has its limitations, as it is known that different nuclei within a single isolate can contain different copies of the SS rRNA gene (Sanders, 2002; Hijri and Sanders, 2005). However, Schußler et al., (2001); (1999) reported that the within-isolate variation of the 18s SS gene is relatively small, and that AMF phylogeny can therefore be based on this gene (Schüßler et al., 2001). Moreover, this method allows for the comparison of the genetic variation of AMF found during this study with that from other ecosystems based on the same gene.

The aim of present study was to seek taxonomically diverse rhizobacteria and to induce a comparative study for their characterisation. This included isolation, screening and inventorisation of the PGPR from the rhizosphere of potato an important commercial crop. In fact the PGPR and also AMF association vis a vis potato crop in central India, especially the Madhya
Pradesh state, stands almost unexplored. PCR based DNA amplification of 16s rRNA gene sequencing and bioinformatic software are employed to ascertain phylogeny of potato related PGPR in rhizo and bulk soil. The aim was also to assess if a consortium of could be developed for the effective growth and yield incremental synergism in the development of potato crop grown in the state of Madhya Pradesh.

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