Rhizospheric bacterial diversity in different *Dalbergia sissoo* Roxb. provenances

Thesis

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By

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"I give thanks and my heart kneels to pray:
God keep me and guide me and go with me today"

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(Samiksha Joshi)
Authoress

August, 2018
Pantnagar

CERTIFICATE

This is to certify that the thesis entitled **"Rhizospheric bacterial diversity in different *Dalbergia sissoo* Roxb. provenances"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Microbiology** and minor in **Molecular Biology and Biotechnology** of the College of Basic Sciences & Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bonafide research carried out by **Ms. Samiksha Joshi**, Id.No. **35974** under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

Pantnagar
August, 2018

<u>CERTIFICATE</u>

We, the undersigned, members of the Advisory committee of **Ms. Samiksha Joshi**, Id. No. **35974**, a candidate for the degree of **Doctor of Philosophy** with the major in **Microbiology** and minor in **Molecular Biology and Biotechnology** agree that the thesis entitled, **"Rhizospheric bacterial diversity in different *Dalbergia sissoo* Roxb. provenances"** may be submitted in the partial fulfillment of the requirements for the degree.

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Introduction

INTRODUCTION

Forests represent largest and highly productive ecosystems on earth. They are spread over 40 million km^2 and represent 30% of the total global land area **(Keenan *et al.*, 2015).** They are both sinks (80% of carbon stored in land vegetation and 40% of carbon in soils) and sources (25% of GHG are released because of deforestation) of carbon. In India forest stored carbon is about 106 t ha^{-1} **(FAO, 2010)**. Moreover, forest provides timber, food, fuel and bioproducts. They also play important role in ecological functions such as, nutrient cycling, water and air purification, providing wild life habitat, soil conservation and diverse floral species. Forest vegetation typically grows in multiple layers and all contribute to ecosystem processes.

Dalbergia sissoo Roxb. common name shisham is a leguminous forest tree species within the genus *Dalbergia*, family Fabacae. It is found either as a pure crop or mixed forest with *Acacia catechu* (common name Khair). It is a native of Indian Sub-continent and grows naturally in Afghanistan, Pakistan, Nepal, Bangladesh and India. In India, its forest is spread throughout sub-himalaya upto an altitude of 1000 m abmsl as well as in Central and Peninsular India. It has also been introduced in various countries throughout the world but is known as invasive species in Australia and Florida state of USA. It is a pioneer tree species that is drought resistant, highly light demander and frost tolerant. Its timber is highly valued and important for country's economy. This tree species is preferred for roadside plantations and agroforestry systems. It is also used for fuel wood, fodder and shade. *D. sissoo* leaf extract is antipyretic, anti-inflammatory and analgesic **(Hajare *et al.,* 2000)** and recommended for eye ailments **(Hajare *et al.,* 2001).** Its bark has antioxidant properties **(Kumari and Kakkar, 2008).** The wood and bark are also used for anal disorders, dysentery, burning sensations, dyspepsia, blood diseases, leucoderma, and skin ailments. It is one of the principal nitrogen fixing tree (NFT) species recommended for plantation programmes in dry regions for soil and water conservation. Since, the profit from *Dalbergia sissoo* timber is higher than from other agricultural products farmers prefer the shisham plantations on their lands.

The isolated incidence of disease in natural and plantation forests of shisham have been reported since long **(Bakshi, 1954).** But from the last three- four decades sudden and systemic death of this tree species in the plantation as well as in natural forests has been observed **(Naz, 2002).** The shisham decline is the complex phenomenon. A few studies have highlighted abiotic environmental factors as one of primary cause of shisham decline **(Manion, 1981 and Sah *et al.,* 1999)**. However several others have identified pathogenic infections as a major cause of shisham mortality **(Javaid, 2008).** Amongst various diseases affecting *Dalbergia sissoo* tree, quick wilting and dieback are the most damaging **(Bajwa *et al.,* 2003)**. Fungi and bacteria both are known to be associated with diseased shisham trees. The fungi implicated in shisham disease and decline includes *Fusarium solani* **(Bakshi, 1954)**, *Ganoderma lucidum* (**Sharma *et al.,* 2000**), *Phytophthora cinnamomi* **(Gill *et al.,* 2001)**, *Botryodiplodia theobromae* (**Khan *et al***., **2004**), *Talaromyces pinophilus* and *Talaromyces verruculosus* **(Dasila *et al.,* 2018).** Bacterial strains within genera *Bacillus* and *Pseudomonas* have also been identified from the diseased shisham trees in Bangladesh **(Tantau *et al.,* 2005)**. All the shisham pathogens identified till date are soil-borne. Outbreak of soil borne phytopathogen is largely dependent on soil quality. Soil quality is governed by structure and composition of soil microbial communities.

Soil quality has been assessed through various enzyme activities. Soil enzyme activity is derived from soil microorganisms **(Ladd, 1978).** They are influenced by soil physico-chemical characters **(Amador *et al.,* 1997),** vegetation **(Waldrop *et al*., 2000; Sinsabaugh *et al.,* 2002),** disturbance **(Bolton *et al.,* 1993, Boerner *et al.,* 2000),** succession **(Tscherko *et al.,* 2003),** environmental factors **(Sparling, 1997)** and microbial community structure **(Waldrop *et al.,* 2000; Kourtev *et al.,* 2002).** Further they can provide more comprehensive insight into processes linking microbial population with nutrient dynamics **(Schimel and Weintraub, 2003).** Hence, these play an essential role in formation of stable organic molecules that contribute to the soil ecosystem functioning and affect the tree health and survival. Although soil enzymes serve as an indicator for entire microbial community function **(Bergstrom *et al*., 1998)** they cannot predict the structure and composition of soil microbiota.

Rhizosphere microbiome affects any plant including trees the most. Rhizosphere is a hotspot of microbial activity. Population and activity of microorganisms in

rhizosphere may be 2–20 times higher than in bulk soil. Other microbial activities like respiration, growth, mineralisation potential, enzyme activities, RNA/DNA ratio are also much higher in rhizosphere **(Baldrian *et al.,* 2010)**. Although forests have mixed vegetation, it is the tree species that largely shape the forest microbiome. Tree diversity, identity and genotype significantly affects the total microbial activity **(Augusto *et al.,* 2015)** as well as the composition of the fungal, bacterial and protist communities **(Tedersoo *et al.,* 2016)** in phyllosphere, litter and bulk soil**.** Trees allocate 33-50% of carbon fixed into soil through specific root exudates. Since root exudation pattern varies with tree genotype, it is the tree genotype that shapes its rhizospheric microbial community. Tree genotype and soil microbial composition as well as activity affect litter deposition and decomposition, nutrient uptake and root exudation pattern of trees. This in turn affects soil aggregation and nutrient availability. The tree genotype also determines temperature (the canopy effect), aeration (oxygen consumption), porosity (root development) and water capacity (root uptake) of soil **(Augusto *et al.,* 2002).** Hence, all soil properties vary with tree species (i.e., the tree species effect) and the type of tree stand (i.e., pure vs mixed) (**Augusto *et al*., 2015**). These differences mainly affect soil biota including microbes (**Korboulewsky *et al*., 2016**). Soil microorganisms especially rhizobacteria are key drivers of nutrient cycling. They carryout functions necessary for soil build up of key nutrients in plant usable forms. They mobilize release of potassium, magnesium from rocks, soil minerals, and organic matter **(Uroz *et al.,* 2009)**. Hence, play significant role in tree nutrition. Bacteria-bacteria, plant –bacteria, plant- bacteria-pathogen interactions govern soil structure formation; decomposition of organic matter; toxin removal; and the cycling of various nutrients **(Elsas *et al*., 1997).** Tree is largely dependent on microbial symbionts for growth-limiting nutrients such as phosphorous and nitrogen. Arbuscular mycorrhizae along with nitrogen-fixing bacteria and actinomycetes are responsible for delivering upto 75% of all phosphorus and 80% of all nitrogen acquired by plants in temperate and boreal forests (**van der Heijden *et al.,* 2008).** Since most of the forest soils are nutrient poor and amendments are not practical so mobilization and transfer of nutritive elements is particularly important. Thus are critical to the maintenance of soil health and function in both natural and managed agricultural or forest soils. In addition, they play key roles in suppressing soilborne plant diseases **(Doran *et al*., 1996).**

Forests are subject to multiple disturbances, such as insect outbreaks, fires or wind throws. They are also significantly threatened by a combination of anthropogenic factors such as climate change, environmental pollution and inappropriate management practices **(Gauthier *et al.,* 2015).** Climatic change alters the relative abundance and function of soil microbial communities because biotic component differ in their physiology, temperature sensitivity, growth rates and their function **(Thomson *et al.,* 2015).** Environmental events like soil erosion lead to rapid change in soil bacterial communities (**Tilman *et al.,* 2001**). Soil physico-chemical parameters show seasonal variation. This can consequently modify the structure and composition of bacterial communities (**Lopez-Mond_ejar *et al.,* 2015**). Hence, the composition of soil bacterial community fluctuates with seasonal variations **(Collignon *et al*., 2011, Augusto *et al.,* 2015)**. Soil pH was highlighted as another major factor influencing the structure of bacterial communities, followed by both the availability of organic (C, N, P) and inorganic (nutritive and toxic cations) nutrients **(Thomson *et al.,* 2015).** Indeed, bacterial communities inhabiting the most nutritive and weatherable minerals (e.g., apatite) exhibited less diversity and were enriched in specific taxa such as Betaproteobacteria compared to poorly weatherable minerals **(Uroz *et al.,* 2016).** In conclusion all environmental factors affect forest soil biota and especially the forest microbiome (archaea, bacteria, fungi, protists) which further has an impact on functioning and homeostasis of forest ecosystems (**Uroz *et al.,* 2011**). Hence, it is essential to understand the composition of bacterial, fungal and even archaeal communities.

The soil bacterial communities in forest ecosystems have been characterized using cultivation dependent and cultivation-independent approaches. Although analysis of bacterial community diversity and structure in soil through cultivation based techniques is useful, it gives limited information as there is nutrient bias and only about 1% of the total bacteria are culturable in the laboratory (**Torsvik *et al*., 2002**). Therefore, culture- independent techniques have been used to understand bacterial soil diversity (**Janssen, 2006**). Culture-independent techniques are based on analysis of DNA, RNA, proteins and lipids. The molecular methods used globally for diversity assessment of different cropping systems include, phospholipid fatty acid (PLFA) analysis, terminal-restriction fragment length polymorphism (T-RFLP), single-strand conformation polymorphism (SSCP), and denaturing/temperature gradient gel electrophoresis (DGGE/ TGGE). Using NGS, it is possible to resolve highly complex microbiota compositions

with greater accuracy, as well as to link microbial community diversity with niche function. Next generation sequencing strategies involve high throughput sequencing and, can effectively provide deep insights into complex microbial communities in ecological niches **(Fakruddin and Mannan, 2012).** Metagenomic approach based on 16S rRNA next-generation sequencing can provide insights of entire microbial community structure, composition and its dynamics in relation to soil functions in a given sample in a single run without any cultivation steps **(Kim *et al.,* 2013).** The analysis of enormous metagenomic data generated by next generation sequencing platforms requires several bioinformatic tools. An accurate taxonomic assignment of each microbe is imminent to accurately evaluate the structure, diversity, richness, and function of resident community in a target environment **(De Filippo *et al.,* 2012**).

Several bioinformatic programmes are available for analysis of metagenome sequence data from targeted and shotgun sequencing methods (**Navas-Molina *et al.,* 2013**). One such programme is Meta Genome Rapid Annotation using Subsystem Technology (MG-RAST). It is a freely available (http:// metagenomics.nmpdr.org), fully automated system able to process metagenome sequence data by performing sequence alignment, functional annotation, phylogenetic assignment of operational taxonomic unit (OTU), and comparative metagenomics **(Meyer *et al.,* 2008).** Similarly Quantitative Insights Into Microbial Ecology (QIIME) is an open-source software pipeline (http://qiime.sourceforge.net/) able to perform, sequence alignment, OTUs identification, taxon-based identification of diversity within and between samples and genetrate phylogenetic trees **(Caporaso *et al.,* 2010**). Both tools have been successfully used to analyze a large number of metagenomic 16S ribosomal RNA datasets to assess their ability in the management of targeted and shotgun matagenomic data.

There is need to cultivate microorganisms from soil habitats to better understand their role in soil processes, development of bioinoculants and biopesticides for improving soil health leading to enhanced productivity. The cultivation based and culture independent methods have their own advantages as well as disadvantages in assessing microbial diversity. Hence, culture-dependent and culture-independent methods must be combined to study complete soil microbial communities from any habitat or ecosystem. This will enable us to elucidate comprehensive structure, composition and function of specific microbial communities in relation to overall health and productivity of forests and plantations.

Microbes associated with plants are classified into three groups: beneficial, deleterious and neutral. Rhizobacteria are generally beneficial. They are free living soil bacteria that colonize the rhizosphere and improve the growth and plant yield when applied to seed or crops **(Kumar *et al.,* 2014).** Important rhizobacterial genera are *Pseudomonas, Enterobacter, Bacillus, Variovorax, Klebsiella, Burkholderia, Azospirillum, Serratia*, and *Azotobacter*. In addition arbuscular mycorrhizal fungi are also plant beneficial. The plant growth promoting ability of rhizobacteria is linked with release of various metabolites/ hormones; (a) ability to synthesize phytohormones such as indole acetic acid (IAA), gibberellins and cytokinins (**Marques *et al*., 2010**); (b) enhancing asymbiotic nitrogen fixation (**Khan, 2005**); (c) solubilisation and mineralization of insoluble phosphate and other nutrients (**Jeon *et al.*, 2003; Hayat *et al.,* 2010; Ahemad and Khan, 2012**); (d) antagonism against plant pathogens through production of antibiotics, enzymes, siderophores etc.(e) competition with with pathogenic microbes **(Lucy *et al.,* 2004).** Besides, micro-organisms play crucial role in soil nutrient recycling and are important for maintaining soil fertility (**Glick, 2012**). Additionally, rhizobacteria have been implicated in biological control of pests and fungal phytopathogens (**Russo e*t al*., 2008**).

Since *Dalbergia sissoo* is an NFT, phosphorous plays an important role in rhizobial nodulation and nitrogen fixation so in shisham growth and life cycle. Phosphorous in soil consists of both organic and inorganic form. Soil organic matter accounts for 20-80% of phosphorous. But approximately 98% of soils have inadequate supply of available phosphorus. The major proportion of natural soil phosphorous is easily converted into insoluble complexes such as iron and aluminium hydro-oxides, crystalline and amorphorus aluminum silicate, and calcium carbonate and thus becomes unavailable for plant nutrition. These precipitated forms cannot be absorbed by plants.Several soil microbes are known to convert insoluble organic and inorganic phosphates to orthophosphates. The bacterial genera; *Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium Pseudomonas, Rhizobium, Rhodococcus* and *Serratia* include phosphate solubilising bacteria (PSB) (**Bhattacharya and Jha, 2012**). The PSBs increase the availability of soluble phosphate and can enhance plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc. and by production of plant growth-promoting regulators **(Ponmurugan**

and Gopi, 2006). The main mechanism of mineral phosphate-solubilization by microbes is associated with the release of low molecular weight organic acids **(Kim *et al.,* 1997).** The organic acids, via their hydroxyl and carboxyl groups, chelate the phosphate-bound cations thereby converting it into soluble forms **(Vyas and Gulati, 2009)**. The PSBs produce gluconic and 2-ketogluconic acids through direct oxidation glucose pathway **(Krishnaraj and Goldstein, 2001).** Conversion of glucose to gluconic acid is facilitated by pyrolloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) and gluconic acid oxidation to 2-ketogluconic takes place via the FAD linked gluconate dehydrogenase (GADH) **(Buch *et al.*, 2008)**. Both enzymes are in the outer face of the cytoplasmic membrane, so acids are formed in the periplasmic space, with the resultant acidification of this region and, ultimately, the adjacent medium as well **(Babu-Khan *et al.*, 1995)**.

PSB inoculation in plants improved solubilisation rate of fixed phosphorus in soil leading to increased production **(Zaye and Mottal, 2005)**. Although enormous information is available on microbial community structure composition and diversity associated with several annual plants **(Bashan, 1998)**. However, very limited information is available on rhizospheric bacterial diversity associated with the deciduous tree such as shisham. Moreover bacterial strains from trees (forests or plantations) have neither been isolated nor characterized for plant growth promoting (PGP) traits. Thus exploration of rhizospheric bacterial diversity of *Dalbergia sissoo* from different provenances was undertaken and, the present study **"Rhizospheric bacterial diversity in different *Dalbergia sissoo* Roxb. provenances"** is proposed with following specific objectives.

i) Analysis of soil samples for physico-chemical properties and soil microbial enzyme activities.

ii) Analysis of bacterial diversity in rhizospheric soil samples from different *Dalbergia sissoo* provenances using culture independent approach.

iii) Isolation and screening of phosphate solubilizing bacteria from rhizospheric soil samples.

iv) Functional and molecular characterization of potential phosphate solubilizing bacteria.

Review of Literature

2.1 Forest ecosystems

Forests are an important source of energy and renewable raw material and help to maintain biological diversity, protect land and water resources, mitigate climate changes, provide recreation facilities, improve air quality and alleviate poverty. At the same time they are affected by different factors such as grazing, fire, pest and invasive species. The total forest cover of the country as per Status of Forest Report 2017 is 7,08,273 km^2, which constitutes 21.54 percent of the geographic area of the country. Uttarakhand forest covers an area of 38,000 km^2 which is 71.05% of its geographical area. One of the fast growing tree species in forest ecosystem with great economic importance is *Dalbergia sissoo* which is a multipurpose tree, eco-friendly, widely used for afforestation and of socio-economic importance **(Troup, 1921)**. Khair-sissoo forests cover an area of 242.35 km^2which contributes 0.98% to total area of Uttarakhand forest coverage (Status of forest report, 2017). A survey by the Forest Research Institute (FRI), Dehradun have found that the mortality of shisham has caused a loss of more than Rs 800 -1,000 crore in the Indian sub-continent. Nearly 8 to 10 lakh trees have wilted so far causing a huge loss in India, Bangladesh and Nepal **(Business Standard, 2008)**. These forests are an important source of high quality timber and other resources with great economic importance due to which problem of mortality faced by this tree species needs to be solved.

2.2 Shisham tree system

Dalbergia sissoo Roxb. belongs to family Fabaceae. It is also known as North Indian Rosewood. It is a fairly large deciduous, nitrogen fixing tree species, a native of Indian sub-continent and Iran. It is commonly known as sisu, shisham, tahli, tali and Irugudujava in India, shewa in Afghanistan and Jag in Iran. This species is generally distributed in the sub-Himalayan tract upto 1200 meters of altitude **(Revathi *et al.*, 2013).** It can tolerate temperature range of 4–45°C, pH in the range of 5-7.7; withstand average annual rainfall up to 2,000 mm and a dry spell of 3–4 months. *D. sissoo* is a pioneer species generally found as a pure patch along the banks of the rivers, stream

and loose alluvial grounds. However, growth is slow in poorly aerated sites, such as those with heavy clay soils. It has tap root system. Seedlings are intolerant of shade.

Flowering in the *D. sissoo* starts profusely within 9 months. The small bisexual flowers are borne on small branches from the leaf axis. There is less information about its breeding and pollination biology. This species is either self or insect pollinated. Leaf flushing is followed by flowering. As mature leaves fall, young flower buds appear along with new leaves. There is pod formation at maturity. Mature pods remain attached to the tree for 7-8 months. The pods are dispersed by wind and water. The reproduction is by seeds, nodal segments **(Datta and Datta, 1983)** or axillary buds **(Dwara *et al.,* 1984)**. Of these micropropagation by nodal segments or axillary buds is preferred for generating planting material. It regenerates where sunlight and moisture are plentiful.

2.3 Shisham mortality

Dalbergia sissoo is best known internationally as a premier timber species. It is also a most planted trees species in India because of its highly valued timber. It is commonly used in rural medicine and preparation of agricultural implements **(Bhattacharya *et al.,* 2014)**. Since 1950s, the natural forests and plantations of shisham in almost all the countries where it is grown are facing large-scale mortality. Tree mortality can be initiated by two processes either endogenic (senescence and intraspecific competition) or exogenic (natural, abiotic and biotic). In recent years, the problem of shisham mortality has been reported from many parts of the country. Slowly this problem reached very dangerous dimensions and the problem spread over many states of India and even in other countries of Southeast Asia.

2.4 CAUSES OF SHISHAM DECLINE

2.4.1 Biotic and Abiotic Factors

Amongst various factors, dieback disease is responsible for the mortality of about 70% of shisham trees **(Khan *et al.,* 1999)** in both natural forests and plantations. Biotic and abiotic factors both are involved in mortality of *Dalbergia sissoo* trees. Mycologists have reported 62 pathogenic species of fungi associated with mortality of shisham. Out of these sixty two species only few have been studied on their pathological aspects in detail. However, after the recent epidemic of shisham decline diseases, many pathologists

conducted studies to investigate for the cause of menace. Soil borne phytopathogens are reported as primary cause of dieback in shisham trees. The major pathogenic microorganisms are *Fusarium*, *Ganoderma*, *Phellinus* and *Meloidogyne*. The root system in fungi infected trees is completely destroyed. The leaves become yellow and later on turn brown. The, twigs start dying from tip progressing downwards. Under severe conditions, the branches start drying one after the other causing the death of the entire tree (**Pathan *et al*., 2007**). **Tantau *et al*. (2005)** identified bacterial isolates of genera *Bacillus* and *Pseudomonas* in die-back affected shisham trees in Bangladesh. Many abiotic stress factors are also known to be responsible for the initiation and severity of shisham decline diseases. Highest mortality percentage of 75–80% was reported along the canal banks due to high moisture contents causing waterlogging of soil (**Bajwa *et al*., 2003**). The high soil moisture level increases the severity of this disease. Additionally, abiotic factors such as non-judicious irrigation schedules, imbalances in soil physical properties (soil, air, moisture, texture etc.) and nutrient deficiencies can also cause large-scale mortality. The abiotic factors like EI Nino southern oscillation also favours spread of the disease **(Collins *et al*., 2010).**

2.5 Soil Physicochemical Properties

Foresters take into consideration chemical and physical properties of soils to assess the capacity of sites to support productive forests. Many soil chemical properties (e.g. via nutrient and carbon supply) directly influence microbiological processes and these processes, together with soil physical-chemical attributes determine (1) the capacity of soils to hold, supply, and cycle nutrients (including carbon), and (2) the movement and availability of water. Soil organic matter (SOM) or organic carbon (SOC) is considered an important attribute of soil health because of its relation to soil physical, chemical as well as biological properties **(Reeves, 1997).** It is commonly recognized as one of the key chemical parameters of soil quality. Through its role in aggregate stability, it influences soil porosity, and thus gas exchange reactions and water relations. Many chemical reactions that influence nutrient availability (e.g. chemical form, adsorption, precipitation) are affected by soil pH. Hence, soil pH provides little direct information as to which soil process is critically affected by it and in turn, critically affects the productive capacity of a soil. Another most significant soil property is soil texture which monitors water and nutrient retention, oxygen exchange and uptake. Electrical

conductivity is a measure of ion concentration and nutrient imbalances (Na dominance in sodic soils). In forest soils, it usually help identify, the sites where highly concentrated soil solutions are known or suspected to inhibit forest growth and productivity and subsequently in reclamation of such soils **(Burger *et al.,* 1994)**.

2.6 Soil Enzyme Activity: An Indicator of Soil health

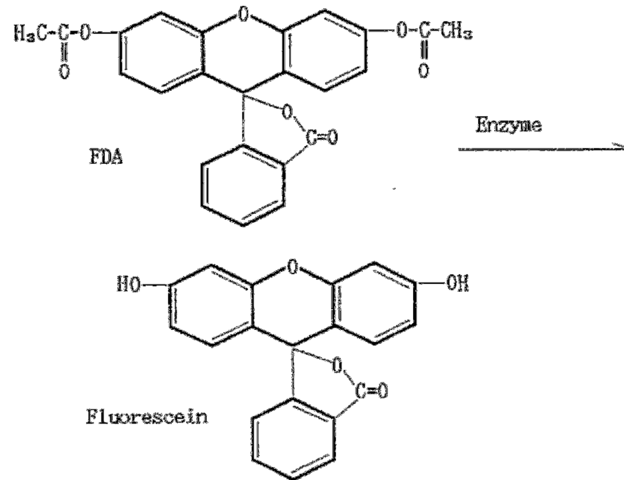
Soil microorganisms are key drivers of nutrient cycling. They are involved in a wide range of biological processes including the transformation of insoluble soil nutrients **(Babalola and Glick, 2012)**. Soil microbes are intimately associated with decomposition of organic matter within ecosystems and hence make nutrient available for plant uptake. Some are capable of solubilizing and mineralizing phosphate and fix nitrogen. Rhizospheric and bulk soil microbial communities are dynamic. The changes in microbial community structure are root exudate mediated **(Nelson and Mele, 2007)**. Different forms of carbon inputs such as sugars, amino acids, and organic acids constitute rhizodeposits and act as substrates to soil microbial communities. Variation in rhizodeposition is considered to be an important driver of microbial community development which in turn determines soil enzyme activities. Soil enzyme activities can be used as an evaluation index of microbial activity and soil fertility **(Monreal and Bergstrom, 2000).** They are indicators of the presence of a particular type of microbial community in the soil. Levels of soil microbial enzymes are significantly related to total nitrogen, phosphorus and organic carbon in soils **(Aon and Colaneri, 2001)**. Since microbial enzymes; phosphatase and urease play an important role in the cycling of phosphorus and nitrogen **(Garcia *et al.*, 2002)**, microbiological processes in soils **(Caldwell, 2005)**, their activity can be used as an indicator of the soil health **(Dick, 1997)**. Among all enzymes, FDA, dehydrogenase, phosphatases and urease are the most frequently evaluated soil enzymes **(Burns *et al.*, 2013)** as they are indicators of microbial population (FDA), microbial respiration (dehydrogenase), phosphorus cycle (phosphatases) and nitrogen cycle (urease) **(Aon and Colaneri, 2001)**. Biological processes are necessary for maintaining the capacity of a soil to recycle different minerals nutrients **(Schjønning *et al.*, 2004)**. Recycling of nutrients is crucial for the continuation of photosynthesis and nutrient mineralization for plants and microbes. Thus, healthy soils have the capacity to keep all the biological processes working in a sustainable way. Microbial indicators are sensitive to changes in soil environment **(Masto *et al.*, 2009)**, and hence provide an early forecast of any change or disturbance.

Table 2.1: Soil enzymes that play significant role in maintaining soil health

Sr.No.	ENZYME	ROLE IN SOIL HEALTH	REFERENCE
1.	FDA	Hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases, (among the major decomposers, bacteria and fungi). Provides an estimation on the whole microbial activity in an environmental sample	**Schnuerer and Rosswall, 1982**
2.	Dehydrogenase	Microbial respiration, integral part of intact cells but does not accumulate extracellularly in the soil	**Casida *et al*., 1964; Tabatabai, 1982; Trevors, 1984**
3.	Phosphatase	P cycles	**Eivazi and Tabatabai, 1977; Dick *et al*. 2000**
4.	Urease	Nitrogen cycle, released by microorganisms and plants, extracellular	**Rotini, 1935; Alef and Nannpieri, 1998**

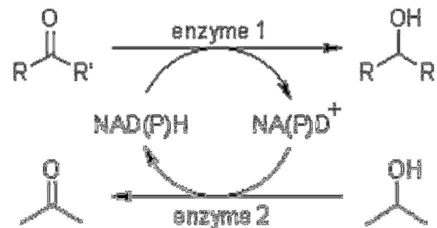
2.6.1 Fluorescein diacetate hydrolysis

Fluorescein diacetate is a substrate to three major enzymes (protease, esterase, and lipase) and hence its hydrolysis act as broad spectrum indicator of soil health. The amount of fluorescein released after the hydrolysis of fluorescein diacetate (FDA) was directly proportional to the microbial population **(Swisher and Carroll, 1980; Schnurer and Rosswall, 1982)**. Fluorescein diacetate is a colorless compound hydrolyzed by, both free (exoenzymes) and membrane-bound enzymes **(Stubberfield and Shaw, 1990)** and releases, fluorescein, a colored product.



2.6.2 Dehydrogenase

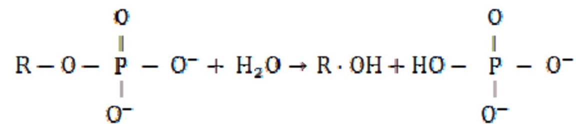
It is a broad spectrum enzyme (oxidoreductase), a constituent of live organisms and responsible for microbial respiration in soil **(Trevors, 1984)**. Dehydrogenases participate in respiration and cause biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors **(Sebiomo *et al.*, 2011)**. Hence, are very important for soil health.



2.6.3 Phosphatases

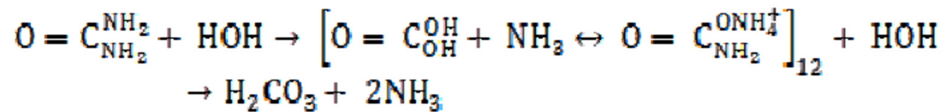
Phosphatases are a broad group of enzymes that are able of catalyzing the hydrolysis of esters and anhydrides of phosphoric acid. They are of two types; alkaline and acid phosphatases. In soil ecosystems, these enzymes play important roles in P cycles **(Speir and Ross, 1978)** act as good indicators of soil fertility **(Eivazi and Tabatabai, 1977; Dick *et al.*, 2000)**. In case of P deficiency in the soil, acid phosphatase is secreted from plant roots to enhance the solubilization and remobilization of phosphate, thus allowing the plant to cope with P-stressed conditions **(Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Versaw and Harrison, 2002)**. Alkaline phosphatases act by removing phosphate groups. The enzyme activities in soil

ecosystems regulate nutrient uptake and plant growth hence the studies of their dynamics are crucial and imminent. Legumes secrete more phosphatase enzymes than cereals (**Yadav and Tarafdar, 2001**). This may be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. **Li and co-workers (2004)** reported that chickpea roots also secrete a greater amount of acid phosphatase than maize. The activity of acid and alkaline phosphatase could be correlated with organic matter of the soil (**Aonan and Colaneri, 2001).** Soil pH influences the rate of production, release, and stability of this enzyme.



2.6.4 Urease

Enzyme urease is responsible for the hydrolytic conversion of urea into carbon dioxide and ammonia. Owing to this property, it has an applied importance in the N-economy of soil and is closely associated with the transformation, biological turnover and bioavailability of nitrogen (**Liang *et al.,* 2003**). Urease activity is also an important factor for survival of ammonium oxidizing bacteria in forest and agricultural soils (**Swensen and Bakken, 1998**).



2.7 Role of microorganisms in forest ecosystem

Forest microorganisms play an important role as decomposers, symbionts, or pathogens, influencing the C turnover and retention and the availability of other nutrients (**Trivedi *et al*., 2013**). Microbial communities mediate biogeochemical cycles, and an understanding of their role in ecosystem processes is essential for the prediction of the forest response to future environmental conditions (**Graham *et al*., 2016).** The ability of fungi to produce a wide range of extracellular enzymes efficiently degrading dead plant biomass make them as essential decomposers in forest soils (**Eichlerova *et al*., 2015**). Moreover, mycorrhizal fungi play a pivotal role in nutrient acquisition and significant transport of carbon in forest soil (**van der Heijden *et al*., 2015**). Bacteria

represent another important, though less explored, integral part of the microbial community in forest soils. Forest soils are among the most diverse microbial habitats on Earth, with bacteria the most abundant group of microorganisms (**Nacke *et al*., 2012**). The root exudation by trees or mycorrhizal hyphae enhances the carbon availability in the rhizosphere consequently affecting the microbial abundance and extracellular enzyme activity as compared to the bulk soils (**Collignon *et al*., 2011**).Five major phyla, *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* are reported to be abundant in most soils (**Lauber *et al.,* 2009**). Bacteria are the major natural agents responsible for nitrogen fixation and other ecosystem processes such as mineral weathering leading to the release of various inorganic nutrients (**Uroz *et al*., 2011**). In addition to pH, several other factors such as organic matter content, biotic interactions (especially the effect of vegetation), climate conditions, and nutrient availability, seems to be most important driver of bacterial community composition in soils (**Lauber *et al*., 2009**). Spatial variation of these factors are mainly responsible for the presence of hot spots with increased abundance and activity around plant roots (**Kuzyakov *et al.,* 2015**). Type of root exudates and composition of microbial communities vary with plant species (**Nguyen, 2003**), while coexistence of multiple plant species may result in complex of soil microbial communities due to increased heterogeneity of root exudates (**Stephan *et al.,* 2000**). Knowledge of rhizosphere effects on nutrient cycling associated with different species is fundamental for characterizing nutrient acquisition of different tree species and for interpreting the influence of tree species on soil processes. It is well established that microbial abundance, composition and diversity can fundamentally alter soil processes that lead to changes in soil nutrient availability. Therefore, there is a strong need for more studies on rhizosphere soil microbial diversities and abundance.

2.8 Culturable approach for microbial community analysis

Microbial communities are a direct indicator of soil health (**Torsvik and Øvreas, 2002; Islam *et al*., 2011**). Till date studies on microbial communities are limited as the 99% of the microbes present in soil is uncultivable (**Lakay *et al*., 2007**). There are many traditional techniques available to trap this hidden treasure. The most traditional method for assessment of microbial diversity is total viable count on selective and differential media. The above methods are inexpensive and provide information about active and cultivable heterotrophic microbial population. However,

this has its own drawbacks viz., is time- consuming, has low sensitivity towards less abundant microorganisms and too selective for fastidious and slow growing organisms**.** Hence, these techniques are inefficient to draw a complete picture of microbial communities residing in an environment. These methods are biased towards fast growing bacteria and spore forming fungi **(Dix and Webster, 1995)**. The major limitations of these methods comprise the difficulties in isolation of bacteria or spores from soil particles or biofilms, selecting specific growth media **(Tabacchioni *et al*., 2000)**, and specific growth conditions (pH, temperature, light). In conclusion, cultural methods cannot completely reflect the total diversity of a microbial community.

2.8.1 Disadvantages of culturable approach

2.8.1.1 Spatial Heterogenity

One of the problem associated with microbial diversity studies is spatial distribution of the microorganisms (**Trevors, 1998b**). To overcome, sampling should be done using multiple spatial scales and sampling intervals ranging from 2.5 cm to 11 m (**Franklin and Mills, 2003**). According to **Grundmann and Gourbiere** (**1999**) soil sampling must be done on a smaller scale and more number of samples collected for analyzing diversity of microorganisms within the microhabitats in soil. Another problem with this approach is that soil is not uniform and heterogeneous. Soil contains many hot spots or microhabitats where bacteria and fungi are highly aggregated in soil. For example rhizosphere region of soil shows two-fold increase in bacterial numbers over bulk part of soil (**Curl and Truelove, 1986**). Conventional approaches used currently are found to be inaccurate, and they hardly indicate comprehensive profile of microbial diversity in soil.

2.9 Culture independent approach

As only 0.1-1% microbe can be recovered and characterised using culture based techniques **(Amann *et al*., 1995),** Culture-independent techniques were employed for characterization and exploitation of microbes in an environment. Almost four decades ago, retrieval of 16S rRNA genes was pioneered (**Olsen *et al.*, 1986**). Since then, the culture independent molecular techniques have been used to study bacterial communities **(Huang *et al*., 2006)**. The polymerase chain reaction (PCR) fringerprinting techniques have revolutionized the characterization of microbial communities.

Molecular techniques enable the differentiation of microbial species and sub-species. Techniques like denaturing gradient gel electrophoresis (DGGE) and fatty acid signatures have brought advancement in our knowledge regarding diversity and composition of soil microbial communities (**Helgason *et al*., 2010**). Significant advances are now possible with DNA-sequencing analyses, with emphasis on metagenomics (**Delmont *et al*., 2012**). Metagenomic approaches also known as cultivation-independent environmental genomics (recovering genetic material directly from environmental sample) promise unprecedented access to community as a whole. These latest methodologies allow rapid and accurate access to microbial diversity and thus facilitate the discovery of new groups of microorganisms (**Hugenholtz, 1998**). The next-generation sequencing (NGS) platforms such as pyrosequencing and Illumina-based sequencing can shed light into the complexities of microbial populations in real time (**Bartram *et al*., 2011**). Next-generation sequencing strategies are high throughput as they can effectively provide deep insights into the complex microbial communities of ecological niches (**Fakruddin and Mannan, 2012**).

Various other techniques for fingerprinting analysis of microbial communities are temperature gradient gel electrophoresis **(Fouratt *et al*., 2003),** terminal-restriction fragment length polymorphism (RFLP) **(Mintie *et al*., 2003),** and single-strand conformation polymorphism **(Bäckman *et al.,* 2003).** Various advantages and disadvantages associated with these techniques are mentioned in **Table 2.2**.

Table 2.2: Advantages and disadvantages of some molecular-based methods to study soil microbial diversity (Kirk *et al.*, 2004)

Method	Advantages	Disadvantages
Mol% Guanine plus Cytosine (G+C)	Not influenced by Polymerase Chain Reaction (PCR) biases Includes all DNA extracted Quantitative Includes rare members of community	Requires large quantities of DNA Dependent on lysing and extraction efficiency Coarse level of resolution
Nucleic acid re-association and hybridization	Total DNA extracted Not influenced by PCR biases Can study DNA or RNA Can be studied *in situ*	Lack of sensitivity Sequences need to be in high copy number for detection Dependent on lysing and extraction efficiency

DNA microarrays and DNA hybridization	Same as nucleic acid hybridization Thousands of genes can be analyzed If using genes or DNA fragments, increased specificity	Only detect the most abundant species Need to culture organisms Only accurate in low diversity systems
Denaturing and Temperature Gradient Gel Electrophoresis (DGGE and TGGE)	Large number of samples can be analyzed simultaneously Reliable, reproducible and rapid	PCR biases Dependent on lysing and extraction efficiency Way of sample handling can influence community, *i.e.* the community can change if stored too long before extraction One band can represent more than one species (co-migration) Only detects dominant species
Single Strand Conformation Polymorphism (SSCP)	Same as DGGE/TGGE No GC clamp No gradient	PCR biases Some ssDNA can form more than one stable conformation
Restriction Fragment Length Polymorphism (RFLP)	Detect structural changes in microbial community	PCR biases Banding patterns often too complex
Terminal Restriction Fragment Length Polymorphism (T-RFLP)	Simpler banding patterns than RFLP Can be automated large number of samples Highly reproducible Ability to compare differences between microbial communities	Dependent on extraction and lysing efficiency PCR biases Type of *Taq* can increase variability Choice of restriction enzymes will influence community fingerprint
Ribosomal Intergenic Spacer Analysis (RISA)/Automated Ribosomal Intergenic Spacer Analysis (ARISA)/ Amplified Ribosomal DNA Restriction Analysis (ARDRA)	Highly reproducible community profiles	Requires large quantities of DNA (for RISA) PCR biases

2.9.1 Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (q-PCR) is a method by which the amount of the PCR product can be determined, in real-time, and is very useful for enumerating microorganisms, and investigating gene expression **(Labrenz *et al.,* 2004)**. Often abbreviated to q-PCR, this method does not rely on any downstream analysis such as electrophoresis or densitometry and is extremely versatile, enabling multiple PCR targets to be assessed simultaneously.

The increase in fluorescent signal during the exponential phase of reaction is directly proportional to the amount of PCR products generated. The intensity of the reporter dye emission is monitored and the cycle number at which fluorescence signals reach to the threshold is recorded and referred as threshold cycle (Ct). The plot of fluorescence against cycle number is then generated by the real-time PCR instrument. Two major types of quantification achieved through the q-PCR are absolute and relative quantification. Absolute quantification is achieved by establishing a standard curve based on known amounts of the target template, thus allowing the determination of the concentration of the unknown samples. The quantification of bacteria in real time is extensively used in the disease diagnosis to determine bacterial or viral load. It is also extensively used in the microbial ecology to determine the abundance of the desired group of microorganisms in the environmental samples.

Several studies have used 16S rDNA q-PCR for determining the bacterial load in the soil ecosystems **(Kleyer *et al.,* 2017; De-Smets *et al.,* 2016)**. Moreover, it has been also used for the quantification of target taxa. For example; *Staphylococcus aureus*, *Bacillus anthracis* **(Makino and Cheun, 2003)** and *Legionella* spp. **(Wellinghausen *et al*., 2001)** are quantified using genus-specific target genes.

2.9.2 Metagenomics

"Metagenomics" is defined as the functional and sequence-based analysis of the collective microbial genomes present in an environmental sample. It allows the study of micro-organisms in their natural environments, without isolation and lab cultivation of individual species **(DeLong, 2002).** This has enabled the study of uncultivable microorganisms; furthermore, it provides system-level insights into the composition, structure, and functioning of microbial communities in various environments

(Warnecke *et al*., 2007) including soil, fresh and marine aquatic habitats, faeces, oral cavity, rumen, glacier ice and cold deserts **(Riesenfeld *et al*., 2004; Xu and Gunsolley, 2014; Kodzius and Gojobori, 2015; Kolte *et al*., 2017)**. Thus metagenomics, has enormous potential in microbial diversity analysis.

Soil is one of the most challenging systems for microbiologists in term of microbial diversity and community size. The soil microbial communities might be an almost unlimited resource of new genes encoding useful products. Understanding the heterogeneity, temporal and spatial dynamics of complex soil microbial communities in soil system is still one of the major challenges in soil metagenomics. Prokaryotes are the most abundant component of the soil biomass.

There are various NGS sequencing platforms each with their own advantages and disadvantages. They are Roche 454 sequencing, Ion Torrent, Pacific Biosciences, SOLiD and Illumina MiSeq. Although 454 (Roche) pyrosequencing had been widely utilized in soil microbial diversity but low sequencing cost and high depth coverage, Illumina sequencing technology is preferred now (**Caporaso *et al*., 2011**). Moreover, sample throughput is enhanced using bar coding which provide a pre-eminent view of microbial composition than in case with other sequencing technologies (**Caporaso *et al*., 2012)**. High taxonomic resolution of Illumina platform helps in identifying rare microbes in different environmental habitats. Several soil bacterial diversity studies have been carried out through Illumina Miseq **(Hong *et al*., 2015)**. Major steps used in the Illumina sequencing are library preparation, cluster generation, sequencing and data analysis. For bacterial community analysis 16S rDNA is amplified with the primers having the adapter at both 5' and 3' ends. Thereafter cluster formation is achieved through the bridge PCR. In sequencing reversible terminator based method is used which detects single bases as they are incorporated into DNA template strands. Thereafter data can be analysed through the different software packages based on the type of analysis required. This DNA-based analysis technique allows simultaneous screening of a large number of microbes in multiple environment samples. Many large-scale metagenomics projects have been undertaken to investigate various aspects of the microbial composition, *e.g.* Human Microbiome Project (http://commonfund.nih.gov/hmp), International Census of Marine Microbes (http://icomm.mbl.edu), and Earth Microbiome Project (http://www.earthmicrobiome.org) for bacterial diversity analysis, thousands of 16S rRNA sequence datasets have been generated through these

community efforts as well as individual projects. An accurate taxonomic assignment of each microbe in a target environmental habitat is needed to evaluate the biodiversity, the structure, the richness, and the role of the residing community in a given environment (**De Filippo *et al.*, 2012**).Therefore, there is a critical need to develop and evaluate efficient and accurate computational algorithms to analyze massive data collected from various biological and ecological environments. Two approaches are commonly used to characterize microbial communities through 16S rRNA sequences: taxonomy-dependent methods and OTU-based methods (**Cai *et al.,* 2011**). The OTU-based methods are especially useful in analyzing less characterized microbial communities

Huge amount of high throughput metagenomic data produced is analyzed through application of bioinformatic tools. One such software is QIIME (Quantitative insights into microbial ecology) that is an open-source software pipe line for interpretation of raw sequence data (http://qiime.sourceforge. net/) and its visualization as network analysis, histograms of within- or between-sample diversity and analysis of whether 'core' sets of organisms are consistently represented in certain habitats (**Knight *et al*., 2007**). QIIME also performs various functions such as choosing operational taxonomic units (OTUs), sequence alignment, inferring phylogenetic trees and taxon-based analysis of diversity within and between samples.

2.10 Plant Growth Promoting Rhizobacteria

The rhizosphere, that is, the narrow zone surrounding and influenced by plant roots, is one of the most complex ecosystems on Earth (**Raaijmakers *et al*., 2009**). It is a hot spot for organism activity, population and diversity. Organisms found in the rhizosphere include bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods. Of these, micro-organisms are most abundant. They are beneficial as well as deleterious. The beneficial bacteria promote plant growth and health. They include nitrogen-fixing bacteria, mycorrhizal fungi, biocontrol microorganisms, mycoparasitic fungi, and protozoa. Different bacterial genera are vital components of soils. Among them crucial are the plant growth promotory rhizobacteria, which helps in plant growth enhancement through various mechanisms. These include potential to produce different plant growth regulators such as indole acetic acid (IAA), gibberellins and cytokinins (**Marques *et al*., 2010),** improving efficiency of asymbiotic nitrogen fixation **(Khan, 2005),** solubilization of inorganic phosphate and

mineralization of organic phosphate, and several other nutrients **(Jeon *et al*., 2003)**, antagonistic activity against phytopathogens through siderophore production, antibiotic production, synthesis of enzymes and fungicidal compounds and competition with detrimental microorganisms **(Lucy *et al.,* 2004)**. The dominant bacterial isolates belong to genera *Bacillus*, *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Burkholderia*, *Arthrobacter* and *Paenibacillus* **(Zhang *et al.,* 2017).** The rhizobacteria are more versatile in mobilizing, transforming, solubilizing the nutrients in comparison to those from bulk soils. Rhizobacteria are dominantly responsible for recycling the soil nutrients and thus are important for soil fertility (**Glick, 2012**). Therefore it is very essential to characterize *Dalbergia sissoo* rhizosphere micro-organisms. Rhizosphere micro-organisms are affected by climate change

2.10.1 Phosphate solubilizers

Soil is a dynamic system and an ecological niche of constant biological activity, influenced to a great extent by its chemical constituents. N, P and K are major macronutrients limiting the productivity of forest (**Lodhiyal *et al*., 1994**). Phosphorus (P) is an essential plant nutrient and plays a key role in plant growth and development Phosphorus has a specific role in plant cellular metabolism. Phosphorus is the constituent of nucleotides which is one of the building blocks of DNA, hence takes part in transfer of genetic features from one to another generation. It helps in energy storage and energy transfer, as it facilitates sugar breakdown and translocation of nutrient within the plant cell. Phosphorus is constituent of ATP. Therefore, it plays role in regulation of overall cellular metabolism **(Theodorou and Panxton, 1993)**. Adequate P levels encourage vigorous root and shoot growth, promote early maturity, increase water use efficiency and grain yield. The lack of phosphorus limits plant growth by disrupting cell division, photosynthesis, nutrient transport, regulation of metabolic pathways within the plant, and the transfer of genetic characteristics from one generation to another **(Armstrong, 1988)**. Microorganisms play a pivotal role in the phosphorus cycle. On average, soil contains 400–1000 mg kg^{-1} of total P, of which only 1.00–2.50% is available for plant uptake **(Chen *et al*., 2008)**. The insoluble P is present as an inorganic mineral such as apatite or as one of several organic forms including phytate, phosphotriesters and phosphomonesters (**Bagyaraj *et al.,* 2015).** Plants absorb phosphorous only in two forms, the monobasic ($H_2PO_4^-$) and the diabasic (HPO_4^{-2}) ions **(Bagyaraj *et al.,* 2015).** Apart from chemical fertilization, microbial P-solubilization

and mineralization is the only possible way to increase plant available phosphorus. Mineral P in the soil may become unavailable through fixation or adsorption in clay soil **(Halajnia *et al.*, 2009)**. Mineral P may also become unavailable because of precipitation reactions with cations such as Ca-P and Mg-P in alkaline soil or Fe-P and Al-P in acidic soil **(Bayer *et al.*, 2001)**. As a result, the concentration of mineral P in the soil solution rarely exceeds 0.1 $mg\,kg^{-1}$. Most of organic P (20 to 80%) is inert. Plant P uptake can be improved by enhancing P solubility or decreasing P fixation in soil. Most of the phosphate fertilizers applied in agricultural fields to combat the P deficiency are rapidly converted into insoluble complexes in the soil**.** Thus, constant and long term application of phosphate fertilizers is detrimental to environment and unaffordable to the farmers of developing nations.

The availability of soil 'P' is influenced by a number of factors such as nature and content of clay, active ses-quioxides, lime, pH and organic matter **(Selvi *et al.,* 2011).** In most tropical soils, phosphate is predominantly present in the inorganic form of 'P' which belongs to two groups-calcium compounds and iron-aluminium compounds. The calcium is predominant under neutral to alkaline soil condition, while iron and aluminium phosphates are predominant under acidic condition **(Selvi *et al.,* 2011).**

One of the most promising cost-effective and sustainable strategy to increase phosphorous absorption efficiency is the use of phosphorous solubilizing bacteria (PSB). These bacteria solubilize the complex phosphate compounds present in soil into the simpler readily absorbable form. Thus providing sustainable source of phosphorous to the crops. Hence, phosphrous fertilizer input is reduced. PSBs act as component of phosphorus cycle **(Fig. 2.1)**, release phosphorus from insoluble sources by different mechanisms. PSB may act by decreasing the pH of the soil, producing organic (gluconic acid) and mineral acids **(Chen *et al.*, 2006)**, alkaline phosphatases **(Rodriguez *et al.*, 1999)**, production of phytohormones and H^+ protonation **(Xiao *et al.*, 2017)**, anion exchange, chelation and siderophores production which promote P solubilization in soil **(Sugihara *et al.*, 2010)**. The ability of PSBs to release soluble ortho-phosphate (Pi) from rock phosphate holds potential importance for the development of phosphate fertilizer technologies. The bacterial isolates belonging to genus *Pseudomonas, Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium and Sinorhizobium* are effective P solubilizers **(Sharma *et al.*, 2013)**. Most significant phosphate solubilizing bacterial genera reported are *Azotobacter, Bacillus, Beijerinckia,*

Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Microbacterium, Serratia, Rhizobium, Bradyrhizobium, Salmonella, Alcaligens, Chromobacterium, Arthrobacter, Streptomyces, Thiobacillus and Escherichea etc. **(Zhu *et al.,* 2011).** Exploitation of PSBs may reduce addition of inorganic P into soil causing substantial reduction in production cost and environmental damage **(Rajan *et al.*, 1996)** and improve soil health.

There are reports of significant improvement in P availability for plants through PSB inoculation. The use of PSB also enhances seed germination, improves seedling emergence, increase seedling resistance to abiotic stress, prevent plants from disease **(Lugtenberg *et al.,* 2002).** The PSB can also enhance the plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc etc. (**Ponmurugan and Gopi, 2006**). Availability of phosphorus also regulates biological nitrogen fixation (**Mills *et al.*, 2004).** In agricultural ecosystems, biologically fixed N is due to symbiotic association of rhizobia in legume root nodules **(Soltis *et al.*, 1995)** or to free living diazotrophic microbes **(Bellenger *et al.*, 2014)**.

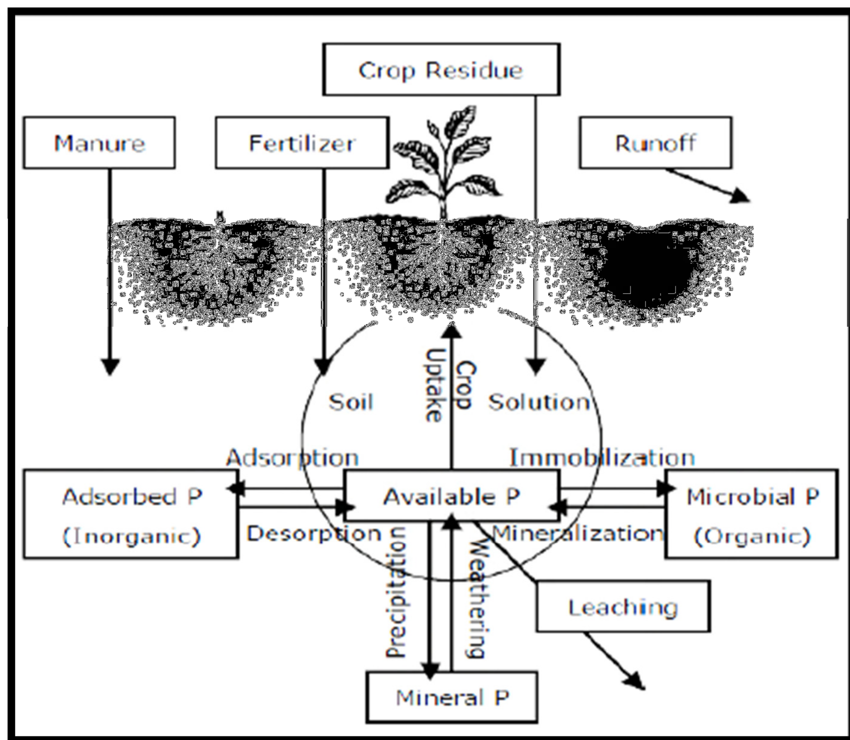


Fig. 2.1: Phosphorus cycle in soil (Hyland *et al.,* 2005)

Mechanism of ‘P’ solubilization-

(A) Organic:

Three enzyme groups are involved in liberation of P from organic compounds.:-

1. Non-specific phosphatases:- This enzyme performs de-phosphorylation of phospho-ester or phospho- anhydride bonds in organic phosphatic compounds.
2. Phytases:- It liberates P from phytic acid.
3. Phosphonatases and C-P lyases enzymes:- causes cleavage of C-P bonds in organophosphate compounds.

(B) Inorganic:

Two theories have been proposed to describe the mechanism of inorganic phosphate solubilization. e.g.:-

1. Acid production theory
2. Proton and enzyme theory

As per acid production theory, phosphate solubilization by PSM takes place by the synthesis of various organic acids such as malic, succinic, glyoxalic, fumaric, α-keto butyric, tartaric, citric, oxalic, 2-keto gluconic and gluconic acid. Release of organic acid leads to acidification of medium **(Puente *et al.,* 2004)** and lowering of pH in culture filtrates **(Singh *et al.,* 2012; Rani *et al.,* 2013).** The amount and type of the organic acid produced varied with the microorganism. The organic acid released in culture filtrate reacts with the insoluble phosphate **(Ahmed and Kibret, 2014).**

According to proton and enzyme theory, esterase type enzymes are presumed to be involved in liberating phosphorus from organic phosphatic compounds. PSMs (phosphate solubilizing microorganisms) are also known to produce phosphatase enzyme along with acids leading to solubilization of P **(Alghazali *et al.,* 1986).**

Factors affecting phosphate solubilization activity of PSMs include, carbon and nitrogen sources in growth medium, availability of free P and salinity. The buffering capacity of soils could limit dissolution of soil phosphates by microorganisms. The variations in growth enhancement are attributed to the differences in the composition and properties of soils, the nature and distribution of soil microflora, and the type of plant **(Kucey *et al.,* 1989).**

The highest solubilization of 'P' was observed in the medium amended with glucose and maltose, followed by sucrose, xylose and galactose. The ammonium as a nitrogen source was better than nitrate for solubilization of phosphorous **(Asea, 1988).**

2.10.2 PSBs as biofertilizer

The interaction between MPS microbes and plants is expected to be of synergistic nature. The MPS microbes direct the release of Pi (inorganic phosphorus) for plant uptake **(Perez *et al*., 2007)**. The phosphate solubilizing microbes also facilitate growth through various other mechanisms **(Fig. 2.2) (Bardin *et al*., 1996; Wani *et al*., 2007; Mittal *et al*., 2008; Yandigeri *et al*., 2011).** The MPS microbes are useful either singly or in consortia as bioinoculants for promoting plant growth whilst keeping the soil health intact. Beneficial effects of the inoculation with PSM to many crop plants have been described by numerous authors **(Tomar *et al*., 1996; Antoun *et al*., 1998; Pal, 1998; Peix *et al*., 2001)**. Dry matter production, P uptake and P content were augmented significantly by the application of PSMs in many legume plants even under temperate conditions **(Singh *et al*., 2005; Chand and Singh, 2006)**. 12-15 per cent yield increase and replacement of 25-28 per cent of phosphate fertilizers was observed in cereals, legumes, potatoes and other field crops on the addition of rock phosphate and inoculation with PSMs **(Arun, 2007).** Use of PSMs can increase crop yields up to 70 per cent **(Verma *et al.,* 2013).** Combined inoculation of arbuscular mycorrhiza and PSB gave better uptake of both native and phosphatic P **(Goenadi *et al*., 2000; Cabello *et al*., 2005)**. Microorganisms with phosphate solubilizing potential enhanced the plant growth by improving biological nitrogen fixation **(Ponmurugan and Gopi, 2006),** seedling length of *Cicer arietinum* **(Sharma *et al*., 2007),** nodule number, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop **(Yazdani *et al*., 2009)**. Application of PSBs alongwith mycorrhizae **(Mehrvarz *et al.*, 2008)** and P fertilizers **(Afzal and Bano, 2008)** resulted in increased biological yield as compared to the sole inoculation of bacteria. Inoculation of Mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley **(Bartholdy *et al*., 2001)**, and *Bacillus megaterium* along with potential N2-fixer *Azotobacter* sp. induced resistance/tolerance against harmful effects of salinity (ranging from 3000-9000 μg/ml) besides significantly improving growth and yield in wheat. For example, increased growth and yield was observed, apple **(Aslantas**

***et al*., 2007**), walnut (**Xuan *et al.,* 2011),** soybean **(Fernandez *et al*., 2007**), sugar beet (**Sahin *et al*., 2004**), maize **(Hameeda *et al.,*2008),** chickpea (**Verma *et al*., 2013**), Wheat **(Shah *et al*., 2001),** peanut (**Taurian *et al.,* 2010**), rice, and tomato **(Walpola and Yoon,2013**).

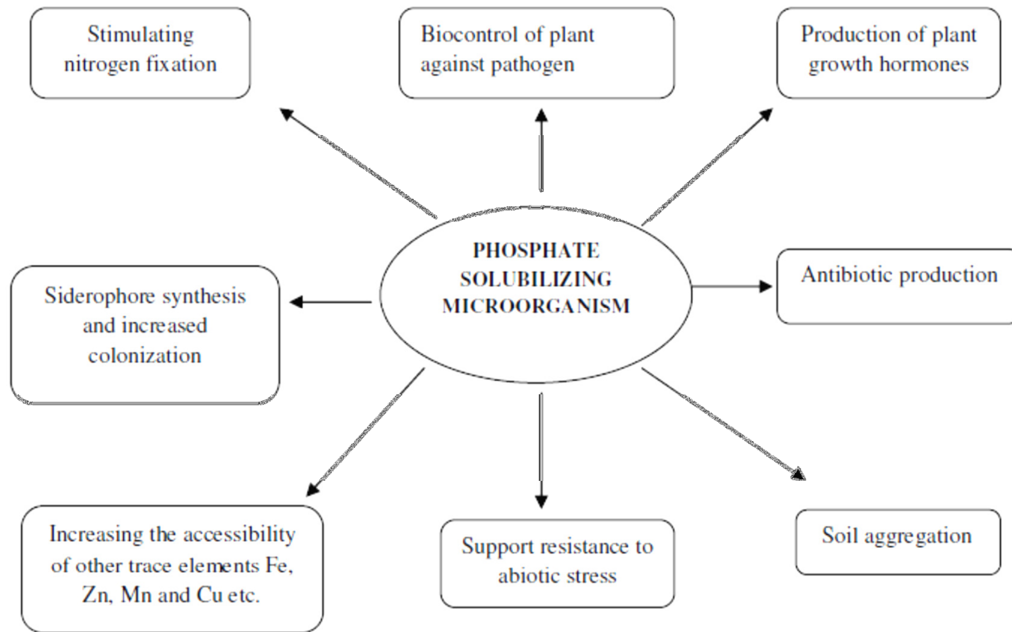


Fig. 2.2: Role of phosphate solubilizing bacteria in plant growth and development (Krishnaraj and Dahale, 2014)

2.10.3 Regulation of P solubilization at genetic level

Phosphate solubilizing microorganisms secrete various organic acids specially gluconic acid. Gluconic acid is synthesized by a mechanism involving direct oxidation of glucose through two key proteins, namely membrane-bound quinoprotein and glucose dehydrogenase (GDH) (**Kim *et al.,* 1997; Patel *et al.,* 2008**) **(Fig. 2.3).** GDH encoded by *gdh* gene requires pyrroloquinoline quinone (PQQ) as a cofactor. The PQQ production is regulated by a *pqq* operon that comprises six genes (*pqq*A, B, C, D, E, and F) in *Klebsiella pneumonia, Enterobacter intermedium* 60-2G and *Rahnella aquatilis* **(Kim *et al.,* 1998; 2003**). PQQ is essential for the formation of holo enzyme which leads to the production of gluconic acid from glucose. *Enterobacter intermedium* 60- 2G strains in which *pqq* genes are inactivated were unable to produce 2-ketogluconic acid (from oxidation of gluconic acid by GDH enzyme) and hence, solubilize hydroxyl-apatite

(Han *et al.,* 2008). Similarly MPS in *S. marcescens* CTM 50650 strain **(Farhat *et al.,* 2009)** is via activation of the direct oxidation pathway of glucose. PQQ serves as a cofactor for several bacterial dehydrogenase enzymes, for example, methanol dehydrogenase and glucose dehydrogenase **(Matushita and Adachi, 1993).** It is derived from two amino acids, tyrosine and glutamate, **(Puehringer *et al.,* 2008).** Genes involved in PQQ biosynthesis have been identified in many microorganisms, where they exist as part of the *pqq* operon. The number of *pqq* genes and the operon structure varies with the microorganism **(Gliese *et al.,* 2010).** The PQQ biosynthetic genes in *K. pneumoniae* are clustered in the operon *pqq*ABCDEF **(Meulenberg *et al.,* 1992),** while in *M. extorquens* AM1, in the *pqq*ABC/DE operon, where the two *pqq* genes, *pqq*C and *pqq*D, are fused, and the *pqq*F and *pqq*G genes form a separate operon with three other genes **(Zhangand Lidstrom, 2003)**. In *P. aeruginosa*, there are six pqq genes constituted as *pqq*ABCDE operon, and *pqq*F is located distal to the operon **(Gliese *et al.*, 2010).** Interestingly, the organization of the *pqq*A, *pqq*B, *pqq*C, *pqq*D, *pqq*E genes in the operon is highly conserved among these bacteria, while the *pqq*F and other potential pqq genes vary a lot **(Choi *et al.,* 2008)**. The functions of some proteins involved in PQQ biosynthesis have been characterized in a few bacteria, though the details of the complete PQQ biosynthetic pathway have not yet been resolved **(Andreeva *et al.,* 2011)**. The 24-amino acid, ribosomally produced, peptide PqqA serves as the precursor of the PQQ molecule **(Goosen *et al.*, 1989)**. *Pqq*B is not directly necessary for PQQ biosynthesis. Its role in *K. pneumoniae* is suggested to be a carrier that facilitates the secretion of PQQ across the plasma membrane into the periplasm **(Velterop *et al.*, 1995).** PqqC is reported to catalyze the last step in PQQ biosynthesis and its structure and mechanism has been well-characterized in *K. pneumoniae* **(Magnusson *et al.,* 2004).** PqqD is proposed to associate with PqqE, however, its function is still unknown. PqqE and PqqF encodes for a radical SAM (S-adenosylmethionine) enzyme with 2 Fe-S clusters, and a putative peptidase respectively **(Velterop *et al.,* 1995).** The functions of PqqE and PqqF are unknown as yet. Although function of *pqq* genes and Pqq proteins are well known, little is known about the mechanisms by which their expression is regulated **(Klinman and Bonnot, 2014)**. The *pqq*C gene from *Pseudomonas putida* KT2440, which is highly conserved in the pqq operon among pseudomonads, is preferentially activated in the pine rhizosphere with low phosphate availability **(Fernandez, *et al.,* 2013)**. **Babu- Khan *et al.* (1995)**

reported 396-base gabY open reading frame from *P. cepacia* and checked its expression in *E. coli* K-12 derivatives which synthesize apo- GDH but not its co-factor PQQ. He reported that in the presence of 1 mM exogenous PQQ, *E.coli* K-12 derivatives, JM109 (pSLY4) and JM109 (pGAB1) synthesizes 10 fold gluconic acid.The PQQ synthase gene from *Erwinia herbicola* was transferred to *Burkholderia cepacia* IS-16 and *Pseudomonas sp*. through broad host range vector pKT230 and plasmid pMCG898 **(Rodriguez *et al.*,2001).** Clones with recombinant plasmid produced higher insoluble phosphate as distinctive source. **Kim *et al.* (1997)** cloned 7 kb fragment from *Rhanella aquatilis* in *E. coli* HB101 and and observed solubilization of hydroxyapatite via GA production. Although *E. coli*¸ is unable to synthesize PQQ due to the lack of corresponding genes **(Matsushita *et al.*, 1997).** However, the heterologous expression of the pqq genes from other species in *E. coli* confer them phosphate-solubilizing activity, suggesting PQQ is an important phosphate-solubilizing factor.

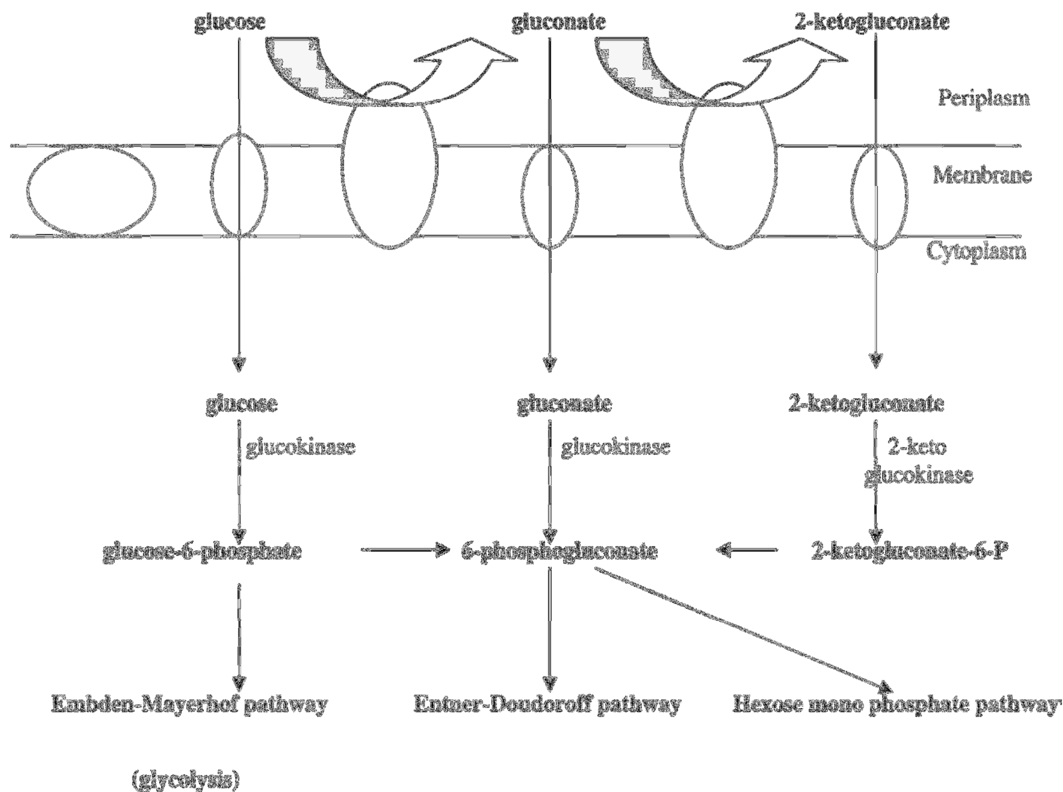


Fig. 2.3: Direct oxidation pathway involved in gluconic and 2-keto gluconic acid production during P solubilization by PSB (Krishnaraj and Dahale, 2014)

In addition to its known function as a cofactor of the GDH enzyme, PQQ is also considered as a vitamin for supporting cell growth and stress tolerance in bacteria and plants **(Si *et al.,* 2016).** Recent studies also reveal its function as a biocontrol agent for plant fungal pathogens **(Choi *et al.,* 2008)**. Therefore, the growing demands of PQQ in agricultural, medical and industrial areas require a continued exploration of highly efficient PQQ producing bacteria. Although PQQ is present in many plants, animals and microorganisms, but, can be synthesized by bacteria alone **(Goodwin and Anthony, 1998)**. The several bacterial strains in genera, *Acinetobacter, Ancylobacter, Gluconobacter, Hyphomicrobium, Klebsiella, Paracoccus, Polyporus, Pseudomonas, Methylobacillus, Methylophilus, Methylovorus, Methylobacterium, Mycobacterium, Thiobacillus, and Xanthobacter* have been identified as high PQQ-producers **(Xiong *et al.,* 2011)**. Interestingly, many high PQQ-producing bacteria are promising inorganic phosphate-solubilizers and have been inoculated alongwith inorganic phosphate fertilizers **(Si *et al.,* 2016).** Though the production of PQQ has been determined in many phosphate-solubilizing bacteria, the correlation between the PQQ producing capacity and their phosphate-solubilizing activity is still unclear and requires further investigation **(Goosen *et al*., 1989; Li *et al.,* 2015).**

Materials and Methods

This chapter includes the description of materials used, experimental procedure and techniques used during the period of experimentation.

3.1 Glass-wares, plastic-wares and Instruments used

All the glass-wares and plastic-wares used during course of study were purchased from Schott Duran, Borosil, Tarsons and Axygen. Various instruments used in this study have been listed in.

3.2 Chemicals and Kits

All chemicals and solvents used in the study were of molecular grade and purchased from standard manufactures *viz*; SRL Pvt. Ltd., Mumbai (India), HiMedia Laboratories Ltd., Mumbai (India), Merck India Ltd., Mumbai (India) and Sigma (USA) etc. Soil DNA isolation kit (Himedia, India) were used for the isolation of soil DNA, HiPurA™ PCR Product Purification Kit was used for the cleaning of the PCR product.

Media Used

(1) Nutrient Agar Medium

(2) Nutrient Broth

(3) King's B Broth

(4) NBRIP Media **(Nautiyal, 1999)**

(5) Pikovskaya's Medium **(Pikovskaya, 1948; Kumar *et al.,* 2017)**

(6) Angle's medium **(Angle *et al.,* 1991)**

Compositions of these media are shown in **Appendix I.** All the media used in this study were made in single distilled water and sterilized through autoclaving at specific period as described by the manufacturer **(Himedia Laboratories Ltd., Mumbai, India).**

3.3 Collection of Soil Samples

Rhizospheric soil samples were collected from natural shisham forests located at Pantnagar; (29° 3’ 0’’ N latitude & 79° 31’ 0’’ E longitude) , Lachhiwala ; (30.2099° N latitude, 78.1342° E longitude) and Tanakpur ;(29.0722° N latitude, 80.1066° E longitude) in Uttarakhand during Dec, 2016. Five quadrants of $10 \times 10 m^2$ were laid down randomly at all the locations to find out the percent mortality. Further, three trees of each forest location were selected within a distance of 1 to 10 m from each other. For each individual tree, the rhizosphere soil were sampled vertically along the base of the plant. Finally, samples of each tree in triplicate were mixed to homogeneity to generate a representative composite sample for further analysis. The homogenized rhizosphere soil samples were kept in sterile plastic bags, maintained in an ice box, transported to the laboratory and stored at -20°C for further analysis. The soil samples were sieved (2mm mesh), homogenised and divided into three parts each dedicated to physicochemical, enzymatic and molecular analysis.

3.4 Physicochemical characteristics of shisham rhizospheric soil

The following soil properties were determined. Soil pH was determined by the slurry method (1:5, soil:distilled deionized water) and measured with an glass electrode of micro processor based pH meter, century CP 931 **(Miller and Donochue, 1992**). Soil electrical conductivity (EC) (1:25, soil:water) at 25°C with digital microprocessor based conductivity meter (Systronic Model 306).Total organic carbon (OC) was determined by wet oxidation method using $K_2Cr_2O_7$ and concentrated H_2SO_4 (**Westerman *et al.,* 1990).** Total nitrogen (TN) was determined by the Kjeldahl digestion method (TKN). Available phosphorous (AP) content was measured by colorimetery after extraction with 0.5 mol l^{-1} $NaHCO_3$ (pH 8.5) for 30 minute **(Olsen *et al.*, 1954).** Available potassium (AK) content was measured with a flame photometer after extraction with 1moll^{-1} NH_4Ac (pH 7.0) for 30 minute (**Yuan *et al.,* 1983).** Minor trace elements like iron and zinc were measured using an atomic absorption spectrometry **(Yao *et al.,* 2003**). The correlation between soil factors within three *Dalbergia sissoo* forests was analysed statistically by two-way ANOVA ($p < 0.05$).

3.5 Soil Enzymatic Assays

To compare the functional potential of the soil microbial communities inhabiting the shisham rhizosphere, we used a combination of enzymatic bioassays.

The soil health was monitored by estimating activities of five enzymes; alkaline phosphatase, acid phosphatase, fluorescein diacetate hydrolysis, dehydrogenase and urease in rhizospheric soil from different provenances. The hydrolysed product of each enzyme was analysed spectrophotometrically and actual activity determined from a standard curve. All assays were conducted in triplicates.

3.5.1 Fluorescein diacetate (FDA) hydrolysis (Inbar *et al.*, 1991)

I. Reagent

(i) Potassium phosphate buffer (60mM, pH 7.6)

Stock solution: - 60 mM, KH_2PO_4 (0.72g in 100ml)

60 mM, K_2HPO_4 (0.852g in 100ml)

Both the solutions were mixed in equal amount and pH was adjusted to 7.6**.**

(ii) Fluorescein diacetate

FDA (2 mg) was dissolved in 1 ml acetone and stored at 4°C

(iii) Stock solution of fluorescein (mg ml^{-1})

Fluorescein (1 mg) was dissolved in 1ml acetone.

(iv) Working solution of fluorescein - 100µg ml^{-1}

II. Preparation of fluorescein standard curve

Different concentration of fluorescein (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml) were prepared from working solution of fluorescein (100µg ml^{-1}) in 50ml flasks. Volume was made upto 50 ml with sodium phosphate buffer (pH-7.6) to which acetone (2.5ml) was added and incubated at room temperature. After 20 min absorbance of samples were read at 490 nm.

III. Procedure

Fluorescein diacetate hydrolysis was determined according to the method of **(Inbar *et al.*, 1991)**. 1 gm of moist field soil taken in Erlenmeyer flask was drenched with 1 ml of FDA solution and 15 ml of buffer. The flasks were shaken for 20 min on a rotary shaker at 25 ºC. The samples were extracted in 10 ml acetone. The extracted samples were filtered and absorbance measured at 490 nm.

3.5.2 Dehydrogenase Activity (Thalmann, 1968)

I. Reagents

(i) Tris buffer (0.1M): 0.1M Tris buffer (1.2 g in 100 ml) of pH 7.6

(ii) TTC stock solution (1% w/v): 1g of 2,3,5- triphenyltetrazolium chloride (TTC) was dissolved in Tris buffer and volume was made upto 100 ml. TTC solution was stored for one day at 4^oC in dark.

(iii) Standard solution of triphenylformazan (100 mg ml^{-1}): 1 mg triphenylformazan (TPF) was dissolved in 1ml acetone.

(iv) Working solution of TPF: 10 mg ml^{-1}

II. Preparation of TPF standard curve

Different concentration of aliquots were prepared from the working solution of TPF (10 mg ml^{-1}) i.e.0, 50, 100, 150, 200, 250, 300, 350 and 400 µg ml^{-1} in 50 ml flasks. Absorbance of the resultant solution was taken at 546 nm using spectrophotometer.

III. Procedure

5 gm field moist soil was placed in Erlenmeyer flask and 5 ml TTC-TRIS buffer was added followed by 24 h incubation at 30ºC in the dark. Thereafter, the sample was extracted with 25 ml acetone. The resulting solution was shaken for 2 h in the dark and filtered. The absorbance of the triphenyl-formazen formed was measured immediately at 546 nm on Perkin Elmer Lambda 35 spectrophotometer.

3.5.3 Alkaline and Acid Phosphatase Activity (Tabatabai and Bremner, 1969)

I. Reagents

(i) **Modified Universal Buffer (MUB)**: 100 mM, pH11 (pH 6 is maintained for acid phosphatase)

(ii) **Calcium chloride ($CaCl_2$, 0.5M)**: 73.5 g $CaCl_2$ was dissolved in 1000 ml of distilled water.

(iii) **Paranitrophenyl phosphate (pNPP, 0.115 M)**: 1.927 g pNPP was dissolved in 50ml of MUB buffer.

(iv) **Stock solution of Paranitrophenol (pNP, 1mg/ml)**: 1mg pNP was dissolved in 1ml MUB buffer.

(v) **Working solution of paranitrophenol**: 200 μg/ml

II. Preparation of standard curve of pNP

Different concentration of pNP (0, 10, 20, 30, 40, 50, 60,70, 80, 90 and 100 μg/ml) was prepared from the working solution of pNP (200 μg/ml) in the test tube. Absorbance was taken at 400nm.

III. Procedure

One gm of moist soil was placed in a 50-ml Erlenmeyer flask, 4 ml of Modified Universal Buffer (MUB), 0.25 ml of toluene; 1 ml of p-nitrophenyl phosphate (PNPP) solution was added and swirled for a few seconds to mix the contents. The flasks were incubated at 37° C. After 1 h, 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide was added. Thoroughly mixed soil suspension was filtered. The absorbance of filterate was read at 400 nm through Lambda 35 UV visible spectrophotometer from Perkin Elmer.

3.5.4 Urease activity (Kandeler and Gerber, 1988)

I. Reagents

(i) **Substrate (Urea) (79.9mM)**: 2.4 g of urea is dissolved in 500 ml distilled water. The solution was prepared fresh.

(ii) **KCl solution (2 M)**: 74.6 g of potassium chloride was dissolved in distilled water and volume was made upto 1000 ml.

(iii) **NaOH solution (0.3M)**: 12 g sodium hydroxide was dissolved in 1000 ml distilled water.

(iv) **Sodium salicylate solution (1.06 M)**: 17 g of sodium salicylate and 120mg sodium nitruprusside was added and diluted upto 100 ml with distilled water. The solution was prepared immediately before use.

(v) **Reagent A**- mix 100 ml of 0.3 M NaOH solution and 100 ml sodium salicylate solution and volume was made upto 1000 ml. The solution was prepared immediately before use.

(vi) Sodium dichloro iso cyanurate (39.1Mm) - 0.1g of sodium dichloro iso cyanurate was dissolved in 100 ml distilled water.

II. Preparation of ammonia standard curve

Standard stock solution- 1 mg NH_4^+-N.ml^{-1} dissolved 38.207 gm of ammonium chloride in distilled water and dilute the volume to 1000 ml with distilled water. Store the solution at 4°C

Working stock solution- 1000 µg NH_4^+-N.ml^{-1}

Aliquots of different concentrations were prepared from the working solution of ammonium chloride (1000 µgml^{-1}) i.e. 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µgml^{-1} in 50 ml flask. Absorbance of the resultant solution was taken at 690 nm using spectrophotometer.

III. Procedure

Five grams of field moist soil was placed in 50-ml Erlenmeyer flasks. It was drenched with 2.5 ml 0.08 M urea solution and incubated at 37 °C. After 2 h incubation 50 ml of 1N KC1 was added and kept on rotary shaker for 30 min. The resulting suspensions were filtered. One ml of filtrate was diluted to 10 ml with DW followed by addition of 5ml Sodium salicylate and 2ml 0.1% sodium dichlorisocyanurate. The samples were incubated at 28±1ºC for 30 min and absorbance read at 690 nm.

The variation in all enzymatic activities of soil based on different origin was determined statistically by analysis of variance (one factor ANOVA).

3.6 Extraction, visualization and quantification of total soil DNA

Total soil DNA was extracted from 0.25 gm of soil samples using the Soil DNA kit (**Himedia, India**), according to the manufacturer's instructions. The purity and homogeneity of DNA samples was checked on 0.8% agarose by agarose gel electrophoresis. 0.8gm agarose (**Himedia, India**) was prepared in 100 ml of 1X TAE buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was carried out up to 2/3rd field of run at 80 volts (5 volts cm$^{\mathbf{-1}}$ being optimum) and subsequently visualized using GelDocMega (Biosystematica). The reagents used for agarose gel electrophoresis are given in **Appendix II**. DNA was quantified spectrophotometrically at 260 nm and stored in TE buffer (10 mMTris, 1 mM EDTA, pH 8.0) at −20 °C till further use.

3.6.1 Real Time Quantification of 16SrDNA in Soil samples

For the enumeration of the total bacteria present in soil samples, quantification of 16SrDNA copy numbers in soil was performed through the real time PCR with following protocol.

3.6.1.1 Extraction of Genomic DNA from *Pseudomonas koreensis* strain AS15

Pseudomonas koreensis AS15 strain was provided by departmental culture collection at G.B. Pant University of Agri. & Tech. Pantnagar. This culture was grown in King's B broth and genomic DNA form this culture was isolated through alkaline lysis method **(Bazzicalupo and Fani, 1995).**

3.6.1.2 Amplification of 16S rDNA from the genomic DNA of *Pseudomonas koreensis* AS15

Genomic DNA of *Pseudomonas koreensis* AS15 was subjected to conventional PCR in a volume of 25µl using universal primer set EUB-341F and EUB 534R (341F- 5′ CCTACGGGAGGCAGCAG 3′ and EUB 534R- 5' ATTACCGCGGCTGCTGG 3') **(Muyzer *et al.*, 1993).** Amplification was performed according to the following reagents and PCR program.

PCR Reagents:

Reagent	Working concentration
10X Buffer	1X
dNTPs (10mM)	200µM
Primer EUB-341F (10µM)	0.1 mM
Primer EUB 534R (10µM)	0.1 mM
Taq DNA Polymerase (3U/µl)	1U
Template DNA	50 ng

PCR program

Steps	Cycles	Temperature	Time (min)
Initial Denaturation	1	95^0C	10
Denaturation		94^0C	1
Annealing	35	58^0C	1
Extension		72^0C	1
Final extension	1	72^0C	10

3.6.1.3 Standard curve preparation

Standard curve was prepared using 16S rDNA amplicons of the *Pseudomonas koreensis* AS15. Amplified product was first purified through the HiPurA™ PCR Product Purification Kit, to remove the unused primers and nucleotides. Thereafter, concentration of the purified amplicons was quantified through the spectrophotometer reading by absorbance values at 260 nm. Ten fold serial dilutions was performed (starting from 30 ng to 0.003 ng) and used as an external standard for quantification by real time PCR. With the help of standard curve (Ct vs concentration in ng) the amount of 16S rDNA in unknown soil DNA samples was quantified by calculating sample Ct value on the standard curve **(Appendix III)**. The copy number was estimated with the following formula:

Number of copies = (amount x $6.022 x 10^{23}$ / (length $x 10^9$ x 650), assuming one copy of ribosomal gene per genome **(Kabir *et al.*, 2003).**

3.6.1.4 qPCR analysis

The qPCR was performed in iCycler iQ™ Multicolor (Bio-Rad Lab, Hercules, CA, USA) instrument using SYBR green chemistry. A set of previously reported universal primers (EUB 341F- 5′ CCTACGGGAGGCAGCAG 3′ and EUB 534R- 5' ATTACCGCGGCTGCTGG 3') were used to perform the real time PCR quantification of 16S rDNA in isolated soil DNA (**Muyzer *et al.*, 1993**). Amplification was performed according to the following reagents and PCR program.

Real Time PCR Reagents for 25 µl reaction

Reagent	Sample Rxn	Standard Rxn
SYBR green supermix (Bio-Rad Lab, USA)	12.5 µl	12.5 µl
Primer- EUB 341F	0.5 µl (0.1µM)	0.5 µl (0.1µM)
Primer- EUB 534 R	0.5 µl (0.1µM)	0.5 µl (0.1µM)
Soil DNA	1 µl (10ng)	-
16S rDNA amplicons	-	1 µl (30ng serially diluted)
Triple Distilled Water	10.5 µl	10.5 µl

qPCR program

Steps	Cycles	Temperature	Time
Initial Denaturation	1	95^0C	3 min
Denaturation		94^0C	1 min
Annealing	35	58^0C	1min
Extension		72^0C	1min

3.6.2. Real Time Quantification of *pqq* C gene in Soil samples

3.6.2.1. Extraction of genomic DNA of *Pseudomonas striata* P-27

The culture of *Pseudomonas striata* P-27 was provided by departmental culture collection at G.B. Pant University of Agri. & Tech. Pantnagar. Cells were harvested from 100 ml grown culture of *Pseudomonas striata* P-27. Genomic DNA was isolated using Alkaline lysis method **(Bazzicalupo and Fani, 1995).**

3.6.2.2. Amplification of *pqq* C gene from *Pseudomonas striata* P-27

Genomic DNA of *Pseudomonas striata* P-27 was subjected to conventional PCR in a volume of 25µl using previously reported primers set (***pqq*C-F**-5′ ATTACCCTGCAGCACTACAC 3′ and ***pqq*C-R-**5′ CCAGAGGATATCCAGCTTGAAC 3′) according to their described protocol (**An and Luke**, **2016**). Amplification was performed according to the following reagents and PCR program.

PCR Reagents:

Reagent	Working concentration
10X Buffer	1X
$MgCl_2$ (25 mM)	0.5 mM
dNTPs (10 mM)	200 µM
Primer pqqC-F (10 µM)	0.2 µM
Primer pqqC-R (10 µM)	0.2 µM
Taq DNA Polymerase (3U/µl)	1U
Template DNA	50ng

PCR program

Steps	Cycles	Temperature	Time (min)
Initial Denaturation	1	95^0C	3
Denaturation		94^0C	1
Annealing	30	55^0C	1
Extension		72^0C	2
Final extension	1	72^0C	10

3.6.2.3. Standard curve preparation

Standard curve was prepared using DNA from *Pseudomonas striata* P-27**.** Ten fold serial dilution series (starting from 50ng) was used as an external standard for quantification by real time PCR. With the help of standard curve the amount of DNA was quantified by software provided by the manufacturer **(Appendix III)** (Bio-Rad Lab, Hercules, USA). The copy number estimation was done with the formula: Number of copies = (amount x 6.022 x 10^{23} / (length x10^9 x 650), assuming one copy of ribosomal gene per genome **(Kabir *et al.*, 2003).**

3.6.2.4. qPCR analysis

The above primers set were also used to perform the Real Time PCR quantification of ***pqq*** **C** gene from soil DNA**.**

Real Time PCR Reagents for 25 µl reaction

Reagent	Sample Rxn	Standard Rxn
SYBR green supermix (Bio-Rad Lab, USA)	12.5 µl	12.5 µl
Primer- pqqC F	1 µl (0.5µM)	1 µl (0.1µM)
Primer- pqqC R	1 µl (0.5µM)	1 µl (0.1µM)
Soil DNA	1 µl (undiluted)	-
DNA amplicons (*Pseudomonas striata* P-27)	-	1 µl (serially diluted)
Triple Distilled Water	9.5 µl	9.5 µl

qPCR program

Steps	Cycles	Temperature	Time (min)
Initial Denaturation	1	95^0C	3
Denaturation		94^0C	1
Annealing	30	55^0C	1
Extension		72^0C	1

3.7. Metagenomic sequencing

The soil microbiota was analysed based on 16S rRNA (V3-V4) gene using Illumina Miseq sequencing platform which generated 250 bp paired-end reads. The V3-V4 region of 16S rRNA genes was amplified using primers V3: 341 F: 5' CCTACGGGAGGCAGCAG 3' and V4: 806 R: 5' GGACTACHVGGGTWTCTAAT 3'.

3.7.1. Sequence quality checking

Raw sequences were processed and checked for different quality parameters. Following quality checks were performed for each sample.

- Base quality score distributions
- Average base content per read
- GC distribution in the reads

3.7.2. Base quality score distribution

Base quality of each cycle for all samples was performed. The quality of left and right end of the paired-end read sequences of the sample was analyzed and the Phred score distribution was calculated. Phred score is a quantitative measure of correctness of any sequence.

3.7.3. Base composition distribution

The composition of nucleotides in the sequence read for each sample and graph was plotted where x-axis represents sequencing cycle and y-axis represents nucleotide percentage. The base composition of left and right end of the paired-end read sequences were calculated.

3.7.4. GC distribution

The average GC content distribution of the sequenced read of the samples was calculated and graph plotted where x-axis represents average GC content in the sequence and y-axis represents percentage of sequences.

3.7.5. Identification of V3-V4 region from paired-end reads

Following steps were performed to extract V3-V4 region from Illumina paired-end sequences.

- **a)** Trimming of spacer and conserved region
- **b)** Building consensus V3-V4 region from trimmed paired-end reads
- **c)** Filters to identify high quality V3-V4 region sequences

Paired-end reads were assigned to samples on the basis of unique barcodes and truncated by cutting off barcode and primer sequence. Usually a paired-end sequence from V3-V4 metagenomics contains some portion of conserved region and V3-V4 region. As a first step the conserved region from paired-end reads were removed. After trimming the unwanted sequences from original paired-end data, sequences were merged using FLASH program (v.1.2.7) to generate consensus V3-V4 region sequence. Multiple filters such as conserved region, spacer and mismatch filters were applied to generate high quality V3-V4 region sequences. While making consensus V3-V4 sequence, all consensus reads were formed with 0 mismatches with an average contig length of ~350 to ~450bp.

3.7.6. Chimera filter and singleton removal

The UCHIME algorithm was used to detect chimera and remove chimera sequences.

3.7.7. OTUs, Taxonomy classification and relative abundance

The entire downstream analysis was done using QIIME (Version 1.9.1) program **(Caporaso *et al*., 2010)**. Pre-processed reads from all the samples were pooled and clustered into Operational Taxonomic Units (OTUs) using Uclust program at 97% similarity. The representative sequences for each OTU were selected by aligning the sequences against Green gene data set using PyNAST program **(DeSantis *et al*., 2006a, b)**. Sequences that did not have 97% identity to any of the reference sequences in the Green genes database were not assigned to OTUs and thus not considered for further analyses. The taxonomic classification of each representative OTU was performed using RDP classifier against SILVA 16S RNA genes database. The phylum and genus distribution for each sample based on OTU and reads were categorized. Further pie-charts were generated using QIIME program.

3.7.8. Statistical analysis

The alpha diversity and beta diversity analysis were subsequently performed using the normalized data. Alpha diversity was applied to analyze species diversity in a sample through three indices: Shannon, Chao1 and observed-species. All of these indices were calculated with QIIME (Version 1.9.1). Community richness was identified using the Shannon and Chao1 estimators. Community diversity was determined using the Shannon indices. The observed species metric indicated count of unique OTUs identified in the sample.

Beta diversity analysis was used to evaluate differences in species complexity among the samples. Beta diversities based on weighted Unifrac approach were calculated by QIIME software (Version 1.9.1). Weighted Unifrac Method was performed to interpret the distance matrix using average linkage by comparing microbial communities between samples. A jackknife test was performed to construct a consensus UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree for all samples in this set.

3.7.9. Venn diagram and Hierarchial clustering

Venn diagram shows the common and unique OTUs and phylum distributed among sample. Venn diagram was prepared using R-package v.3.4.3. The heatmap with hierarchical clustering tree (HCL) shows the relative read abundance of top 20 Genus among samples. The hierarchical clustering score was calculated by Multi Experiment Viewer based on metric calculation using Pearson correlation method. The node and edges are connected by means of distance score between genus and between samples.

3.8. Enumeration of bacteria in soil samples

The soil samples were analysed for aerobic mesophilic count on Angles's and Pikovaskya medium by serial dilution pour plating method. A 100 gm soil was suspended in 1000 ml suspended water/ normal saline and serially diluted up to 10^{-6} dilution. Three dilutions (10^{-2}, 10^{-4}, 10^{-6}) were selected and analysed for population by pour plating on both medium. The plates were incubated at 30°C for 24 hrs and observed for the development of colonies. Further population of bacteria per gram of soil was calculated by formula as following.

$$\text{cfu g}^{-1}\text{ soil sample} = \frac{\text{No. of colonies} \times \text{amount of sample used for plating}}{\text{Amount of sample used for preparing initial dilution} \times \text{dilution factor taken for dilution}}$$

Comparisons of cfu's of three different regions were analyzed statistically, using one-way Analysis of Variance (ANOVA).

3.9. Isolation of phosphate solubilising bacteria

Presence of *pqq* C gene was observed in all the soil samples. All three soils were subjected to serial dilution in normal saline (0.85% NaCl in DW). All 6 dilutions from 10^{-1} up to 10^{-6} were spread on sterile pikovaskya agar plates (**Himedia laboratories Pvt. Ltd.**) and incubated at 30°C for 24 hours. The plates were observed for colonies with halozones around them. The colonies exhibiting clear halo zones were selected and purified. In all eighteen bacterial colonies were isolated on pikovaskya medium plates from Lachhiwala, Tanakpur and Pantnagar soil.

3.9.1. Morphological Characterization

3.9.1.1. Gram's Reaction

A loopful culture was smeared on a clear slide and stained through Gram's staining procedure as per Bergey's Manual of Determinative Bacteriology **(Bergey *et al.*, 1994)**. The slide was observed under oil immersion objective lens (100 X magnification) of compound microscope and observed for Gram's reaction, shape and arrangement of the cells. The reagents used for Gram's staining are given in **Appendix IV**.

3.9.2. Maintenance and preservation of purified cultures

All the bacterial cultures were maintained on nutrient agar medium plates and slants and stored at 4°C. For long-term preservation, bacterial cultures were stored in the glycerol stocks prepared in nutrient broth and kept at -20°C.

3.10. P Solubilization

3.10.1. Qualitative P solubilization

Log phase bacterial isolates was spot inoculated on Pikovskaya's agar plates containing tricalcium phosphate and were incubated at 30± 1°C for 3-4 days. The plates were observed for formation of clear zone around the bacterial growth. Comparative solubilization index was determined by the following formula **(Pikovskaya, 1948; Edi-Premono *et al.,* 1996**).

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter} + \text{ Halozone diameter}}{\text{Colony diameter}}$$

3.10.2. Quantitative estimation of phosphorous

Phosphorous solubilized by bacterial isolates were quantified by **Fiske and Subbarow** (**1925**) method. The method is based on the principle that soluble phosphorous reacts with ammonium molybdate to form phosphomolybdic acid, which is reduced by α-aminonaphtholsulphonic acid. The resulting product is blue coloured in presence of sulfite. The intensity of blue colour is directly proportional to the amount of phosphorous present in the solution and is estimated by spectrophotometer at 640nm.

Reagents used:

i. 10 N H_2SO_4

ii. Molybdate solution: 2.5% ammonium molybdate in 5 N H_2SO_4

iii. Colour reagent: 10 ml of 5% sodium bisulphite

20% sodium sulphite

25g 1-amino-2-naphthol-4-sulphonic acid

iv. 60% Per chloric acid (PCA)

v. Stock of phosphorous (KH_2PO_4): 10 mM

Procedure:

a) Preparation of standard curve

(i) A standard curve of phosphorus was prepared by using 10 mM stock solution of KH_2PO_4 from which different concentrations (100µM, 200µM, 500µM, 800µM, 1mM, 2 mM, 5 mM and 10 mM) were taken in series of test tubes and volume made up to 1 ml with distilled water.

(ii) Then 0.4 ml of 60% PCA, 0.4 ml molybdate solution, 0.2 ml of colour reagent and 4 ml TDW was added sequentially and mixed thoroughly.

(iii) Test tubes are kept at room temperature for 5 min for colour development.

(iv) The resulting blue colour solution was read at 640 nm.

(v) Standard graph was prepared with concentration of phosphorous on x-axis and OD on y-axis (**Appendix V**).

b) Phosphorous estimation

(i) Bacterial isolates were grown in 25 ml NBRIP medium for 72 h at 28±1°C, 120 rpm. Cultures were centrifuged at 5000 rpm for 15 min.

(ii) One ml of culture supernatant was taken in a tube, 0.4 ml of 60% PCA, 0.4 ml molybdate solution, 0.2 ml of colour reagent and 4 ml TDW was added sequentially into culture supernatant. The tubes were left at room temperature for 30 min.

(iii) The absorbance of blue colour solution was measured at 640 nm and amount of phosphorous is estimated from standard graph

3.10.3. Statistical analysis

The experimental data (qualitative and quantitative) were statistically processed using t- test (cochran s approx t-test). All results were expressed as mean ± SEM. F values for which $p < 0.05$ were considered significant **(Fernandez *et al*., 2007).**

3.11. Biochemical characterization

3.11.1. Starch hydrolysis

Amylase activity was assessed by growing the bacteria on Glucose Yeast Extract Peptone Agar (GYP) medium with 0.2% soluble starch pH 6.0 **(Kasana *et al.*, 2008)**. After incubation at 30° C for 2 days, the plates were flooded with 1% iodine in 2% potassium iodide.

3.11.2. Urea Hydrolysis

Bacterial culture were spotted on nutrient agar plates supplemented with phenol red and 2% urea (w/v). The plates were incubated at 30° C for 2 days. The formation of pink colored zone were indicates positive test **(Cappuccino and Sherman, 2002)**.

3.11.3. Presence of Nitrate reductase

Nitrate reductase test was performed by inoculating 0.5 ml of culture suspension into 10 ml of the nutrient broth containing 1% KNO3 and incubated for 5 days at room temperature. After incubation at 28±1° C for 2 days, few drops of sulphanilic acid and α- Napthylamine (5g/1 in 5M acetic acid) was added to the test tubes. Development of red color within minutes was considered as positive and absence of color indicates negative test**.**

3.11.4. Lipase production

The bacterial colonies were inoculated in nutrient agar plates supplemented with 1% (w/v) Tween 80. The isolates that produced lipase were identified by clear/ precipitate formed around the colony.

3.11.5. Xylanase activity

Xylanolytic activity was determined by growing the bacteria in xylanase activity indicator medium (0.5% (w/v) xylan and 1.5% agar (w/v)) **(Farkas *et al.*, 1985).** After the incubation at 28±1° C for 3-4 days period, the plates were flooded with

1% iodine in 2% potassium iodide**.** A clear zone around the bacterial colony indicated positive test.

3.11.6. Protease production

For examining protease activity log phase bacterial isolates were spotted on Skim milk agar plates and incubated at 28^0C for two days .The isolates that produced protease were identified by clear zone around bacterial colony **(Shaheen *et al.,* 2008)**.

3.11.7. Pectinolytic Activity

Pectinolytic activity was determined by growing the bacteria in Pectin Agar medium. After the incubation period, the plates were flooded with 1% iodine in 2% potassium iodide (**Hankin and Anagnostakis, 1975).** A clear zone formed around the bacterial colony indicated pectinolytic activity.

3.11.8. Presence of Catalase

This test was performed to study the presence of catalase enzyme in different isolates. A smear of culture was made on a clean and dry glass slide. A drop of H_2O_2 was added and mixed with smear on slide. The production of gas bubbles and effervescence constituted a positive test **(Aneja, 2006)**.

3.12. Functional characterization of Isolates

3.12.1. Zinc solubilisation

The log phase bacterial cultures were spot inoculated on nutrient agar medium supplemented with ZnO and $ZnCO_3$ (0.1 %). The plates were incubated at 28 ± 1^0 C for 5 days. The plates were observed for zone of clearance around the bacterial colonies. The zone and zone size was correlated to the zinc solubilisation ability of isolates **(Saravanan *et al.*, 2003)**.

3.12.2. Siderophore production

The bacterial cultures were screened for siderophore production on Chrome azurol S (CAS) agar plates **(Schwyn and Neilands, 1987)**. CAS dye (60.5 mg) was dissolved in 50 ml deionised water, and then mixed with 10 ml of a Fe (III) solution (1 mM^{-1} of $FeCl_3.6H_2O$ in 10 mM^{-1} HCl). This mixture was added to 72.9 mg of Hexa-decyl-Trimethyl-Ammonium Bromide (HDTMA) dissolved in 40 ml water. The

resulting mixture was autoclaved, cooled to 50- 60°C and mixed with 300mL nutrient agar media. The CAS agar plates were inoculated with log phase cultures of all 22 bacterial isolates and incubated at 28±1^{0}Cfor 5-6 days. The plates were observed for appearance of orange to yellow halo zones around bacterial colony.

3.12.3. Indole acetic acid (IAA) production

Bacterial isolates were grown in nutrient broth supplemented with 0.01% tryptophan and incubated at 28±2°C for 3 days. The broth was centrifuged at 10,000 rpm for 20 min at 4°C to collect the supernatant. The amount of IAA was qualitatively determined by adding 4 ml of Salkowasky's reagent (1 ml of 0.05 M $FeCl_3$ in 50 ml of 35% $HClO_4$) to 2ml of culture supernatant. Uninoculated broth with Salkowski reagent served as reference. Development of red color indicates positive result **(Patten and Glick, 2002)**.

3.12.4. HCN production

Cyanide production was detected according to method of **(Bakker and Schippers, 1987).** The log phase culture were streaked on plates containing nutrient agar supplemented with 4.4g of glycine per litre. Filter paper dipped in 0.5% picric acid and 2% sodium carbonate solution was placed on the lid of each petriplate. Petriplates were incubated at 28±2°C for 3-7 days. The change of filter paper colour from yellow to orange brown indicated cyanide production. The plates without bacterial inoculums were considered as control plates.

3.12.5. Ammonia Production

Actively growing bacterial isolates were inoculated in 10 ml Peptone water and incubated for 72 h at 27± 2^0 C in a rotatory shaker at 100 rpm. Production of ammonia was tested by adding Nessler's reagent (1ml) to the bacterial culture after 4 days of incubation (**Cappuccino and Sherman, 1992).** Presence of yellow to brown color indicates production of ammonia.

3.13. Determination of antibiotic sensitivity and resistance pattern

Antibiotic sensitivity and resistance of eighteen strains were assayed by the disc diffusion method **(Yao, 2002).** A culture inoculum was prepared by growing cells in nutrient broth for 24 hours at 30°C. One milliliter of actively growing bacterial cultures

was pour plated on nutrient agar plates. Octo disc of different antibiotics at the following concentrations: ampicillin (10mcg), cephalothin (30mcg), chloramphenicol (30mcg), clindamycin (2mcg), erythromycin (15mcg), gentamicin (10mcg), oxacillin (1mcg) and vancomycin (30mcg) were placed on the surface of the medium and left for 30 minutes at room temperature for diffusion of the antibiotics. All antibiotics were purchased from **Himedia Laboratories Pvt. Ltd.** The plates were incubated for 48 hours at 30°C. After incubation, the bacteria were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone.

3.14. Molecular characterization

3.14.1. Extraction of genomic DNA of the strains

Genomic DNA of all isolates was extracted by a modified method of **(Bazzicalupo and Fani, 1995).**

- Nutrient broth inoculated with single bacterial colony was incubated at 28^0 C and 140 rpm till log phase growth was attained.
- Log phase culture of all 22 isolates was pelleted at 10,000 rpm for 5min.
- The pellet was washed with Tris Cl (pH =6.8) at 10,000 rpm for 5 min to remove polysaacharides.
- The supernatant was discarded and 567µl of Tris EDTA was added. The pellet was homogenized on vortex mixer.
- 30 µl SDS and 5 µl Proteinase K was added.
- After gentle inversion, solution was incubated at 37^0C for 1 hour.
- 100 µl of 5M NaCl and 80 µl of 10% CTAB was added and the solution was mixed by gentle inversion and incubated at 65^0C for 15-20 min.
- The equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed by vigorous inversion and centrifuged at 12,000 rpm for 10 minutes.
- Upper aqueous phase was collected and equal volume of Phenol: Chloroform: Isoamylalcohol was added to it.
- It was mixed by vigorous inversion and centrifuged at 14,000 rpm for 10 minutes.

- Upper aqueous phase was collected and 3 µl of RNase was added to it and incubated at 37^{0}C for 30 minutes.
- Double volume of chilled absolute ethanol was added and kept at -20^{0}C overnight.
- The above mixture was centrifuged at 12,000 rpm for 20 minutes and supernatant was discarded.
- DNA pellet was air dried and dissolved in 50 µl Tris – EDTA (pH 8).
- The DNA was electrophoresed in 0.8% Agarose gel at 100V for 45 minutes.
- The gel was visualized under U.V light on gel documentation system (Gel Doc Mega- Biosystematica).

3.14.1.1. Quantification of genomic DNA

DNA sample (10 µl) and 490 µl TE buffer were mixed by inverting the tube. The absorbance was taken at 260 nm and 280 nm through UV/Vis spectrophotometer Lambda 35, Perkin Elmer. The TE buffer acted as blank. The ratio of Abs_{260}nm/Abs_{280} nm provides an estimate of purity of nucleic acid. Pure preparation of DNA has Abs_{260}/Abs_{280} ratio of 1.8 to 2.0.

DNA concentration was calculated by using following formula:

$$\text{DNA concentration } (\mu g/ml) = \frac{Abs_{260} \times \text{Dilution factor} \times 50}{1000}$$

where,

$$\text{Dilution factor} = \frac{\text{Total volume of sample in cuevette}}{\text{Volume of the DNA taken from stock}}$$

3.14.2. 16S rDNA amplification

Amplification of 16SrDNA was done using template DNA of all 22 bacterial isolates recovered from soil of three *Dalbergia sissoo* provenances. The 1492 bp 16S rDNA region was amplified using Primers GM3f (5' TACCTTGTTGTTACGACTT3') and GM4r (5'TACCTTGTTACGACTT3') (**Muyzer *et al.,* 1995**).

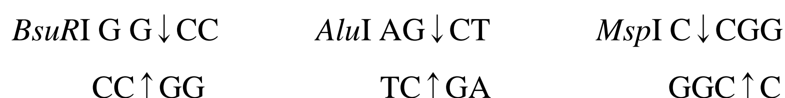
The reaction mixture contained: 5 µl template DNA was taken and to it 45 µl of reaction mixture was added which consisted buffer (100mM Tris –HCL with 15mM

$MgCl_2$), 1µM of each dNTPs and one unit of Taq DNA polymerase. The reaction condition include an initial denaturation of min at 95^0C, followed by 35 cycles of 1 min at 94°C, 1 min at 52°C and 1 min at 72°C with the final extension of 5 min at 72°C. The amplification was carried out in thermal cycler, Gen Amp PCR System 9700 (Applied Biosystems) Amplified DNA was electrophoresed in 1.0% agarose gel at 80 mA for1 hour alongwith λDNA/EcoRI/HindIII Double Digest ladder and visualized under UV gel documentation system (Gel Doc Mega, Biosystematica).

3.14.3. Restriction Analysis of 16S rDNA

The 16S rDNA amplicon of all 18 isolates was digested with three tetracutter restriction endonucleases; *MspI, AluI,* and *BsuRI*. The digestion reaction was set in reaction mixture of 25 µl, which included 20 µl amplicon and reaction mixture included 1X assay buffer for enzyme, 1U/reaction of each restriction endonuclease *Msp*I, ,*Alu*I, and Fast digest *BsuR*I. For digestion with *Msp*I and *Alu*I reaction mixture was kept at 37°C for 2h and *Bsu*RI fast digest for 5 min. Enzymes were then inactivated by adding loading dye and kept at -20°C. The product of restriction digestion was analysed on 2.5% agarose gel electrophoresed at 60V and visualized under UV Gel documentation system.

The recognition sites of the enzyme are as follows:



3.14.4. *In silico* analysis of sequence data

On the basis of ARDRA profiles and morphological characters, isolates were selected and taxonomically identified. The 16S rDNA region of all 18 isolates were sequenced on 3730 DNA sequencer using ABI big dye terminator technology(Central Instrumental facility, Biotech Centre UDSC, New Delhi) using same set of primers that were used in 16SrRNA gene amplification. The gene sequences were subjected to a similarity search using Basic Local Alignment Search (BLASTn) algorithm **(Altschul *et al*., 1990)** at EzBioCloud's database (https://www.ezbiocloud.net/identify) **(Yoon *et al.*, 2017)**.

3.14.4.1. Nucleotide sequence accession number

The 16S rRNA gene sequences of all 22 bacterial isolates from this study have been deposited at NCBI under accession numbers MG966339-MG966355 **(Appendix VI).**

3.15. Amplification of *pqq* C and *pqq* A Gene from Bacterial Isolates

3.15.1 Extraction of Genomic DNA from bacterial isolates

Genomic DNA was isolated from all the isolates using alkaline lysis method (**Bazzicalupo and Fani, 1995)** described in previous section**.**

Agarose gel electrophoresis

Agarose gel electrophoresis was done to evaluate the purity of isolated DNA samples. as per the method mentioned in previous section.

3.15.2 Amplification of PQQ gene from bacterial isolates

The best-characterized mechanism for microbial phosphate solubilization is through secretion of gluconic acid **(Goldstein, 1995)**. The gluconic acid is produced from glucose through the activity of a glucose dehydrogenase (GDH) enzyme that requires the redox cofactor pyrroloquinoline quinone (PQQ). The PQQ coenzyme is synthesized exclusively in microbes, its precise mechanism is not yet completely understood **(Klinman and Bonnot, 2014)**. The genes required for its synthesis comprise a combination of the following: *pqqA*, *pqqB*, *pqqC*, *pqqD*, *pqqE*, *pqqF*, and *pqqG.* In general, the *pqqA*, *pqqB*, *pqqC*, *pqqD*, and *pqqE* genes are conserved and arranged in that particular order in what is typically referred to as the *pqq* operon (*pqqABCDE*) **(Shen *et al.*, 2012)**. Other commonly found genes include *pqqF* and *pqqG*, which can be located either proximal or distal to the *pqq* operon **(Yang *et al.,* 2010)**. Previous studies have proved the requirement of PQQ for the mineral phosphate solubilization ability. The bacterial genomic DNA of selected isolates was subjected to amplification at, Gen Amp PCR System 9700 (Applied Biosystems) in a 20µl volume using PQQ primers set. PQQ primer set (*pqq*A-F: 5'ATGTGGACCAAACCTGCATAC 3' *pqq*A-R:5'GCGGTTAGCGAAGTACATG GT3') and (*pqq*C-F: 5'ATTACCCTGCAGCACTACAC3' *pqq*C-R: 5' CCAGAGGATATCCAGCTTGAAC 3') were used.

Fig. 3.1 Comparison of the *pqq* gene clusters of different microbes. Positions and orientations of the *pqq* genes are indicated by white and colored arrows. The same color represent homologous encoded proteins.

Reagents:

10X Assay Buffer	1X (New England Biolab, MA, USA)
$MgCl_2$	0.5 mM
dNTPs	200µM (New England Biolab,MA, USA)
Taq DNA Polymerase	1U (New England Biolab, MA, USA)
Forward Primer	0.3 µM
Reverse primer	0.3 µM
Template DNA	50 ng

Volume was maintained with autoclaved triple distilled water. Amplification was done using following programme.

PCR program

Steps	**Cycles**	**Temperature**	**Time**
Initial Denaturation	1	94^0C	5 min
Denaturation		94^0C	30 sec
Annealing	30	50^0C	30 sec
Extension		72^0C	1 min
Final extension	1	72^0C	10min

The amplicons along with 50bp DNA ladder, were loaded on 2.0 % ($^w/_v$) Agarose gel which was prepared in 1X TAE buffer (pH 8.0) electrophoretically.

3.16. Phylogenetic analysis

The full 16S rRNA gene sequences corresponding to all eighteen phosphate solubilizing bacteria (PSB) were compared with those available in the National Centre for Biotechnology Information (NCBI) Gen Bank database. Homology studies were carried out with the NCBI Gen Bank BLAST programme **(Altschul *et al*., 1990)**. Multiple sequence alignment and comparison was performed by ClustalW algorithm **(Thompson *et al*., 1997)** with the selected reference sequences**.** The phylogenetic tree was constructed using neibhour-joining (NJ) method **(Saitou and Nei, 1987)** with the help of molecular evolutionary genetic analysis (MEGA) version 7.0 software. Bootstrap analysis of 1000 replicates **(Felsentiein, 1985)** was performed using MEGA version 7.0 **(Kumar *et al.,* 2016).** Percent identity, divergence and conserved sequence alignment all were performed through MEGA alignment.

Results and Discussion

Plant health is closely related to the dynamic balance between the three groups of rhizosphere microorganism; beneficial, deleterious and neutral. According to **García-Salamanca *et al.* (2013),** the taxonomical and functional structures of soil microbial communities are influenced by plant and soil properties. Plants exert, due to root characteristics and exudation pattern, selective pressure on the soil microbial population through modification of the soil physicochemical properties. An increased understanding of composition and dynamics of the *Dalbergia sissoo* rhizosphere microbiome is an essential step to design successful bicontrol strategy against soil borne diseases and safeguard its productivity. However, it is difficult to predict the structure and composition of its microbiota based on environmental parameters such as soil type, climate, and management practices. So, the composition and diversity of bacterial communities in *Dalbergia sissoo* rhizosphere from healthy and diseased natural forests were established using metagenome approach.

Soil and rhizosphere-dwelling bacteria enhance plant fitness and growth through various mechanisms. Enhanced nutrient acquisition from the soil is one of them. Plants acquire phosphorus, essential nutrient, from the soil, most of soil phosphorous is immobilized and becomes unavailable for plant uptake. The PSBs, through solubilization and mineralization of immobilized P, may reduce the addition of inorganic P into soil causing the substantial reduction in production cost and environmental damage **(Rajan *et al.,* 1996)** and improve soil health also. There are reports of significant improvement in P availability for plants through PSB inoculation. Microbial inoculants have also been used for enhancing the growth of *Dalbergia sissoo* seedlings grown under stress conditions **(Bisht *et al.*, 2009).**

Percent mortality at Pantnagar was found to be highest (85%) followed by Lacchiwala (20%) and Tanakpur (25%). So, in this study bacterial abundance and diversity of shisham rhizosphere from Lacchiwala, Tanakpur, and Pantnagar provenances were elucidated through quantitative PCR and metagenomic approach. In addition abundance of potential phosphate solubilizing bacteria associated with shisham rhizosphere was estimated. Several PSBs were isolated and characterized employing a polyphasic approach.

4.1 Soil physicochemical analysis

Soil physiochemical analysis was performed to assess the soil nutrient status and health. Soil texture was silty loam in Lachhiwala and Tanakpur soil whereas silty clay loam in Pantnagar. Soil pH in Pantnagar soil was 6.85 which was comparatively higher than Lachhiwala and Tanakpur where it was 6.00 and 6.12 respectively. Electrical conductivity for Lachhiwala, Tanakpur and Pantnagar soil was 0.11 dsm^{-1}, 0.14 dsm^{-1} and 0.13 dsm^{-1} respectively. Total organic carbon (TC) was found higher in case of Pantnagar (42750 kg/hac) as compared to Lachhiwala (19500 kg/hac) and Tanakpur (25000 kg/hac). Further available phosphorus (AP) content was highest in Lacchiwala (56.48 kg/hac) followed by Tanakpur (46.87 kg/hac) and Pantnagar soil (37.86 kg/hac). Total Kjeldhal Nitrogen (TKN) in Pantnagar, Lachhiwala and Tanakpur was 137.98 kg/hac, 163.07 kg/hac and 100.35 kg/hac respectively while soil potassium was 434.11 kg/hac, 505.34 kg/hac and 520.12 kg/hac respectively. Soil, iron and zinc were significantly higher in Lachhiwala and Tanakpur soil as compared to the Pantnagar soil. The analyses of macro and micro-nutrient contents alongwith some other important parameters (soil type, pH and electrical conductivity) of *D. sissoo* (D.S.) rhizospheric soil from three different provenances are presented in **(Table 4.1)**. Soil nutrient properties were analysed statistically. The ANOVA ($p < 0.05$) results revealed highly significant differences between soil nutrient values at Lachhiwala, Tanakpur and Pantnagar **(Fig. 4.1)**.

Table 4.1: Soil physicochemical properties in three different *D. sissoo* provenances

PROPERTIES	**PANTNAGAR** 29ºN Lat, 79 ºE long **252 mabsl**	**LACHHIWALA** 30.2ºN Lat, 78.1ºE long **508 mabsl**	**TANAKPUR** 29ºN Lat, 80ºE long **245 mabsl**
Soil pH	6.85	6.00	6.12
Electrical conductivity	0.13	0.11	0.14
Soil type	Silty Clay Loam	Silty Loam	Silty Loam
Carbon	42750 kg/hac	19500 kg/hac	25000 kg/hac
Phosphorus	37.86 kg/hac	56.48 kg/hac	46.87 kg/hac
Potassium	434.11 kg/hac	505.34 kg/hac	520.12 kg/hac
Nitrogen	137.98 kg/hac	163.07 kg/hac	100.35 kg/hac
Iron	11 kg/hac	12.5 kg/hac	22.6 kg/hac
Zinc	0.2 kg/hac	9.3 kg/hac	11 kg/hac

NOTE: Each value is the mean of three replicates. Data was analysed statistically at the 5% ($p<0.05$) level of significance

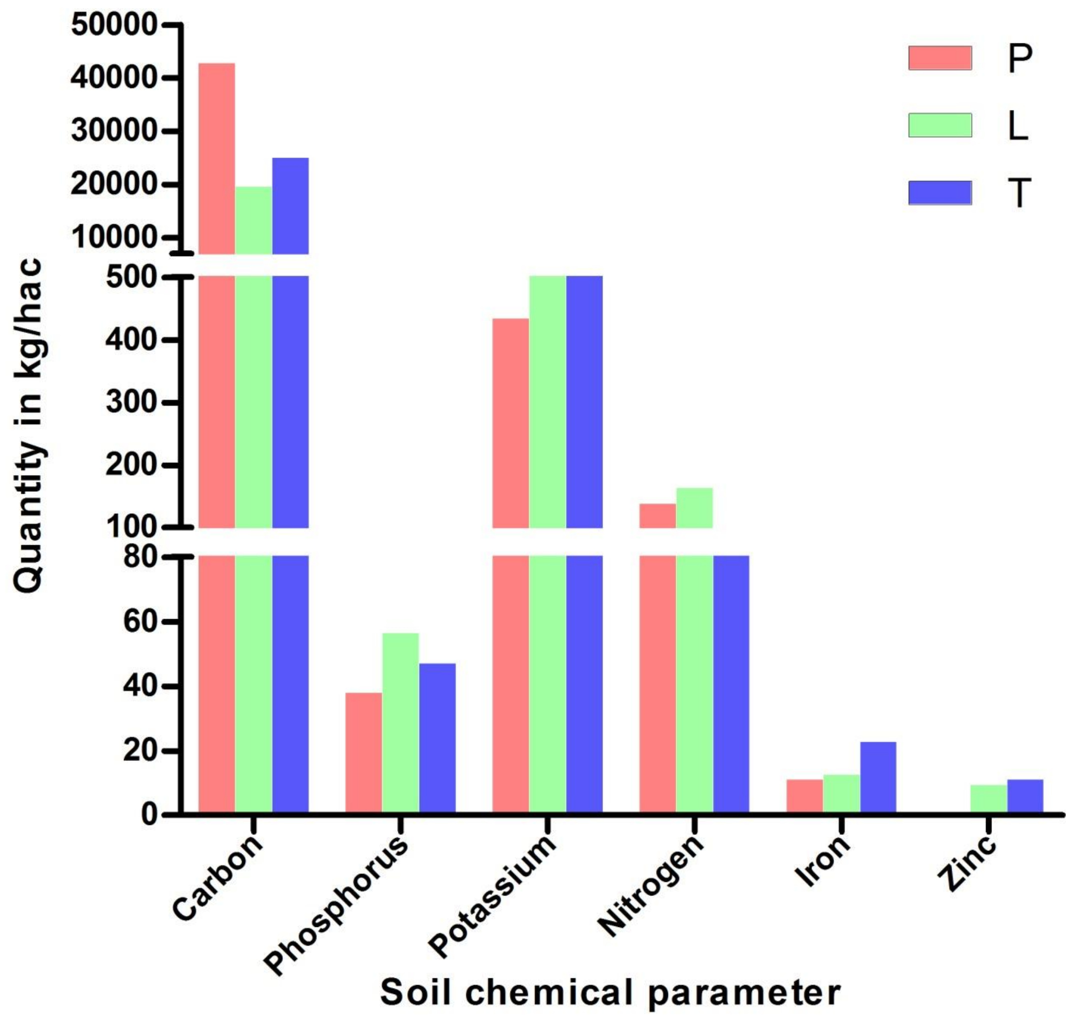


Fig. 4.1: Soil physicochemical properties at three different *Dalbergia sissoo* provenances

Disease conducive soil of Pantnagar was reported to be silty clay loam with high pH, high carbon, low phosphorus, low potassium and low micronutrients (Fe and Zn) content in comparison with other two samples (disease suppressive). *Dalbergia sissoo* thrives well on loose sandy soil but suffer adversely from root disease in stiff and clayey soil **(Bakshi, 1957)**. The stiff and clayey soil leads to asphyxiation of the feeding roots and were subsequently colonised by wilt fungus *Fusarium solani*. **Negi *et al.* (1999)** observed that sissoo growing in stressful sites allocated more carbon to its roots (60%) and less to foliage (40%) compared to those in normal sites (root 51%, foliage 49%). The reason behind the D.S. mortality in Pantnagar may be the deficiency of micro and macronutrients in soil. Micronutrients are reported to play an important role in plant functioning, provide healthy environment for the growth of beneficial microbial community in rhizosphere region **(Ghosh *et al.*, 2017)**. Similarly macronutrients N, P and K are involved in disease resistance and tolerance **(Dordas *et al.*, 2008)**. Hence, low phosphorous in Pantnagar soils might be responsible for promoting disease incidence and spread. Moreover slightly high pH of soil could also be the cause for mortality in Pantnagar D.S. forest. Present findings are similar to those by **Negi *et al.*, 1999** that higher pH hinder the availability of phosphorous to the plants and caused disease in plants. Nitrogen content was moderate in all the three regions. According to **Bisht *et al.* (2009)** D.S. through N_2 fixation has positive effect on soil fertility.

4.2 Soil enzyme activities

Figure 4.2 a-e shows alkaline phosphatase, acid phosphatase, florescein diacetate hydrolysis, dehydrogenase and urease activities of *Dalbergia sissoo* rhizospheric soils from shisham forests at three different locations. Rhizosphere fluorescein diacetate hydrolysis activity varied from 291.2 µg fluorescein g^{-1} h^{-1} soil at Lachhiwala forest to 372.6 µg fluorescein g^{-1} h^{-1} soil for Tanakpur forest **(Fig. 4.2a)** and a medium range was reported at Pantnagar (325 µg fluorescein g^{-1} h^{-1})**.** Dehydrogenase enzyme levels were two fold higher in case of Tanakpur forest (4300µgTPFg^{-1} h^{-1}) as compared to Lachhiwla forest (1880 µg TPF$g^{-1}h^{-1}$) while least activity was reported in the case of Pantnagar forest (1770 µgTPF$g^{-1}h^{-1}$) **(Fig. 4.2b)**. Lachhiwala forest also tended to have highest activity for acid phosphatase enzyme (1109.6 ug PNP g^{-1} h^{-1}) whereas the activity was comparable at Tanakpur (654.5 ug PNP g^{-1} $h^{-1}$and Pantnagar forest soil (574.8 ug PNP g^{-1} h^{-1}) **(Fig. 4.2c)**.

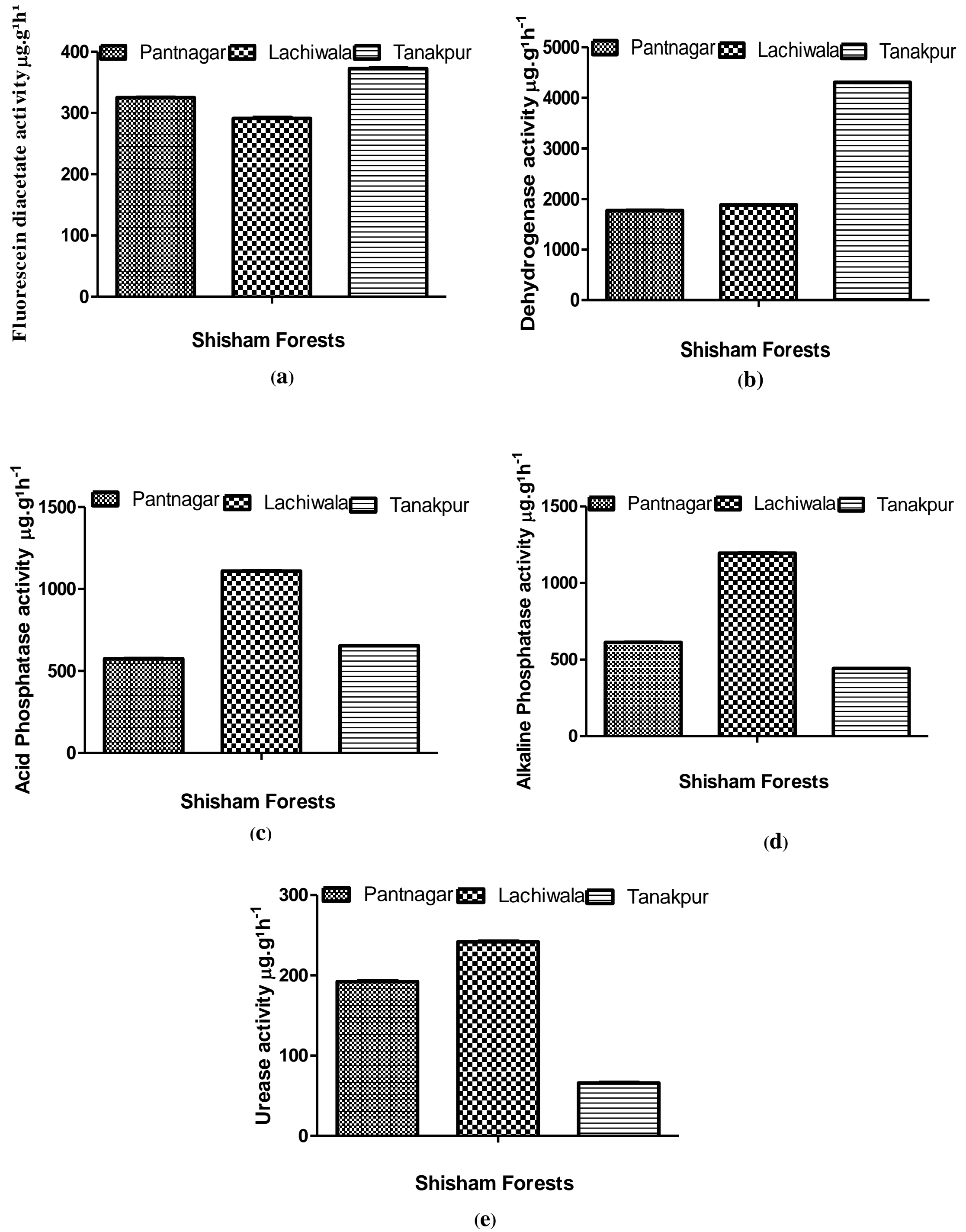


Figure 4.2: Enzyme activities in rhizospheric soil of three different *Dalbergia sissoo* provenances (a) Fluorescein di acetate (b) Dehydrogenase (c) Acid phosphatase (d) Alkaline phosphatase (e) Urease. Graphs are plotted with mean values (n=3) plus standard error

The highest alkaline phosphatase activity was found in *D. sissoo* rhizosphere from Lachhiwala forest (1196.2 ug PNP g^{-1} h^{-1}) followed by Pantnagar forest (613.87 µ g PNP g^{-1} h^{-1}) and lowest in case of Tanakpur forest (442.8 µg PNP g^{-1} h^{-1}) **(Fig. 4.2d)**. The maximum urease activity was observed in the *D. sissoo* rhizosphere soil from Lachhiwala forest (241.93 µg$NH_4^+$$g^{-1}$$h^{-1}$) followed by Pantnagar forest (192.25 µg$NH_4^+$$g^{-1}$$h^{-1}$). The minimum urease activity was observed in rhizosphere soil from Tanakpur forest (65.74 µg$NH_4^+$$g^{-1}$ h^{-1}) **(Fig. 4.2e)**. There was a significant difference ($p< 0.05$) between the enzyme activities of D.S. rhizosphere soils from three provenances **(Table. 4.2).**

Table 4.2: Soil enzyme activities in three different *Dalbergia sissoo* provenances

	Values represent mean ± SD*					
Enzyme activity	**Locations**	**Acid Phosphatase µg PNP g^{-1} h^{-1}**	**Alkaline Phosphatase µg PNP g^{-1} h^{-1}**	**Fluorescein diacetate µg florecein g^{-1} h^{-1}**	**Dehydrogenase µg TPF g^{-1} h^{-1}**	**Urease µg N g^{-1} h^{-1}**
	P	574.8±1.15	613.04±0.83	325±0.57	1770±1.15	192.25±0.67
	L	1109±0.66	1196.23±0.63	291.2±1.15	1880±1.15	241±0.56
	T	654.5±0.30	442.8±0.33	372.6±0.57	4300±0.57	65.78±0.65

NOTE: Each value is the mean of three replicates . Data was analysed statistically at the 5% ($p<0.05$) level of significance
SD*: standard deviation

Soil nutrients and soil enzyme activities are closely related. Soil organic carbon (SOC), nitrogen, phosphorus, potassium and other elements significantly affect the activities of the soil enzymes **(Sahrawat, 1983; Burke *et al*., 2011)**. In the present study Fluorescein diacetate (FDA) and Dehydrogenase activity correlated with culturable microbial population or respiratory metabolism **(Wolinska, 2012).** The dehydrogenase and FDA activities were higher in D.S. rhizosphere from Tanakpur where the aerobic bacterial population was also highest. Soil phosphatase activity is pH sensitive, depends on the number and diversity of soil resident microflora **(Nannipieri *et al.,* 2003)**. The acid and alkaline phosphatase, and urease activities were higher in D.S. rhizosphere from Lachhiwala. This is in contrast to the observation of **Nannipieri and co-workers (2003)**. The possible explanation is that only aerobic culturable bacterial count was determined; soil enzyme activities are the measure of total microbial population. One of the reasons for higher acid and alkaline phosphatase activity could be that phosphatase enzyme activities are proportional to the ds DNA and

ATP content of soil **(Renella, 2006).** Another reason is that soil pH at Lachhiwala is 6.00 which validly proves maximum phosphatase activity there. Urease is an important enzyme involved in soil nitrogen (N) cycling. It catalyzes the hydrolysis of organic N to inorganic forms, the former using urea-type substrates and the latter ammonia or ammonium ion substrates **(Cookson, 1999). Gianfreda *et al.* (2005)** found a significant positive correlation of urease and phosphatase with available nitrogen and phosphate. This could be the reason for higher AN and AP content in disease suppressive soil of D.S. forests. Thus BNF by *sissoo* trees positively contributed towards urease activities **(Bisht *et al.*, 2009)**. Increased enzyme activities indicate an increase in carbon and nutrient leakage in the soil **(Naseby and Lynch, 1998)**. The higher carbon promotes colonization of pathogens whereas higher nutrient availability could promote rhizosphere colonization of beneficial microbes. Hence it is not the individual carbon and nutrient content but the ratio that affects the rhizosphere microbiome.

4.3 Metagenomic DNA

The complex genomic DNA molecules from all the soil samples were isolated, quantified **(Table 4.3)** and visualized successfully as intact band on 1% agarose gel **(Fig. 4.3).**

Table 4.3: Quantification of metagenomic DNA in rhizospheric soil samples

Sr. No.	Sample name	260/280 ratio
1.	Lachiwala	1.70
2.	Tanakpur	1.80
3.	Pantnagar	1.70

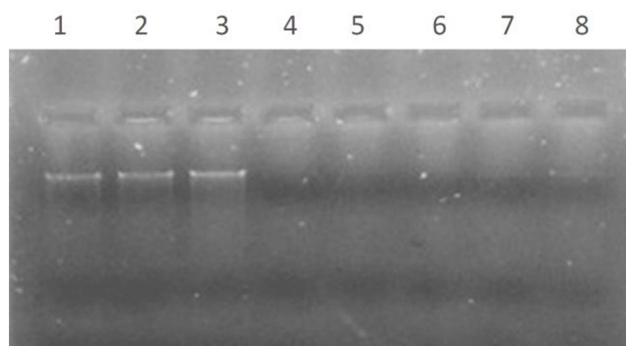


Fig 4.3: Metagenomic DNA isolated from rhizospheric soil samples of three provenance

4.4 Real Time Quantification

4.4.1 Real Time Quantification of 16S rDNA from soil DNA

The 16S rDNA was quantified in rhizospheric soil samples collected from Lachhiwala, Tanakpur and Pantnagar **(Kumar *et al*., 2014).**The result showed that the 16S rDNA copy number ranged from 4.92×10^{10}, 8.10×10^{9} and 1.73×10^{9} copies per gram of soil at Pantnagar, Lachhiwala and Tanakpur respectively (**Table 4.4a**). The 16S rDNA copy number was highest for Pantnagar followed by Lachhiwala and Tanakpur respectively **(Kumar *et al.,* 2014).**

Table 4.4 (a): Quantification of 16S rRNA gene in rhizospheric soil of three *Dalbergia sissoo* provenances

S. No.	Sample ID	Copy Number of 16S rRNA gene in soil
1.	P	4.92×10^{10}
2.	L	8.10×10^{9}
3.	T	1.73×10^{9}

Differences in 16S rRNA gene abundance suggested that bacterial abundance in particular habitat is affected not merely by altitude but also by physiological and edaphic soil factors like pH, temperature, nutrient availability, local flora and fauna. Another important factor, governing microbial abundance and diversity is plant type which directly influences the root exudation pattern. The composition of root exudates is affected by plant species as well as plant development stage **(Di Cello *et al*., 1997; Siciliano *et al*., 1998; Yang and Crowle, 2000; Dunfield and Germida, 2001).** Since root exudates are used as carbon sources by microorganism. Hence, root exudation pattern govern the structure of rhizosphere microbial community. Moreover various components of root exudates are differentially utilized by microorganisms thus leading to difference in abundance and diversity of rhizosphere communities (**Rumberger *et al.*, 2004; Orlando *et al.*, 2007**). Thus one possible explanation for the result obtained in this study could be difference in D.S. root exudation pattern within three provenances.

4.4.2 Real time quantification of *pqq* C gene from soil DNA

Upon real time quantification of the partial *pqq* C gene from D.S. rhizosphere soils at all three provenances, the copy number ranged from 5.56×10^{4} to 6.98×10^{5}

copies per gram of soil. Maximum *pqq* C gene abundance was observed at Pantnagar (6.98×10^{5}) followed by Lachhiwala (1.30×10^{5}) and Tankapur (5.56×10^{4}) **(Table 4.4 b).** Although phosphate-solubilizing bacteria widely exist in the soil ecosystem, their populations vary with soil properties (chemical and physical properties, phosphate and organic matter content) **(Kim *et al.,* 1998a)**. Phosphate-rich soils contain fewer phosphate-solubilizing bacteria, which are also low in their diversity **(Azziz *et al.,* 2012)**. Hence, Pantnagar soil with least plant available phosphorus content had high abundance of *pqq* C gene.

Table 4.4 (b): Quantification of *pqq* gene in rhizospheric soil of three *Dalbergia sissoo* provenances

S. No.	Sample ID	Copy Number of *pqq* gene in soil
1.	P	$6.98X10^{5}$
2.	L	1.30×10^{5}
3.	T	5.56×10^{4}

4.5 Metagenome sequencing targetting partial V3-V4 region of 16S rDNA

Only about1% of the total 10 billion microorganisms and a thousand different species present in a gram of soil **(Knietch *et al.*, 2003)** are assessable through cultivable techniques **(Schloss and Handelsman, 2003)**. Moreover, cultivation of microbes is biased for media and growth conditions **(Delmont *et al.*, 2011)**. Hence, cultivation dependent methods are complemented by culture-independent methods for microbial diversity analysis. Culture-independent techniques are based on DNA isolation from environmental samples directly and sequencing of the complete or specific region of metagenome **(Serkebaeva *et al.*, 2013)**. As bacteria are the dominant and most diverse group among the microbial communities of any environment, the metagenomic analysis of variable 16S rDNA region is widely used for characterization of bacterial communities in an environment **(Delmont *et al.,* 2011a, b)**. The raw reads obtained after sequencing, are compared with the reference sequences from different databases like Greengene **(Desantis *et al.*, 2006)** and ribosomal database project **(Cole *et al.,* 2009)** for bacterial identification.

4.5.1 Statistics of metagenomes

Targeting the hypervariable V3-V4 16S rDNA region, a total of 8,40,853 (Lachhiwala), 7,48,477 (Tanakpur) and 9,58,426 (Pantnagar) 250 bp sequence **(Fig. 4.4; Fig. 4.5, Table 4.6)** length reads using Illumina MiSeq platform were obtained for three forest samples. An average of 8, 49,252 reads were obtained for each sample. It was observed that the reads have GC content in the range of nearly 50%. The average GC content distribution of the sequenced reads of the samples is shown in **(Fig. 4.6)** More than 80% of the total reads have phred score greater than 30 (>Q30; error-probability >=0.001) **(Table 4.5).** While making consensus V3-V4 sequence, the passed reads aligned to each other with 0 mismatches with an average contig length of ~350 to ~450bp (**Fig. 4.7**). From all of the samples, a total of 24, 91, 431 high-quality sequences (809,458 for Lachhiwala, 725,772 for Tanakpur and 956,201sequences for Pantnagar) were obtained after filtering the low-quality reads, chimeras, and attachment sequences. A summary of reads that passed each filter can be found in (**Table 4.7).** The effective sequence reads comprised 14, 07,880 OTUs **(Fig. 4.8)** which after singleton removal remained to 17, 2195 OTUs for all samples (**Table. 4.8**).

4.5.2 Taxonomic analysis of operational taxonomic unit at phylum level

In all 89.33% of the 16S rRNA sequence reads obtained from Illumina high throughput sequencing belonged to 10 bacterial phyla. The 10 most dominant bacterial phyla are Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Chloroflexi, Planctomycetes, Nitrospirae, Firmicutes and Candidate division WS-3. The relative abundance of eight dominant phylum varied among Lachhiwala, Tanakpur and Pantnagar and was distinct in each metagenome **(Fig. 4.9).** Proteobacteria was the most abundant phylum in *Dalbergia sissoo* (D.S.) rhizospheric soil from Lachhiwala and Tanakpur accounting for 46-48% (average 47%) of the total valid reads whereas Acidobacteria was dominant in D.S. rhizospheric soil from Pantnagar with 43% relative abundance. The distribution of remaining seven dominant phyla varied in all the three metagenomes. In Lachhiwala Acidobacteria (15%) was second most abundant followed by Bacteroidetes (11%), Actinobacteria (5%), Verrucomicrobia (4%), Chloroflexi(4%), Planctomycetes (4%), and Nitrospirae (1%). In Tanakpur soil the bacterial phyla in order of relative abundance are Firmicutes (21%) Acidobacteria (6%), Actinobacteria (5%), Bacteroidetes(5%), Verrucomicrobia (4%), Planctomycetes (2%) and Chloroflexi whereas

Table 4.5: Raw read summary

Sl.No	Sample	Run	Read orientation	Mean read quality (Phred score)	Number of reads	% GC	% Q < 10	% Q 10–20	% Q 20–30	% Q > 30	Number of bases (MB)	Mean read length (bp)
1	L2		R1	36.88	840853	54.71	0.05	4.93	3.39	91.63	210.21	250.0
			R2	34.51	840853	54.83	0.3	10.19	6.65	82.86	210.21	250.0
2	P2		R1	36.53	958426	57.22	0.05	5.87	3.9	90.18	239.61	250.0
			R2	34.07	958426	57.39	0.3	11.41	7.38	80.9	239.61	250.0
3	T1		R1	36.88	748477	53.54	0.05	4.9	3.4	91.65	187.12	250.0
			R2	33.67	748477	53.73	0.3	12.4	7.91	79.39	187.12	250.0

Table 4.6: Base composition distribution of the samples

Sample Name	Base Composition (%)			
	A	C	G	T
L	22.67	27.65	27.12	22.39
P	21.55	28.94	28.37	20.97
T	23.11	27.20	26.44	23.07

Table 4.7: Read summary and Pre-processing reads statistics

Sample Name	Total Reads	Passed Conserved Region Filter	Passed Mismatch Filter	Consensus Reads	Chimeric Sequences	Preprocessed Reads
Lachhiwala	840853	840853	839299	839299	29841	809458
Pantnagar	958426	958426	956201	956201	0	956201
Tanakpur	748477	748477	746951	746951	21179	725772

Table 4.8: Summary of Singleton OTUs

Total Reads	2491431
Total OTUs Picked	1407880
Total Singleton OTUs	1235685
Total OTUs after Singleton removal	172195

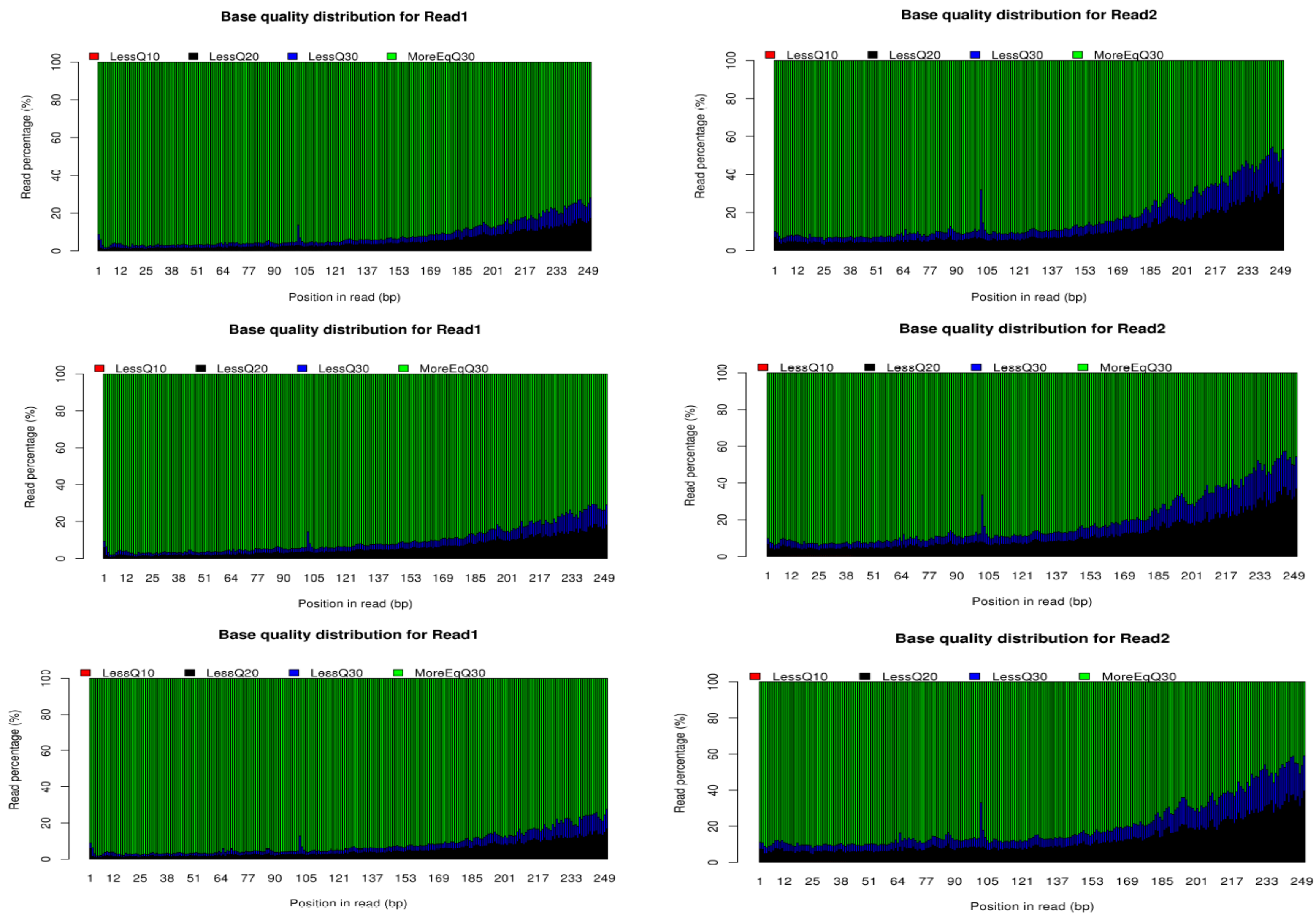


Fig.4.4: Base quality distribution of (a) sample L (R1), (b) sample L (R2), (c) sample P (R1), (d) sample P (R2) c) sample T(R1), (d) sample T (R2)

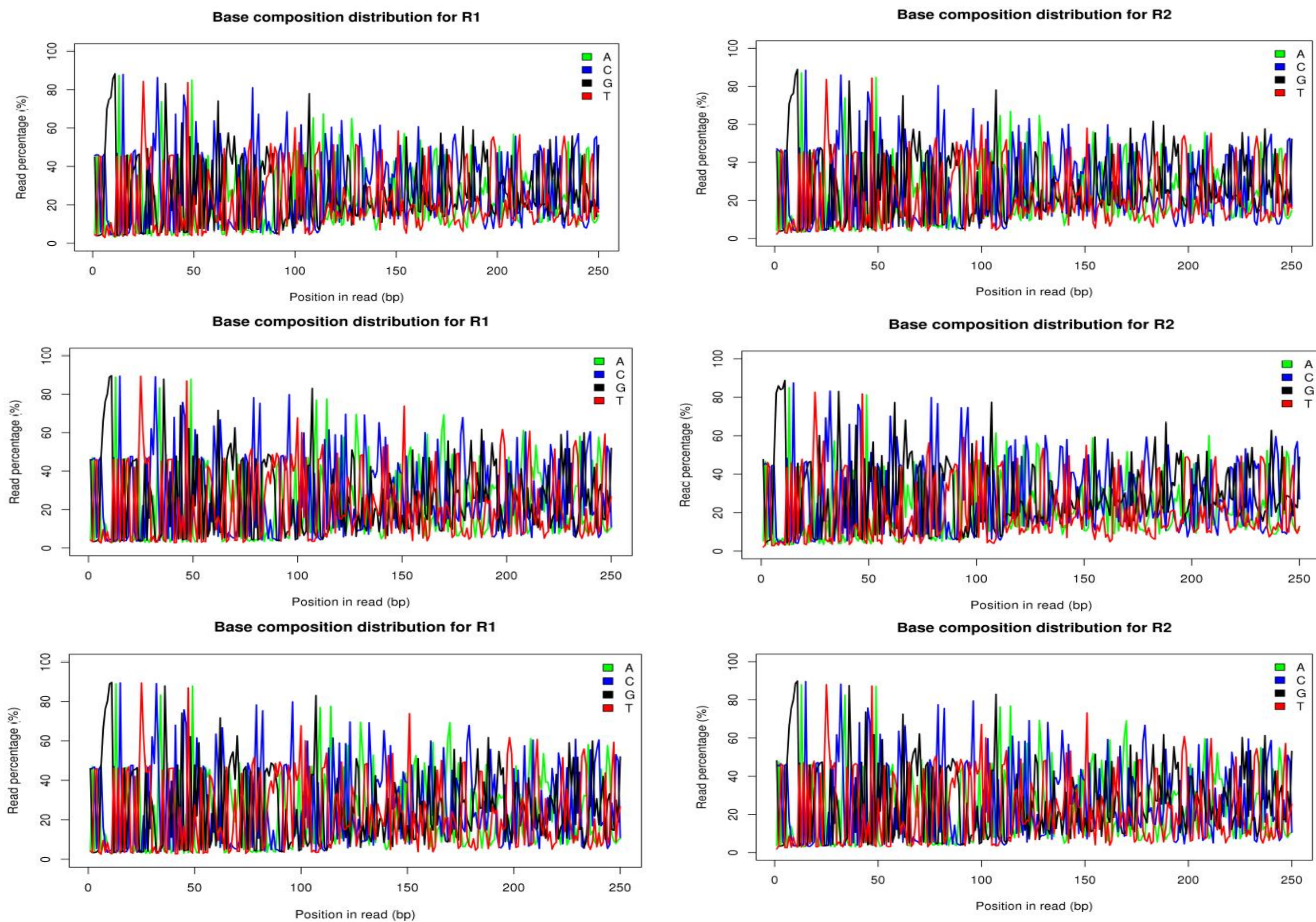


Fig. 4.5: Base composition distribution of (a) sample L (R1), (b) sample L (R2), (c) sample P (R1), (d) sample P (R2) c) sample T (R1), (d) sample T (R2)

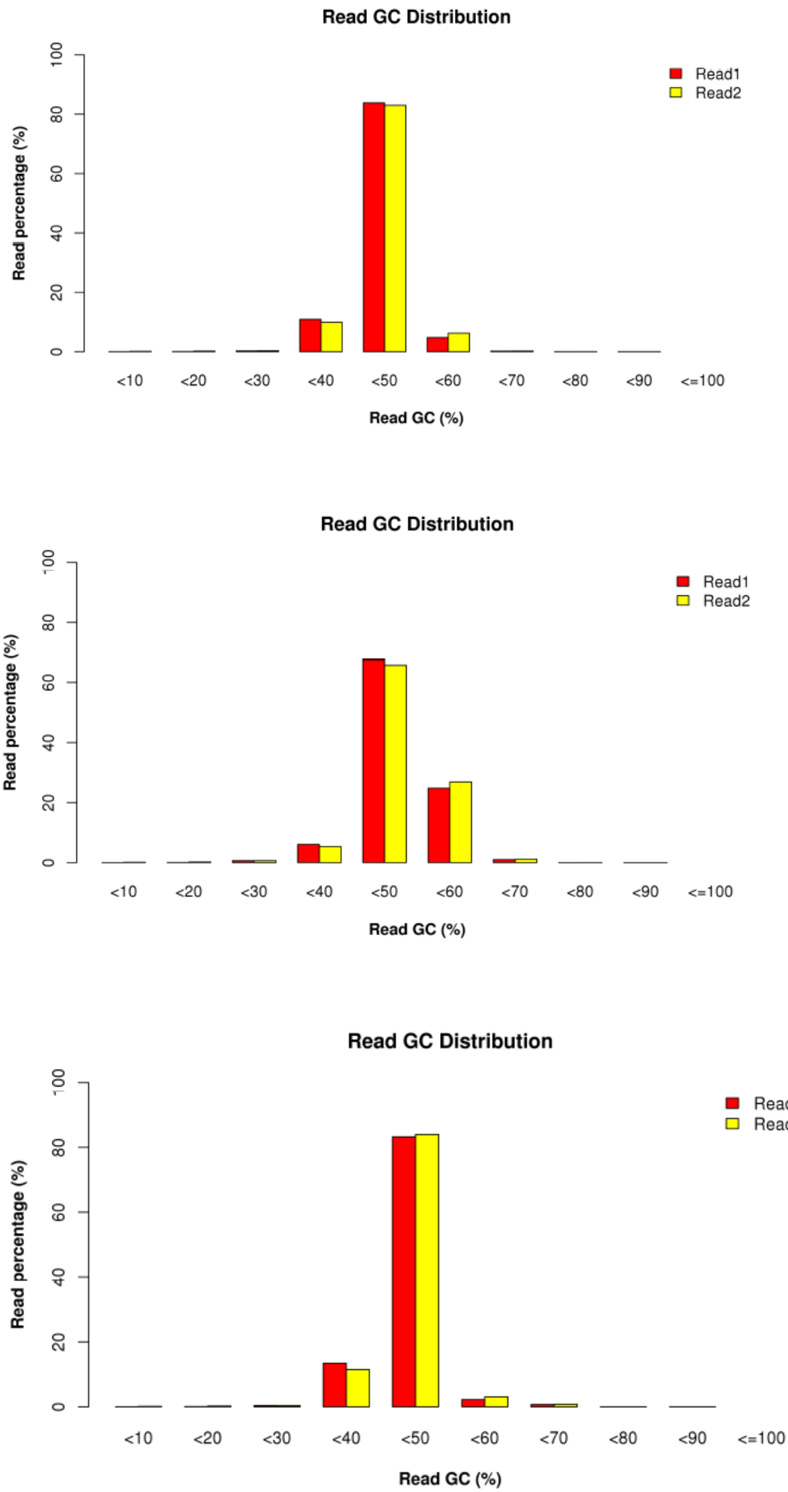
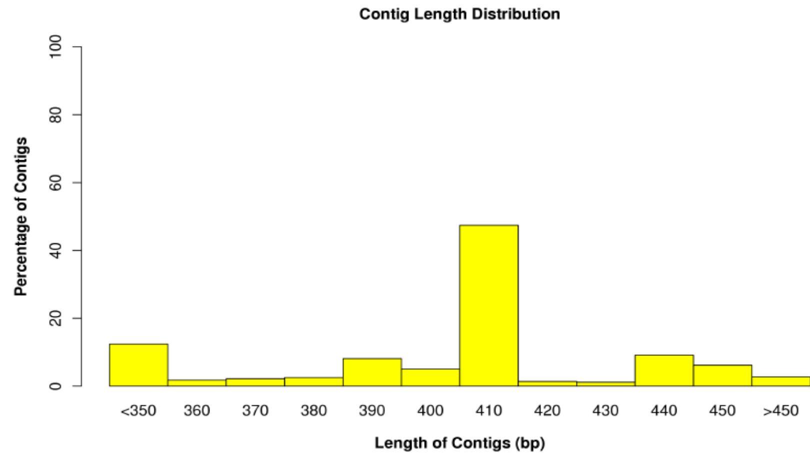
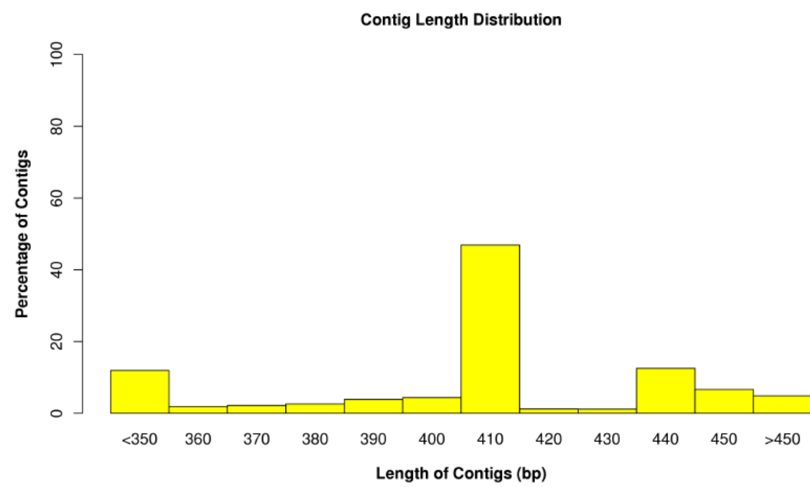


Fig. 4.6: GC distribution of sample (a) L sample (b) P sample (c) T sample





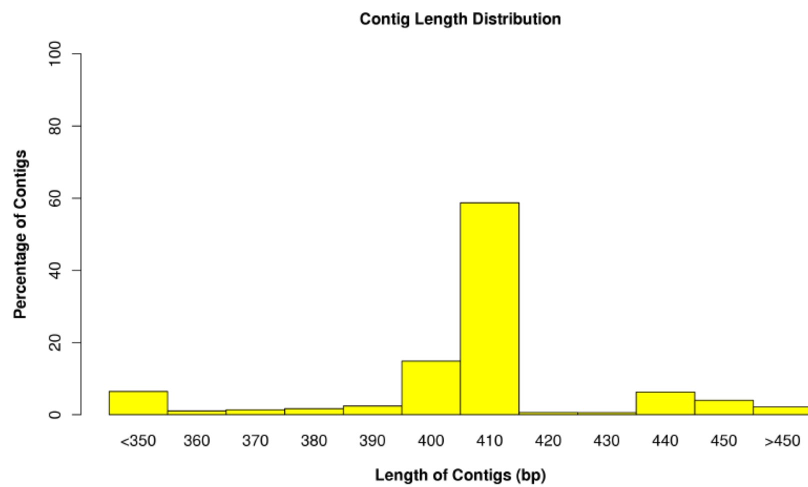


Fig. 4.7: (a) Contig Length distribution of sample L (b) Contig Length distribution of sample P (c) Contig Length distribution of sample T

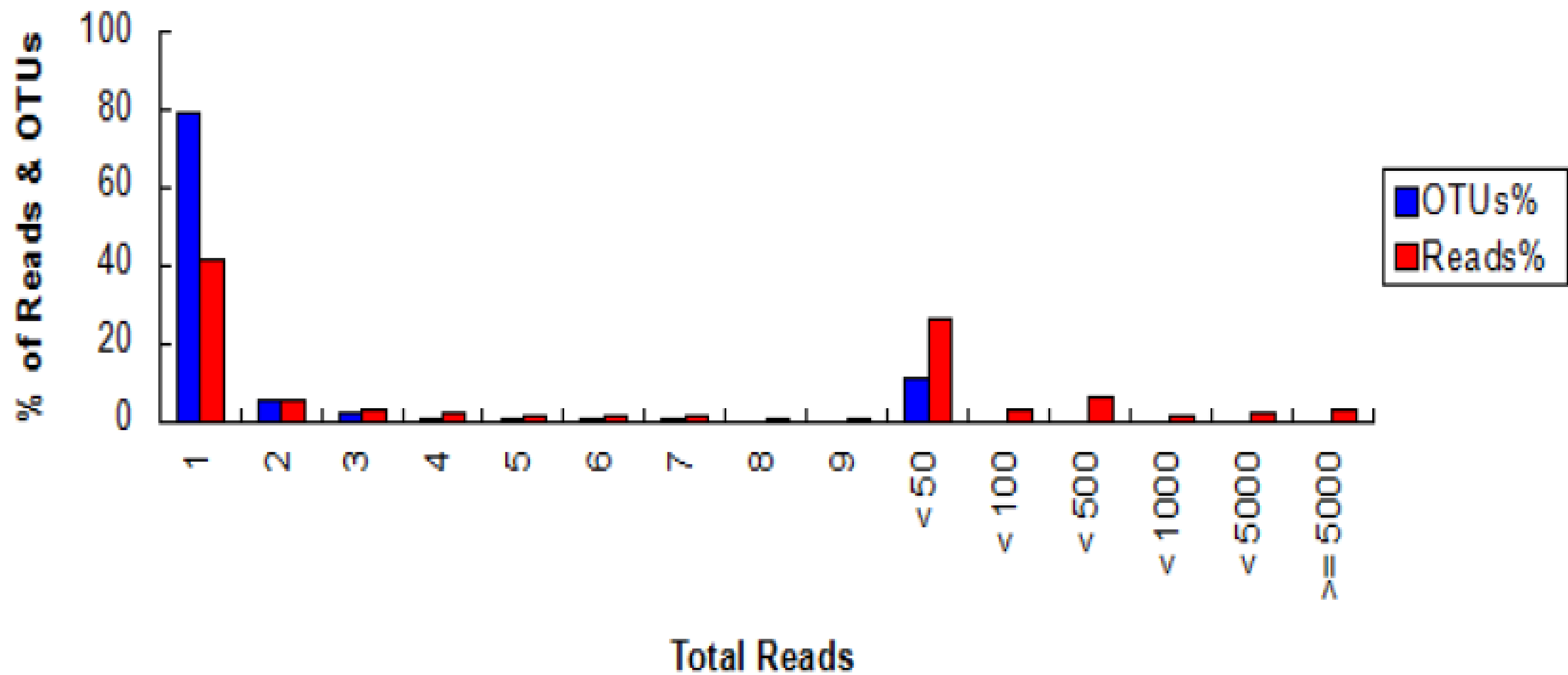


Fig. 4.8: Shows a graphical representation of reads and OTU proportion. The blue bar represents percentage of total OTUs in the read-count groups. The red bar represents percentage of total read contributed by the OTUs in the read-count group

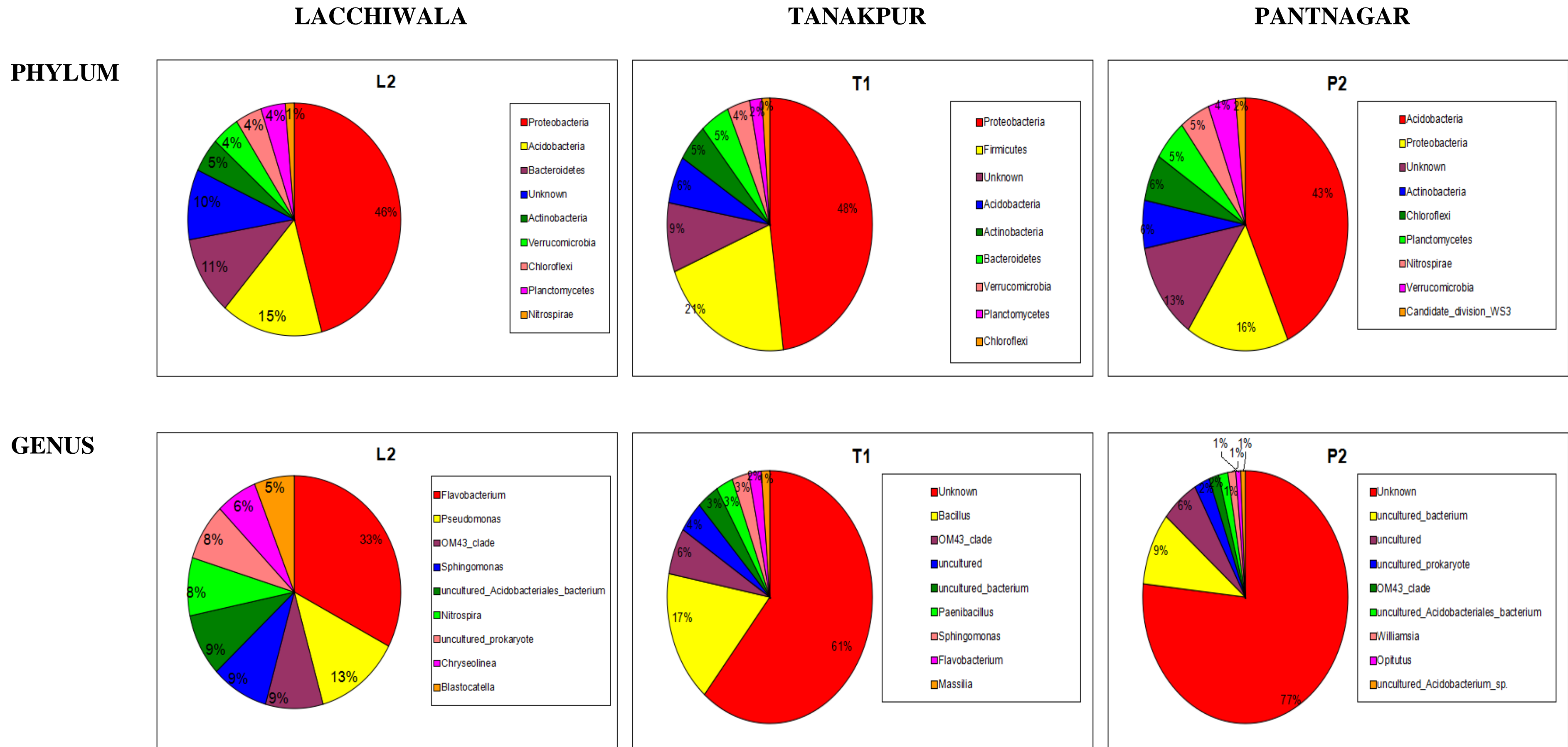


Fig. 4.9: Relative abundance of dominant bacterial phyla in the D.S rhizospheric soil metagenome from three different provenances namely

in Pantnagar relative abundance of phyla is as following; second most dominant Proteobacteria (16%), Actinobacteria (6%), Chloroflexi (6%), Planctomycetes (5%), Nitrospirae (5%), Verrucomicrobia (4%) and Candidate_Division _WS3.

4.5.3 Taxonomic classification of OTU's at Genus level

At the genus level **(Fig. 4.9)**, *Flavobacterium* (33%) amongst Bacteroidetes, *Pseudomonas* (13%), *Sphingomonas* (9%) and *Nitrospirae* (8%) from Proteobacteria and uncultured *Acidobacteriales* (9%) from Acidobacteria were identified, in D.S rhizospheric soil at Lachhiwala. Additionally, *OM_43 Clade* (9%), *Uncultured prokaryote* (8%), *Chryseolinea* (6%) and *Blastocatella* (5%) were also identified. In Tanakpur D.S rhizospheric soil samples most of the sequences (61%) remain unknown. Gram positive Bacilli (17%) belonging to *Firmicute*s were the second most abundant genera after unknown bacteria and identified as potential indicators at the genus level. Others *OM_43 Clade* (6%), *Uncultured* (4%), *Uncultured Bacterium* (3%), *Paenibacillus* (3%), *Sphingomonas* (3%), *Flavobacterium* (2%) *and Massilia* (1%) were present in small proportion. Disease conducive soil of Pantnagar constitutes 94% members to unknown or uncultured category. Only a few genera such as *OM_43 Clade* (2%), *Uncultured Acidobacteriales* (1%), *Uncultured Acidobacterium sp*. (1%), *Williamsia* (1%) and *Opitutus* (1%) were identified at Pantnagar.

4.5.4 Differences in bacterial communities in *Dalbergia sissoo* rhizosphere samples from three provenances

The total number of unique bacterial OTUs detected in all three samples was 4,83,795 where 3,46,228 were associated with Lachhiwala, 39,464 with Tanakpur and 55,784 OTUs only with Pantnagar, while 5,971 were shared by all the three provenances. Unique OTUs shared between the two healthy regions, Lacchiwala and Tanakpur were 10,281 whereas wilted region Pantnagar shared 18,230 unique OTUs with Lachhiwala and 7,837 with Tanakpur **(Fig. 4.10a)**. The total number of unique bacterial taxa amongst *Dalbergia sissoo* rhizosphere soil at all three provenances were 46. Out of which, 35 phyla were common. Three bacterial taxa Lentisphaerae, Candidate_division_WS6 and Candidate_division_OP3 (representing 19 OTUs, 0.027%) were shared at Tanakpur and Lachhiwala representing healthy D.S. rhizosphere. Phyla Deinococcus-Thermus and SAR were shared between Tanakpur and Pantnagar whereas Caldiserica, Candidate_division_SR1, JL-ETNP-Z39, NPL-UPA2,

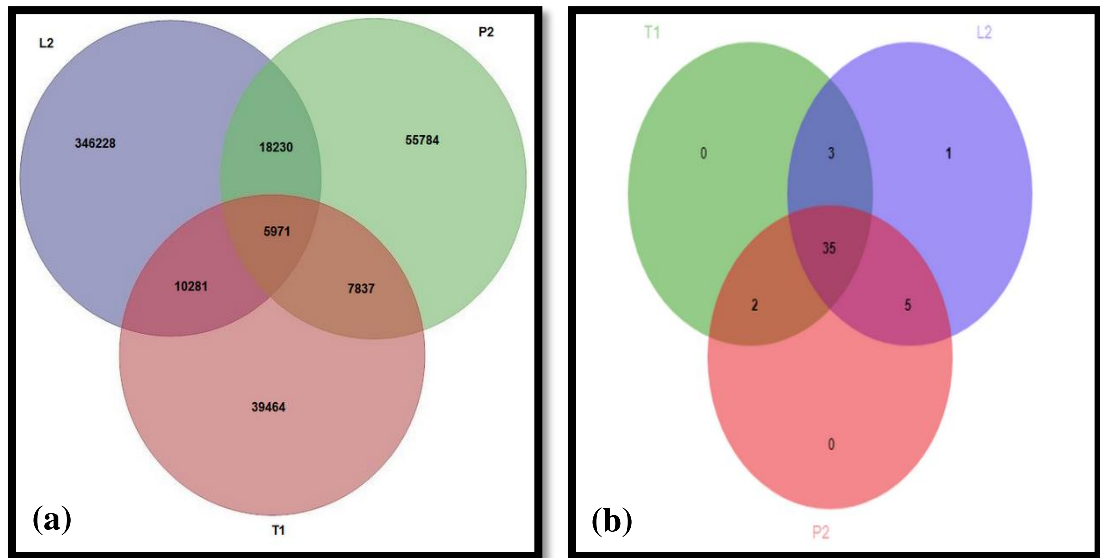


Fig. 4.10: Venn diagram showing the common and unique (a) OTUs (b) Phylum among three *Dalbergia sissoo* provenances

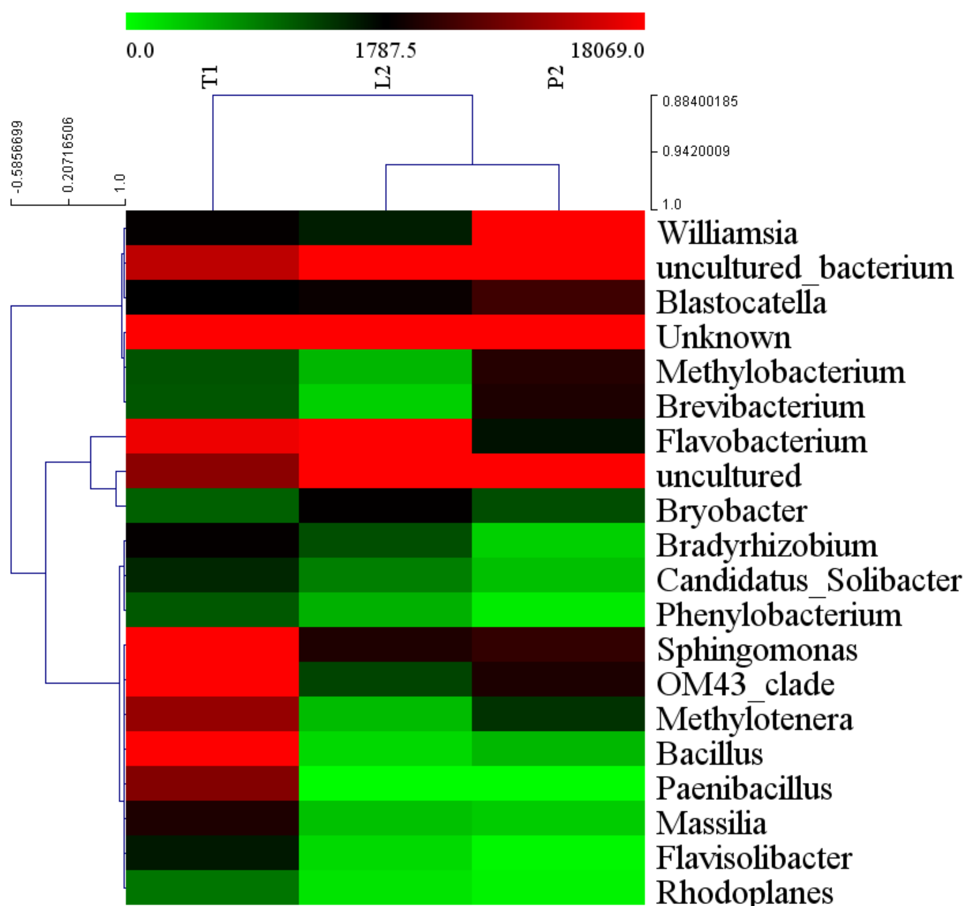


Figure 4.11: Heatmap and hierarchical cluster analysis of bacteria based on the relative abundances of dominant genera from three different *Dalbergia sissoo* provenances

and Thermotogae between Lachhiwala and Pantnagar. A single phylum Deferribacteres was exclusively present in D.S. rhizosphere from Lachhiwala **(Fig. 4.10b)**. The relative abundance of top 20 dominant genera in three different samples are shown in heatmaps and the hierarchical cluster. The distinction between healthy and wilted stands was attributable to the differential abundance of bacterial genera *Flavobacterium, Bradyrhizobium, Candidatus_ Solibacter, Phenylobacterium, Flavisolibacter* and *Rhodoplanes* in D.S. rhizosphere. These genera were relatively more abundant in healthy stands (Tanakpur and Lachhiwala) **(Fig. 4.11)**. However, genera *Williamsia, Blastocatella, Methylobacterium,* and *Brevibacterium* were relatively abundant in D.S. rhizosphere soil from Pantnagar where there was widespread tree mortality.

4.5.5 α diversity in rhizospheric soil samples from three different *Dalbergia sissoo* Provenances

The bacterial diversity of soil samples was analysed by calculating the alpha diversity indices **(Table 4.9).** The coverage of samples was from 67.19% to 88.86%. The total number of OTUs ranged from 63553 to 87822 across all three samples. The OTUs obtained from each sample were shown in rarefraction curves **(Fig 4.12)**. The rhizospheric sample from Pantnagar provenance had highest number of OTUs as well as highest number of Chao1 index (species richness) whereas rhizospheric soil from Tanakpur provenance has lowest number of OTUs as well as lowest number of species. The highest Shannon diversity appeared in rhizospheric sample from Pantnagar whereas lowest appeared in Tanakpur. The Shannon diversity (abundance and evenness) at Lachhiwala provenance was comparable to that of Tanakpur. The count of unique OTUs was highest at Pantnagar soil followed by Lachhiwala and Tanakpur **(Table 4.9).**

Table 4.9: Illumina miseq reads, number of OTUs, estimated OTU richness and diversity indices in rhizospheric soil from *Dalbergia sissoo* provenances at Lachhiwala, Tanakpur and Pantnagar

Taxon	Sample	Reads		Number of OTUs	Coverage (%)	Alpha diversity		
		Raw	Number of effective sequences			Shannon	Chao	Observed species metrices
Bacteria	L	840853	839299	69110	88.86	13.49	66462.34	58913
	T	748477	746951	63553	67.19	13.14	59231.84	56519
	P	958426	956201	87822	77.93	14.12	80562.70	71953

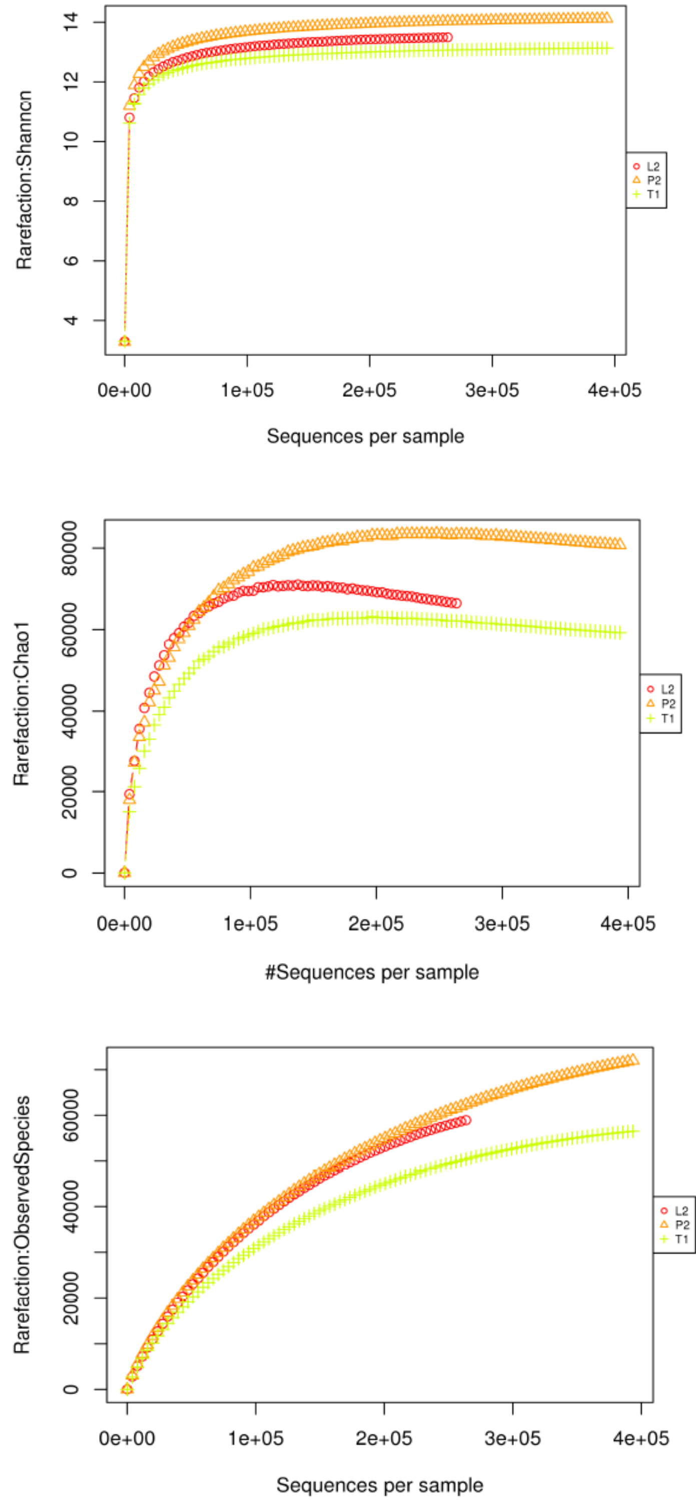


Fig. 4.12: (a) Shannon curve: Alpha diversity was computed using Shannon metrics. (b) Chao1 curve: Alpha diversity was computed using Chao1 metrics. (c) Observed species curve: Alpha diversity was computed using observed species metrics

4.5.6 β diversity analysis

Additionally, weighted (based on the abundance of taxa) UniFrac distance metrics were applied to estimate the β-diversity of samples **(Fig 4.13).** Three samples formed two clusters. The disease-conducive rhizospheric sample from Pantnagar forest formed independent cluster whereas D.S. rhizospheric soil from disease suppressive Tanakpur and Lacchiwala forests aligned in same cluster.

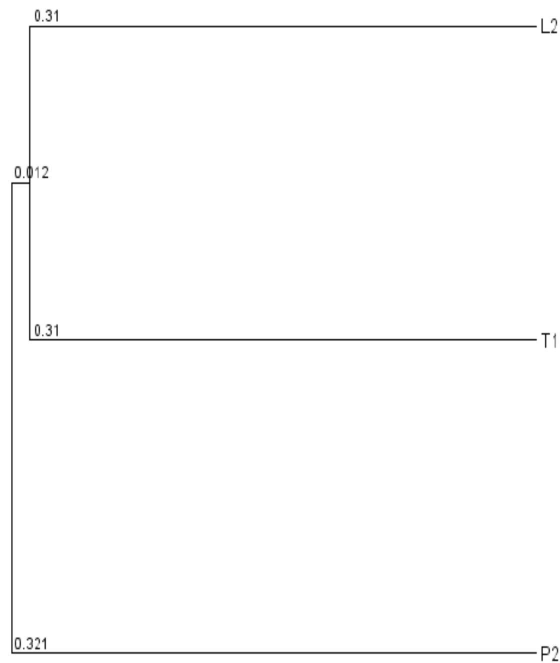


Fig. 4.13: Phylogenetic analysis of bacterial community in *Dalbergia sissoo* rhizosphere in three provenances using weighted unifrac approach (Beta diversity)

Belowground microbiota, in particular soil/rhizosphere/root associated microbial communities affect fitness, development and productivity of trees. Moreover specific members of the microbial communities associated with perennial trees interact with soil fauna. The interactions within different groups of belowground microbiota regulate nutrient cycling, ecosystem functioning and in turn influence tree growth and health. The negative interaction or abundance of pathogens in the soil/ rhizosphere may lead to various diseases and subsequently tree mortality. Most of the previous studies about tree disease have only focused on single host pathogen interaction. The high throughput Illumina sequencing method employed in this study overcame this limitation and obtained thousands of sequences simultaneously to identify as many

bacteria as possible. The soil examined, harboured a diverse bacterial community. At phylum level, most of the bacterial taxon were present in rhizospheric soil from all three provenances but at genus level the diversity and relative abundance of bacterial taxa varied from one sample to other. This suggests that though all the samples are from geographically linked region, bacterial composition at genus level varies. The probable reasons for this variation could be differences in soil nutrient composition that influences its structure and ecological conditions. Hence, soil resident bacterial communities are diverse, a total of 1,72,195 OTUs have been detected in the present study. The different bacterial types and their complex interactions may affect the diversity and abundance of microbes. These microbes can be plant beneficial or plant deleterious/pathogenic. Based on the performance of trees, the soils from provenances were classified as disease suppressive or disease conducive. Soils from Tanakpur and Lachhiwala provenances were considered disease suppressive as no incidence of shisham mortality was reported. Soil from Pantnagar D.S. forests was disease conducive as large scale sissoo mortality was prevalent there.

Dalbergia sissoo rhizospheric bacterial communities from Lachhiwala, Tanakpur, and Pantnagar were analyzed through metagenome sequence analysis to determine the cause and factors responsible for differential performance of trees at three provenances. The species richness and abundance indices were higher at Pantnagar soil (disease conducive) than Tanakpur and Lachhiwala (disease suppressive). Beta diversity estimated through weighted Unifrac distance metrices showed that bacterial diversity (based on OTUs) at all three provenances were closely related. But even then, the relative abundance of OTUs belonging to particular phyla can act as biological indicators of soil and tree health. Proteobacteria and Firmicutes were most abundant phyla at Lachhiwala and Tanakpur whereas Acidobacteria was abundant at Pantnagar. Betaproteobacteria, Gammaproteobacteria, and Firmicutes have been identified as taxa associated with disease suppression **(Mendes *et al.,* 2011)** because several species and strains belonging to these taxa are antibiotic producers. On the other hand α Proteobacteria are known to play a key role in carbon, nitrogen and sulphur cycling **(Rampelloto *et al.*, 2013)**. Several genera within Proteobacteria and Firmicutes such as *Pseudomonas, Flavobacterium, Bacillus* **(Bhattacharyya and Jha, 2012)**, *Paenibacillus* **(Cheong *et al.,* 2005)**, *Sphingomonas* **(Enya *et al.,* 2007)**,

Nitrospirae (**Isobe *et al*., 2014**) and *Massilia* (**Ofek *et al*., 2012; Poupin *et al.,* 2013**) were identified in D.S. rhizosphere **(Vacheron *et al*., 2013; Vessey, 2003).**

These include most common plant growth promoting rhizobacteria (PGPR). *Pseudomonas* and *Bacillus sps* are also considered as promising BCAs because of their ability to produce antibiotics (**Moeinzadeh *et al.,* 2010**). DAPG producing fluorescent psuedomonads are known to suppress soil-borne phytopathogenic diseases (**Raaijmakers and Weller, 1998**). Thus, their presence in rhizosphere can be linked to disease suppression. Several effective biocontrol agents for *Fusarium* wilt belong to these two groups **(Cao *et al*., 2011).** *Bacillus* sp. produce antibiotics, surfactin, and iturin into the rhizosphere and plays a vital role in plant disease suppression and growth promotion (**Kinsella *et al.,* 2009).** *Flavobacterium* is reported as the most significant phosphate solubilizing bacteria (**Mehnaz and Lazarovits, 2006**). Strains of Sphingomonadaceae were more prevalent in tobacco wilt suppressive soil (**Kyselkova´ *et al.,* 2009**). Besides acting as BCAs to winter pathogen (*Fusarium* sp.) under greenhouse conditions (**Wachowska *et al*., 2013**), certain strains of family Sphingomonadaceae are associated with nitrogen fixation (**Adhikari *et al.,* 2001**). This explains the prevalence of Sphingomonas, in the shisham rhizosphere from healthy stands. The occurrence of *Nitrospirae* in a healthy sample indicates good nitrification resulting in enhanced nitrogen uptake by trees (**Daims *et al*., 2015**). *Massilia* species exhibit production of siderophore and indole acetic acid and siderophore, and antagonism towards *Phytophthora infestans* (**Poupin *et al.,* 2013**). In Pantnagar, D.S. rhizospheric soil was abundant in genera like *Williamsia, Opitutus, OM-43 Clade* and *uncultured Acidobacteriales. Acidobacterial* abundance is positively correlated with organic carbon availability (**Jones *et al.*, 2009; Navarrete *et al.,* 2013b**). Moreover, its abundance in tropical soils indicates decreased soil Ca and Mg content and the increase in soil pH. High soil pH is linked to the reduced availability of different macro- and micro-nutrients (**McBride, 1994**). Hence, mortality in Pantnagar D.S. forest stand. The ratio between Proteobacteria and Acidobacteria (P/A) may indicate the general nutrient status of soils (**Smit *et al.*, 2001**). Low P/A ratio is indicative of oligotrophic, while high of copiotrophic soils. Therefore, low Proteobacteria and high Acidobacteria in Pantnagar soil indicate a poor nutrient status of the soil which could be one of the reasons for D.S. mortality. In contrast, the presence of Phyla Lentisphaerae,

Candidate_division_WS6, Candidate_division_OP3, and Deferribacteres at Lachhiwala and Tanakpur could be linked to healthy plantations there. A high relative abundance of Phylum Lentisphaerae has been correlated to low *Fusarium* disease incidence in banana (**Shen and co-workers, 2014**). In addition, the relative abundance of Lentisphaerae was positively correlated with total nitrogen (TON) and ammoniacal nitrogen (NH4-N) contents of soil. Bacteria affiliated to candidate division WS6 well known in anaerobic sediment environment but their biochemical and metabolic functions are not deciphered as yet **(Dojka *et al.,* 2000)**. Hence, the strange enrichment of candidate division WS6 at healthy sites needs to be studied. Similarly, bacteria belonging to candidate division OP3 are highly orthologous with Deltaproteobacteria. The candidate division OP3 has been reported in both oxic and anoxic peat layer probably indicating facultatively anaerobic metabolism **(Glockner *et al*., 2010)**. Members of this group are likely to be complex at the molecular level and possess complex metabolism. The studies regarding its complex metabolic process might reveal ecological interaction in rhizosphere/bulk soil ecosystem and role in maintaining healthy plantations. Phyla Deferribacteres is uniquely present and is known to consist of plant growth promoting bacteria used as commercial bioinoculants. For example, bioformulation as Deferribacteres as one component has been used for seed treatment, incorporated into the soil and nutrient solution in hydroponic systems as PGP and BCA for vegetable crops **(Bettiol, 2014)**. The OTUs classified as *Flavobacterium, Bradyrhizobium, Candidatus_Solibacter, Phenylobacterium, Flavisolibacter,* and *Rhodoplanes* were associated only with the healthy rhizosphere. The possible explanation is that all the mentioned genera include validated PGPRs. Bacterial genus, *Flavobacterium* is ePGPR (existing in rhizosphere) **(Ahmed and Kibrat, 2014)** whereas *Bradyrhizobium* is iPGPR (residing inside nodule) and symbiotically fix atmospheric nitrogen **(Bhattacharya and Jha, 2012)**. While investigating the effect of rhizomicrobiome on plant biomass a positive correlation between the occurrence of *Flavisolibacter, Lutimonas* and *Geodermatophilacae* and plant biomass of soyabean and alph-alpha have been observed **(Xiao *et al*., 2017)**. Similarly, the abundance of phylum Proteobacteria, Bacteriodetes and Actinobacteria mainly genera *Burkholderia, Flavisolibacter* and *Pseudomonas* dynamically changed in relation to different growth stages of maize **(Yang *et al.,* 2017)**. Moreover, during comparative analysis of bacterial communities

associated with organic and conventional farming systems Proteobacteria mainly genera *Rhizobium, Bradyrhizobium, Mesorhizobium, Burkholderia, Stenotrophomonas, Pseudomonas, Sphingomonas,* and *Rhodoplanes* has been documented in organic farming systems, all are plant growth promoting rhizobacteria (PGPB) (**Upchurch *et al.*, 2008**). Several of these are atmospheric N_2 fixing and may play a pivotal role in forest ecosystems as less nitrogen is available there. Hence the presence of the above bacterial OTUs in Lachhiwala and Tanakpur can be related to disease suppressive soil in both the provenances. However entire microbiome cannot be linked to disease suppressive soils. Thus identifying individual bacteria with multiple plant growth promoting attributes and biocontrol potential is more realistic and practically relevant approach. But bacterial community analysis using metagenome were crucial in identifying an initial framework of disease suppressive indicators.

Comparison of the microbial community in the rhizospheric soil of diseased and healthy shisham was systematically assessed and improved our understanding of microecological changes including wilt disease of shisham. Further, this is the first investigation to shed light to hitherto distribution of unexplored rhizospheric bacterial diversity from three D.S. provenances of Uttarakhand region. The characterization of D.S. rhizospheric microbial community structure at phylum and genus level could be useful in screening and identification of potential antagonists. Presence of large numbers of unidentified OTUs indicates the presence of novel bacterial diversity in D.S. rhizosphere which should be further studied with more high- resolution metagenomics techniques.

4.6 Culturable bacterial population count in rhizosphere soil

Total population as enumerated on Angle's medium in *Dalbergia sissoo* rhizospheric soil of of Tanakpur, Lachhiwala and Pantnagar was $2.76x10^{4,}$ $1.87x10^{4}$ and $1.96x10^{4}$ cfu g^{-1} of soil. However count of phosphorus solubilizing bacteria was $1.20x10^{4}$ cfu g^{-1}, $1.55x10^{4}$ cfu g^{-1} and 1.06 $x10^{4}$ cfu g^{-1} soil at Tanakpur, Lachhiwala and Pantnagar respectively (**Table 4.10; Fig 4.14).** The total number of bacterial count recorded in both medium were statistical significant ($P>0.05$) i.e. between Tanakpur, Lachhiwala and Pantnagar. These results were different from the results of molecular analysis. One possible reason could be that cultivation-based approach is nutrient biased and only 1% of the total naturally occurring bacteria are isolated in the

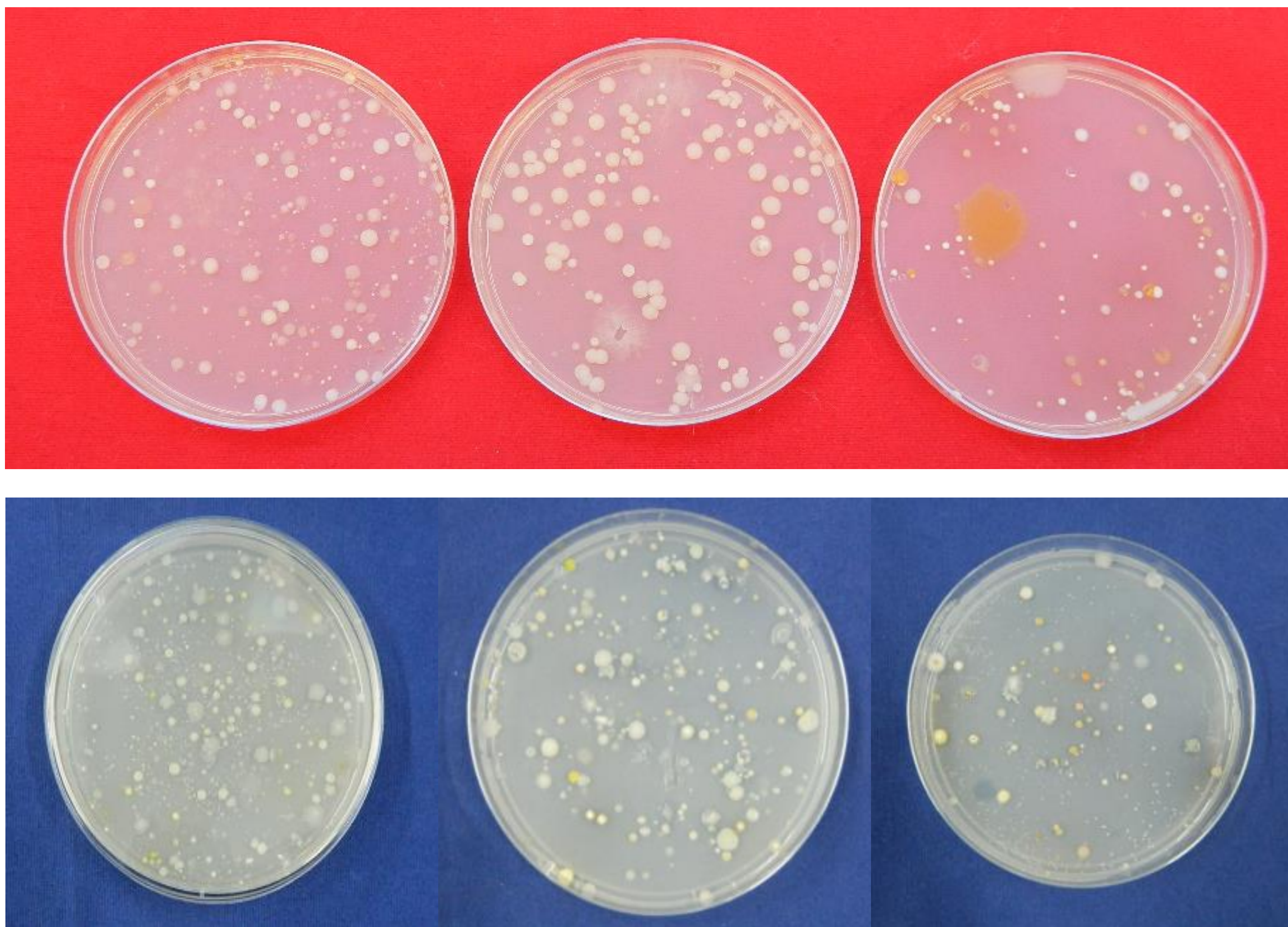


Fig. 4.14: Enumeration of bacterial population from soil in different medium

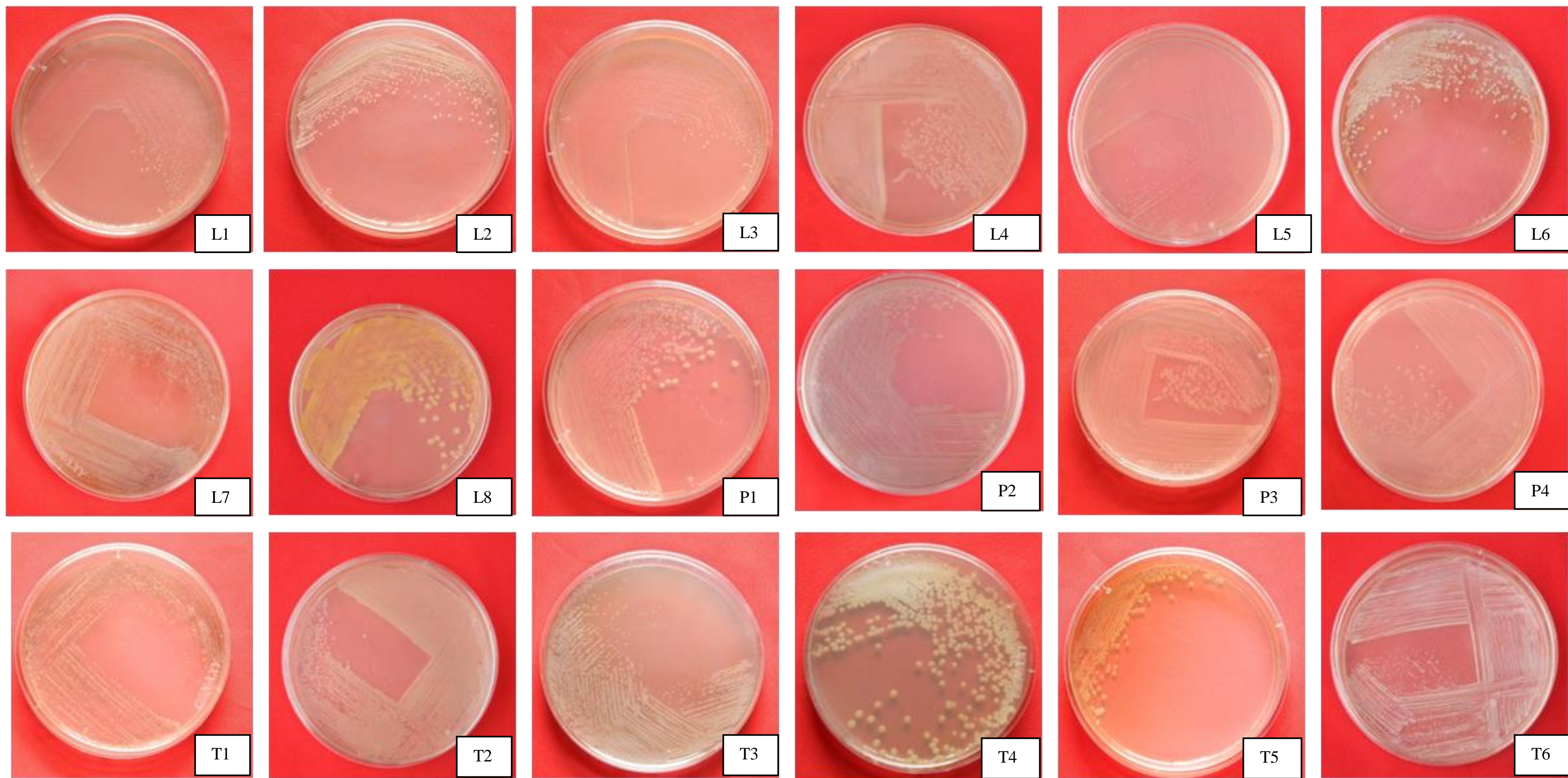


Fig. 4.15: Streaked plates of eighteen bacterial strains recovered from *Dalbergia sissoo* rhizosphere at three different provenances

laboratory (**Torsvik and Overseas, 2002**). However, to have complete glimpse of bacterial diversity associated with soil, culture-independent approaches based on sequencing of 16S rRNA genes were used **(Janssen, 2006)**.

Table 4.10: Enumeration of bacterial population in soil samples on two different medium

Medium (population count)	Pantnagar	Lachiwala	Tanakpur
Angels medium	$1.96X10^{4}$	$1.87X10^{4}$	$2.76X10^{4}$
Pikovaskya medium	$1.06 X10^{4}$	$1.55X10^{4}$	$1.20X10^{4}$

NOTE: Each value is the mean of three replicates. Data was analysed statistically at the 5% ($p<0.05$) level of significance

The present study revealed that PSBs were present at all three D.S. provenances, however their population level varied. This is mainly due to the soil abiotic factors. In a earlier study **Kucey (1983)** detected PSB in almost all soils tested, although varies with soil conditions, climatic factors and cropping history. This large variation in the distribution of PSB in different soils may be due to the differences in organic carbon content of the soil **(Yadav and Singh, 1991).**

The direct relation between soil nutrient status and indigenous living microflora necessitates the study of microbial population directly. Serial dilution method is old but still reliable method to deduce total viable population. Microorganisms make very crucial part of the soil to maintain self-sustainability of soil ecosystem. Microbial population of soil system is a direct indicator of soil health. Population densities of microorganisms in the rhizosphere depends on the physicochemical composition of the rhizospheric soil, changes in soil pH, water potential, partial pressure of oxygen and physical and chemical characteristics of plant exudation (**Griffiths *et al.*, 1999**).

4.7 Phosphorus solubilising bacteria (PSB) recovered from three *Dalbergia sissoo* provenances

In all 18 PSBs, eight from Lacchiwala, four from Pantnagar and six from Tanakpur were recovered on Pikovaskya agar plates from D.S. rhizospheric soil of different provenances **(Fig. 4.15).**

4.7.1 Qualitative screening of p solubilisation potential of bacterial isolates

In all eighteen tricalcium phosphate solubilising bacteria were recovered from Lachhiwala, Tanakpur and Pantnagar respectively. All eighteen bacterial isolates exhibited zone of solubilization in the range 1.16 to 4.75 cm on pikovaskya agar plates **(Fig 4.16a, b).** The isolates from Lachhiwala provenance depicted higher phosphorus solubilising index as compared to Tanakpur and Pantnagar**.** Highest P solubilising index (PSI) was detected in L4 and lowest in T4.

4.7.2 Quantitative estimation of p solubilisation potential of bacterial isolates

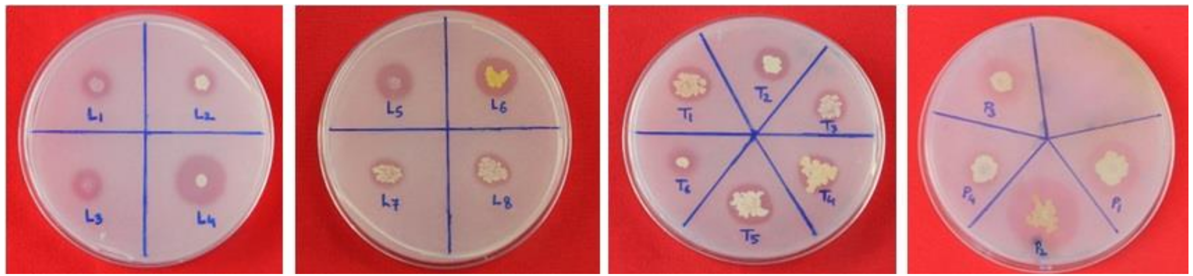
Amongst all eighteen bacterial isolates, L4 solubilized highest amount of phosphorus (891.38 μg/ml) and T4 (285.78 μg/ml) solubilized lowest amount of phosphorus **(Fig. 4.16c).** The solubilizing index of PSBs as detected on pikovaskya agar plates positively correlated with amount of p solubilized in NBRIP liquid medium. Moreover, a correlation between the qualitative and quantitative test could be established for all the tested strains. Correlation analysis indicated that the values of PSI and the amount of soluble P in liquid NBRIP medium containing $Ca_3 (PO4)_2$ shared a highly significant relationship (t value =15.30069) which indicates that the strains with the highest potential to solubilize $Ca_3(PO4)_2$ in liquid media were the same as the ones that exhibited the largest halos.

4.8 Cultural characteristics

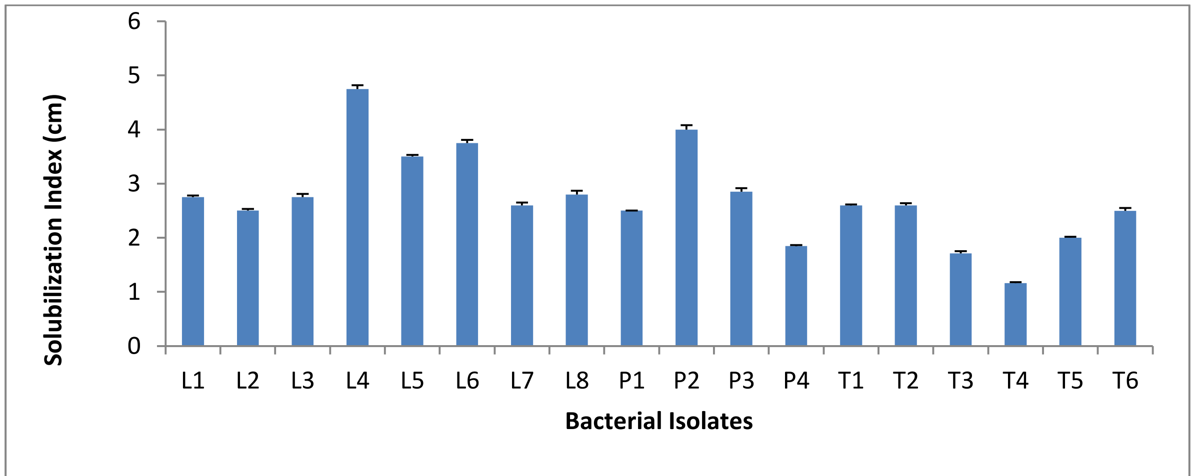
All the isolates were observed for the cultural characteristics on Nutrient agar medium. For all isolates colonies appeared on NA plates within 48 hours of incubation at 30 ºC. The gram's reaction and cell morphology for all bacterial isolates have been summarized in **Table 4.11.**

4.9 Functional characterization of PSB recovered from *Dalbergia sissoo* rhizosphere

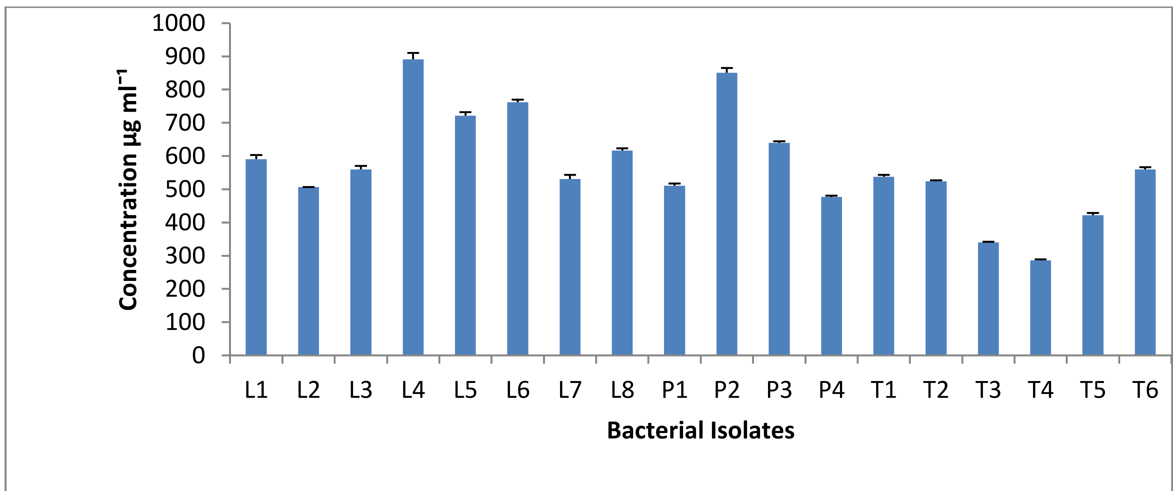
A considerable worldwide research has focused on the exploration of varied agro-ecological niches for the existence of native beneficial micro-organisms. **(Lugtenberg and Kamilova, 2009; Dastager *et al.,* 2010)**. In the current study, rhizobacteria positive for phosphate solubilization were isolated from rhizospheric soil of three different provenanace. These were screened for various enzyme activities and plant growth promotory properties. All PSBs exhibited one or more of enzyme activities; amylase,



(a)



(b)



(c)

Fig. 4.16: Phosphorous solubilization potential of bacterial isolates recovered from *Dalbergia sissoo* Roxb. rhizosphere at Lachhiwala, Pantnagar and Tanakpur (a) Halo zone around bacterial colonies on Pikovskya agar plate (b) Qualitative estimation for tricalcium phosphate solubilization by bacterial isolates (c) Quantitative estimation for tricalcium phosphate solubilization by bacterial isolates in NBRIP-BPB broth medium (pH 7.0) at 30˚C

Table 4.11: Cultural characteristics and description of sites from where bacterial isolates have been recovered

S. No.	Isolate Code	Gram Reaction	Cell Morphology	Location
1	L1	Negative	Small rods	Lachhiwala
2	L2	Positive	Cocci	Lachhiwala
3	L3	Negative	Small rods	Lachhiwala
4	L4	Negative	Small rods	Lachhiwala
5	L5	Negative	Small rods	Lachhiwala
6	L6	Positive	Filamentous	Lachhiwala
7	L7	Positive	Filamentous	Lachhiwala
8	L8	Negative	Small rods	Lachhiwala
9	P1	Negative	Small rods	Pantnagar
10	P2	Negative	Small rods	Pantnagar
11	P3	Negative	Small rods	Pantnagar
12	P4	Negative	Small rods	Pantnagar
13	T1	Positive	Filamentous	Tanakpur
14	T2	Negative	Small rods	Tanakpur
15	T3	Positive	Filamentous	Tanakpur
16	T4	Positive	Coccii	Tanakpur
17	T5	Positive	Filamentous	Tanakpur
18	T6	Positive	Cocci	Tanakpur

Table 4.12: Biochemical characterization of phosphorus solubilizing bacteria from three *Dalbergia sissoo* provenances

Geographical Region	Strains	Amylase			Urea Hydrolysis			Nitrate reduction			Lipase activity			Xylanase activity			Protease activity			Pectin Hydrolysis			Catalase production		
		-	+	++	-	+	++	-	+	++	-	+	++	-	+	++	-	+	++	-	+	++	-	+	++
Lachhiwala	L1	■			■				■		■			■					■			■			■
	L2	■			■					■	■			■				■		■					■
	L3	■			■				■		■			■			■			■					■
	L4	■					■	■			■			■			■			■					■
	L5	■			■				■		■			■					■			■			■
	L6	■			■			■			■			■			■			■			■		
	L7		■		■			■					■			■		■		■			■		
	L8		■		■			■				■				■		■		■					■
Pantnagar	P1	■			■				■		■				■		■					■			■
	P2	■				■				■			■	■					■	■					■
	P3	■			■				■		■			■			■					■			■
	P4	■			■				■		■				■		■					■			■
Tanakpur	T1	■			■			■			■					■	■					■	■		
	T2	■					■		■		■			■			■			■				■	
	T3		■		■			■				■			■		■			■			■		
	T4	■			■			■			■				■		■			■					■
	T5			■	■			■				■				■		■		■				■	
	T6	■					■	■			■			■			■			■				■	

urease, nitrate reductase, lipase, xylanase, protease, pectinase and catalase **(Fig.4.17a; Table 4.12).** Among the eighteen isolates, four L7, L8, T3 and T5 were positive for amylase production. Urease test was found positive for L4, P2, T2 and T6. All the isolates except L4, T1, T3, T4, T5 and T6 exhibited nitrate reduction. Out of eighteen PSBs, five; L7, L8, P2, T3 and T5 were positive for lipase activity. Only eight isolates, L7, L8, P1, P4, T1, T3, T4 and T5 were positive for xylanase production. Five isolates from Lachhiwala (L1, L2, L5, L7 and L8), one each from Pantnagar (P2) and Tanakpur (T5) were positive for protease production. Six of eighteen isolates L1, L5, P1, P3, P4 and T1 were positive for pectinase enzyme production. Except L6, T1 and T3, all the isolates were able to produce catalase as Production of gas bubbles and effervescence was observed after addition of drops of H_2O_2.

The PGPRs stimulate plant growth through various direct and indirect mechanisms. The direct mechanism include production of hormones for example IAA, enzymes (protease, amylase, urease, pectinase and xylanase etc.), metabolites (ammonia and HCN production), acquisition of macro and micro nutrients from soil i.e. solubilization and mineralization of complex organic and inorganic P compounds and chelation of iron and zinc through siderophores. Indirect mechanisms include inhibition of growth of phytopathogens through production of antibiotics or induced systemic resistance in plants **(Richardson *et al.,* 2009)**. Amongst 18 PSBs, seven isolates were able to solubilise Zinc. Zinc solubilization efficiency was highest in L3, L5, P2 and T2 and lowest in L4, P3 and P4. Eight isolates were positive for siderophore production. Size of orange halo was maximum for L7, L8, T1and T3 and minimum for L1**.** IAA production was maximum by L4, P3, T1, T2 and T4 and least by L1, L5, L6, L7, L8, P1, P4, T3 and T5. All the isolates except P2 were negative for HCN production. Ammonia production in peptone water marked by color change from yellow to orange was found positive for all isolates except L6, L7, T1 and T3. Hence, all bacterial isolates exhibited multiple PGP traits in addition to inorganic P solubilization **(Table 4.13; Fig 4.17b).**

Microorganisms with amylase, pectinase, xylanase, lipase and protease activity were not only helpful in organic matter decomposition and plant growth promotion, but also are important in the disease suppression **(Kavamura *et al.,* 2013).** Bacterial strains producing hydrolytic enzymes such as protease, lipase, pectinase, amylase were reported

Table 4.13: PGPR properties of phosphorus solubilizing bacteria from three *Dalbergia sissoo* provenances

Geographical Region	Strains	Zinc solubilisation			Siderophore production			Indole acetic acid production			HCN production			Ammonia production		
Lachhiwala		-	+	++	-	+	++	-	+	++	-	+	++	-	+	++
	L1	■				■			■		■					■
	L2	■			■			■			■				■	
	L3			■	■			■			■					■
	L4		■		■					■	■					■
	L5			■	■				■		■					■
	L6	■			■				■		■			■		
	L7	■					■		■		■			■		
	L8	■					■		■		■				■	
Pantnagar	P1	■			■				■		■					■
	P2			■	■			■				■				■
	P3		■		■					■	■					■
	P4		■		■				■		■					■
Tanakpur	T1	■					■			■	■			■		
	T2			■	■					■	■					■
	T3	■					■		■		■			■		
	T4	■			■					■	■				■	
	T5	■			■				■		■				■	
	T6	■			■			■			■				■	

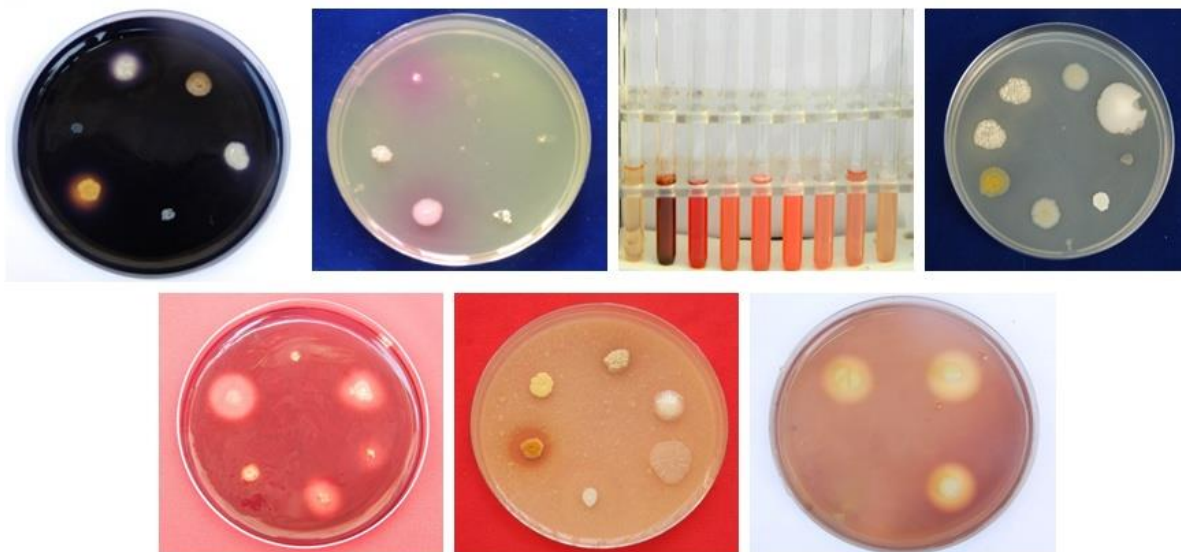


Fig. 4.17 (a): Enzymes and metabolites produced by isolates (a) Amylase (b) Urease(c) Nitrate reduction (d) Lipase (e) Xylanase (f) Protease (g) Pectinase

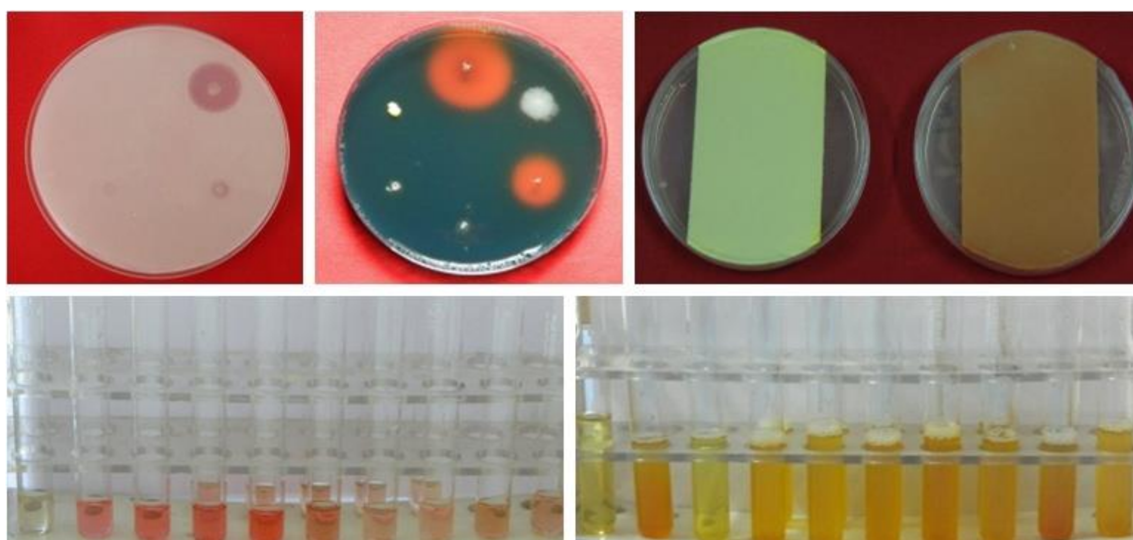


Fig. 4.17 (b): Plant growth promoting properties of isolates (a) Zinc solubilisation (b) Siderophore production (c) IAA production (d) HCN production (e) Ammonia production

to inhibit the growth of pathogenic fungi *F. culmorum* and *F. oxysporum.* **Nielson and Sorensen (1999)** demonstrated that *P. fluorescens* antagonistic to *R. solani* and *Pythium ultimum*, and produced lytic enzymes. The bacterial strains with amylases, pectinases, xylanase and protease production have high commercial value as these constitute a group of industrial enzymes which shares a major part of world enzyme market **(Azad *et al.*, 2009)**.The enzyme urease catalyses the hydrolysis of urea to CO_2 and NH_3 with the concomitant rise in soil pH. Thus, involved in regulation of N supply to plant **(Fazekašová *et al.*, 2012)**. Nitrate reductase activity (NR) which catalyzes the reduction of NO_2^- to N_2O under anaerobic conditions. Nitrate reductase is an adaptive enzyme synthesized only in the presence of NO_3^- ions. Hence, its activity is commonly used as an indicator for ability of plants to utilize NO_3^- from the soil **(Barford and Lajtha, 1992).**

In soil, both macro and micronutrients undergo a complex dynamic equilibrium between soluble and insoluble forms. The equilibrium is strongly influenced by the soil pH which can be shifted by the microbiota ultimately affecting their accessibility to plant roots for absorption. The beneficial effect of PGPR in maintaining adequate levels of mineral nutrients especially the P in crop production had been previously reported **(Rodriguez and Fraga, 1999; Saravanan *et al.*, 2007)**. In our study, all 18 bacterial isolates were efficient phosphate solubilizers. The ability of PGPR strains to solubilize insoluble P and convert it to plant available form is an important characteristic under conditions where P is a limiting factor for crop production. The soil phosphate solubilizing bacterial strains can increase the availability of phosphorus to plant by mineralizing organic phosphorus compounds and converting inorganic phosphorus into more available form **(Bar-Yosef *et al.,* 1999)**. Phosphate solubilization is mainly due to the production of microbial metabolites including organic acids. The production of organic acid decreases the pH and makes phosphorus available **(Puente *et al.*, 2004; Sahin *et al.*, 2004).** The presence of P-solubilizing microbial population in soils may be considered a positive indicator of use of microbial biofertilizers.

Most natural soils are deficient in soluble zinc. However, rhizospheric microorganisms can solubilise Zn in plant available forms **(Saravanan *et al.*, 2003).** Out of eighteen isolates recovered in present study, seven isolates were positive for Zn solubilisation. Production of organic acids is the prominent mechanism for Zn solubilisation by rhizobacteria **(Pietr *et al.*, 1990)**.

Siderophores may enhance plant growth by mobilizing metal cations including Fe and Cu (**Gururani *et al.,* 2012**). Indirectly stimulate P solubilization and disease suppression **(Wahyudi *et al.,* 2011; Hamdali *et al.,* 2008).** Applied P fertilizers readily form complexes with soil cations such as Fe, Ca and Al. Siderophore positive PGPRs scavenge Fe^{3+} from complex compounds under iron starvation condition and thus indirectly release P in soil **(Sharma *et al.*, 2013).** Moreover, they deprive phytopathogen from iron and hence lead to disease suppression. Out of 18 isolates, five isolates exhibited yellow to orange halo zone on CAS amended NA plates were positive for siderophore production.

Phytohormone production is also the plant beneficial trait. IAA production by bacteria enhances the root system which inturn increases plant nutrient uptake **(Ribeiro and Cardoso, 2012).** In the present study, out of 18 isolates, 14 were able to produce IAA in varying quantity. The IAA production varies among different bacterial species and strains. It is also influenced by culture conditions, growth stage and substrate availability (**Sridevi and Veera, 2007**).

Although HCN plays an important role in disease suppression **(Ramette *et al.,* 2003**). It sometimes is deleterious to plants (**Bakker and Schippers, 1987; Alströmand Burns, 1989).** In the current study only one isolate was positive for HCN production.

Production of ammonia helps in plant growth directly by supplying nitrogen to plants **(Marques *et al.,* 2010)** and indirectly by suppressing plant pathogens **(Minaxi *et al.,* 2012**).

4.9.1 Intrinsic Antibiotic Resistant (IAR) pattern of shisham associated rhizobacteria

Rhizobacterial strains differed considerably in resistance to antibiotics **(Table 4.14).** To investigate the sensitivity of bacteria to routinely used antibiotics, and to find naturally resistant antagonistic bacteria, all were checked for sensitivity to eight antibiotics. Bacteria L5 and T2 were resistant to all eight antibiotics (tested bacteria grew well on plates containing antibiotic). Out of eighteen isolates most of the bacteria exhibited resistance towards ampicillin (10mcg), cephalothin (30mcg), chloramphenicol (30mcg), clindamycin (2mcg), erythromycin (15mcg), oxacillin (1mcg) and vancomycin (30mcg) except gentamicin (10mcg). The results revealed that L2, L6, L7, T1, T3, T4 and T5 were sensitive to all antibiotics (**Table 4.14; Fig 4.18**).

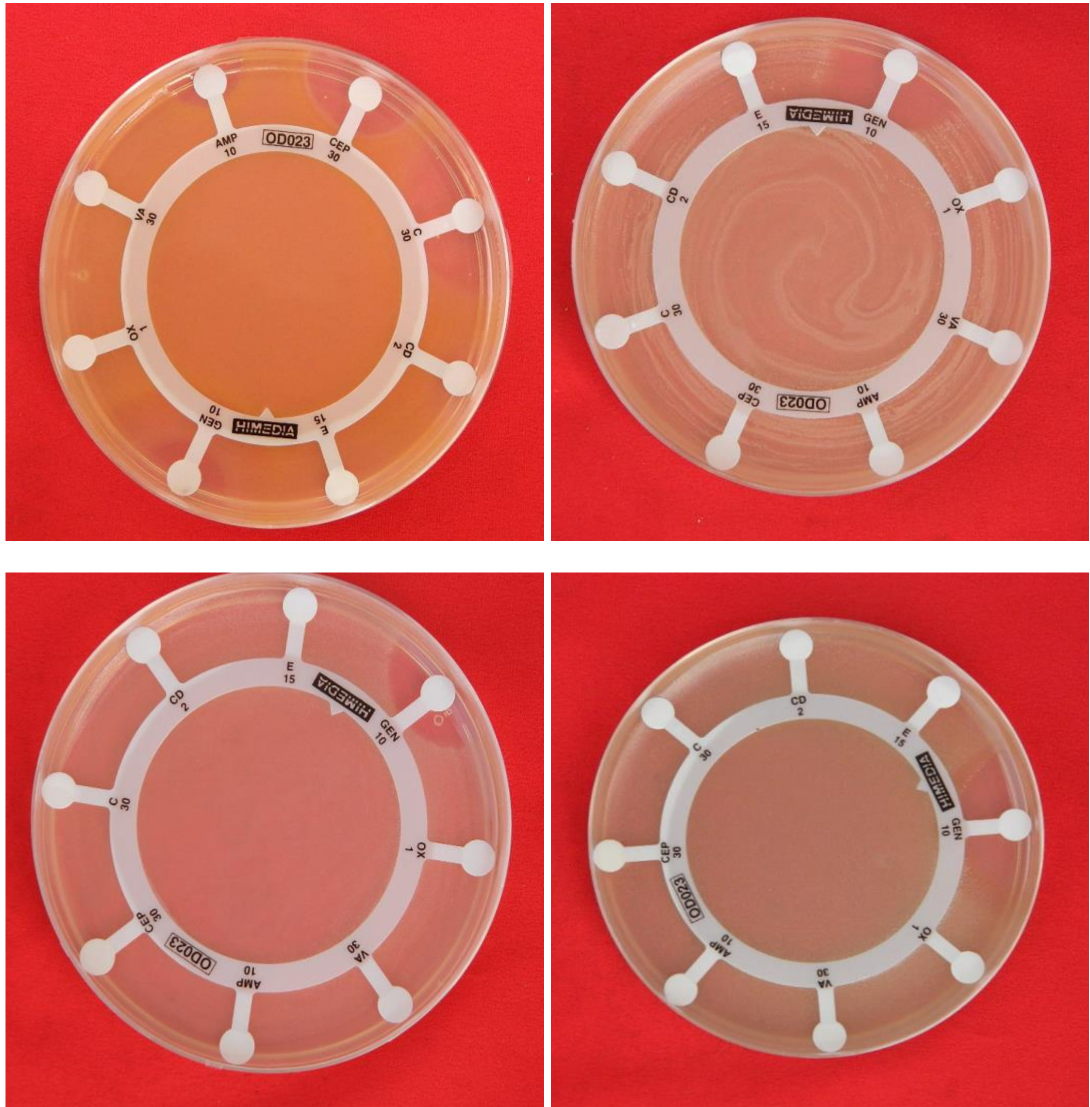


Fig 4.18: Antibiotic sensitivity of bacterial isolates towards different antibiotics

Table 4.14: Antibiotic sensitivity profile of phosphorus solubilizing bacteria from three *Dalbergia sissoo* provenances

Geographical Locations	ID	Ampicillin (10 mcg)	Cephalothin (30 mcg)	Chloramphenicol (30 mcg)	Clindamycin (2 mcg)	Erythromycin (15 mcg)	Gentamicin (10 mcg)	Oxacillin (1 mcg)	Vancomycin (30 mcg)
Lachhiwala	**L1**	R	R	R	R	R	S	R	R
	L2	S	S	S	S	S	S	S	S
	L3	R	R	R	R	R	S	R	R
	L4	R	S	R	R	R	S	R	R
	L5	R	R	R	R	R	R	R	R
	L6	S	S	S	S	S	S	S	S
	L7	S	S	S	S	S	S	S	S
	L8	S	S	S	S	S	S	R	R
Pantnagar	**P1**	R	R	R	R	R	S	R	R
	P2	R	R	R	R	R	S	R	R
	P3	R	R	R	R	R	S	R	R
	P4	R	R	R	R	R	S	R	R
Tanakpur	**T1**	S	S	S	S	S	S	S	S
	T2	R	R	R	R	R	R	R	R
	T3	S	S	S	S	S	S	S	S
	T4	S	S	S	S	S	S	S	S
	T5	S	S	S	S	S	S	S	S
	T6	S	S	S	R	R	S	S	S

Bacteria acquire resistance to antibiotics either through a genetic mutation or horizontal transfer of antibiotic resistance genes **(Spain and Alm, 2003).** Furthermore, the increased use of antibiotics in agriculture and healthcare is also contributing to antibiotic resistance in bacteria. Bacteria that acquire resistance to antibiotics have added ecological advantage to survive in an environment affected with multiple stresses including antibiotics.

4.10 Molecular characterization

4.10.1 Amplification of 16S rDNA gene

PCR amplification of 16S rDNA gene region of all eighteen phosphate solubilizing isolates recovered from shisham rhizosphere of different provenances resulted in a distinct band of 1492 bp **(Fig. 4.20).**

4.10.2 Restriction analysis of amplified 16S rDNA gene region

When restriction analysis of PCR amplified 1492 bp 16S rDNA gene region of all eighteen PSB isolates with three restriction endonucleases *Alu* I **(Fig. 4.21a)**, *BsuI* **(Fig. 4.21b)** and *Msp* I **(Fig. 4.21c)** was performed, 1-4 well resolved bands of 1000-100 bp were observed on electrophoresis in 2.5% agarose gel.

Restriction pattern with enzyme *Alu I*

When 16S rDNA amplicon was digested with restriction enzyme *AluI*, 2 to 4 well resolved bands of 700bp to 100 bp were observed in all eighteen isolates. Restriction endonuclease *AluI* resolved all 18 strains into eight different genotypes **(Fig. 4.21a)**.

Restriction pattern with enzyme *Bsu I*

The restriction pattern of amplified 16S rDNA region with restriction enzyme *AluI* resulted in 2 to 4 well resolved bands in a range from 1000bp to 100 bp. The restriction with *Bsu I* resolved all 18 strains into six different genotypes **(Fig. 4.21b)**.

Restriction pattern with enzyme *Msp I*

The restriction profiles obtained with *Msp* I enzyme resulted in one to three well resolved bands in a region from 200bp to 600bp. All eighteen isolates were distinguished into eight genotypes **(Fig. 4.21c)**.

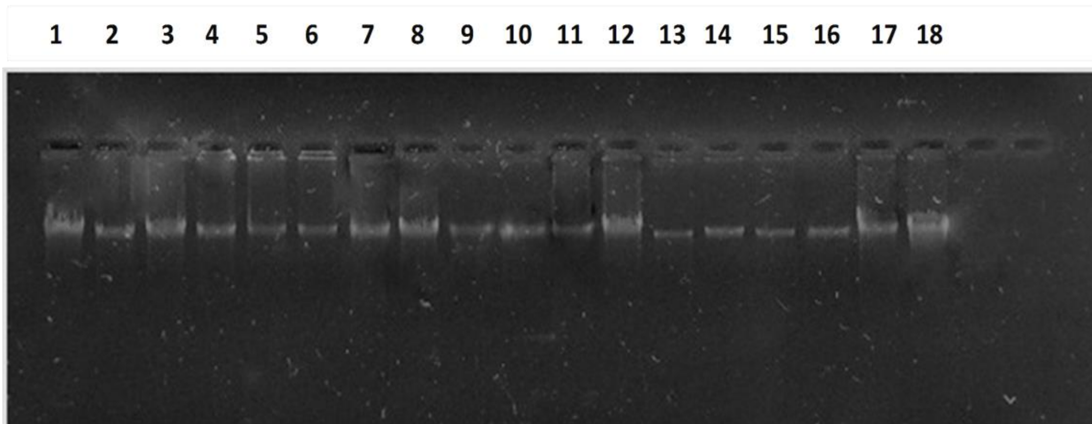


Fig. 4.19: Genomic DNA of the bacterial cultures. Lanes: 1. L1; 2. L2; 3. L3; 4. L4; 5. L5; 6. L6; 7. L7; 8. L8; 9. P1; 10.P2; 11.P3; 12.P413.T1; 14.T2; 15.T3; 16.T4; 17.T5; 18.T6

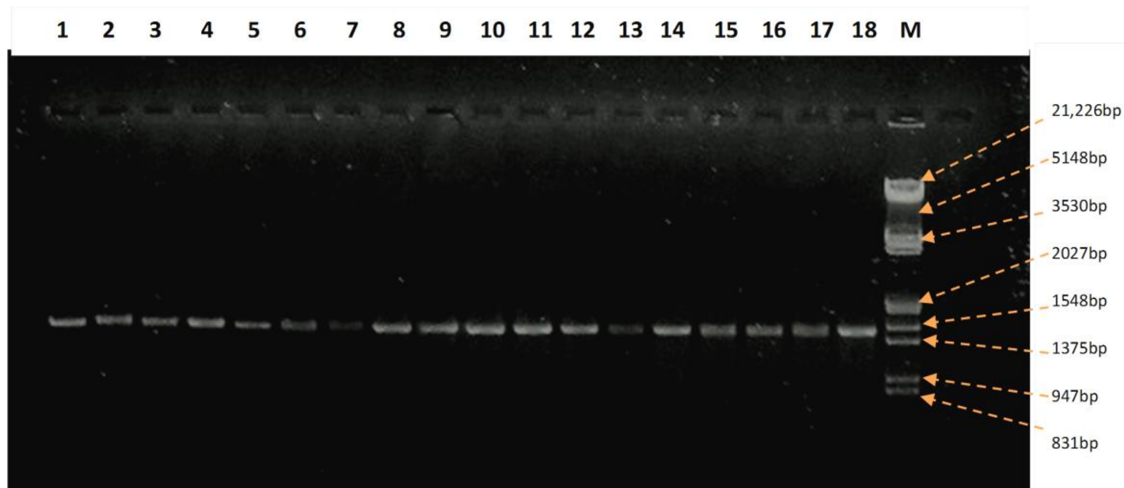


Fig. 4.20: 16SrRNA amplification of the bacterial cultures. Lanes1. L1; 2. L2; 3. L3; 4. L4; 5. L5; 6. L6; 7. L7; 8. L8; 9. P1; 10.P2; 11.P3; 12.P4; 13.T1; 14.T2; 15.T3; 16.T4; 17.T5; 18.T6 lane 19= M= Lambda DNA/Eco RI/Hind III Double digest ladder

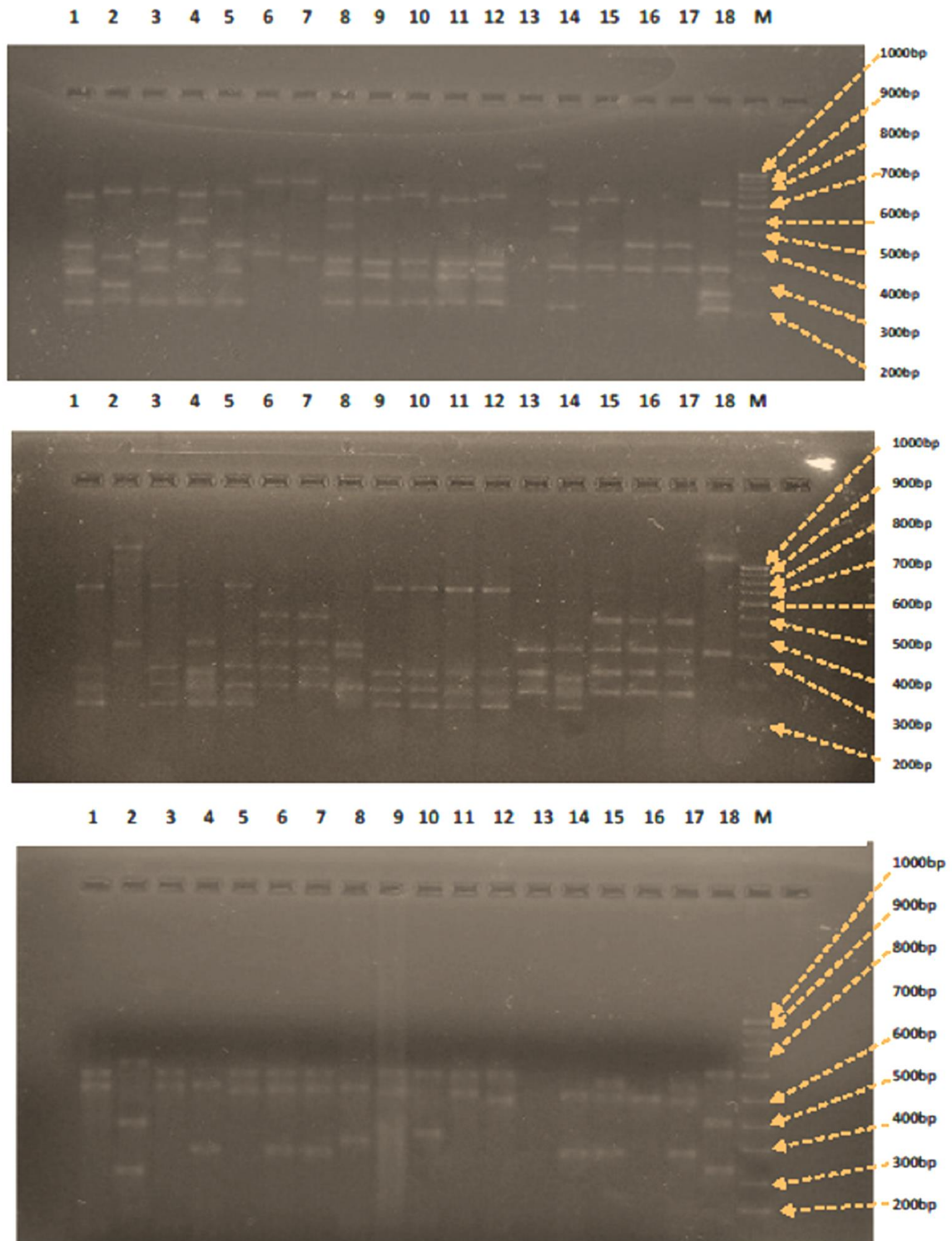


Fig. 4.21 : Restriction profile of 16S rDNA using (a) *Alu*I, (b) *BsuR* I and (c) *Msp*I. Lane 1-18= Isolates, lane 19= M= stepup 100 bp ladder

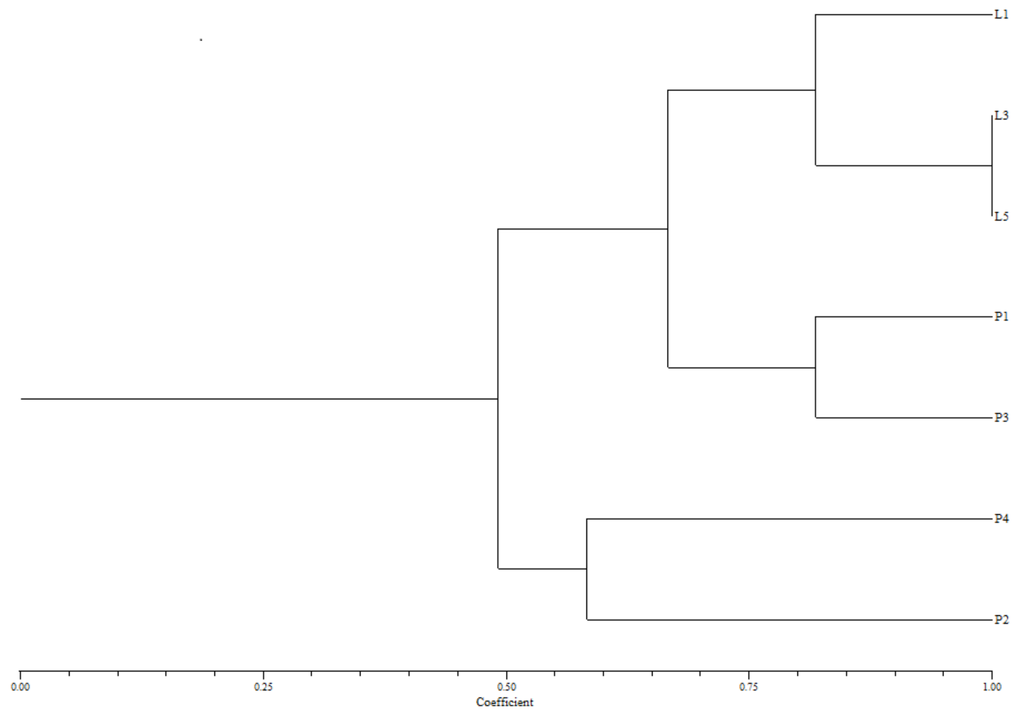
4.10.3 A combined UPGMA dendogram based on ARDRA profiles with *Alu* I, *BsuI* and *Msp* I

An unweighted pair group means average (UPGMA) dendogram calculating Jaccard's coefficient was constructed based on analysis of the ARDRA profile of 16S rDNA region with *Alu* I, *BsuI* and *Msp* I through NTSYSpc version 2.0 software. Restriction profile was interpreted on the basis of bands developed **(Fig. 4.21)**. Similar banding patterns obtained after combination of the three independent digestions were grouped. The isolates depicted higher polymorphism with *Alu*I and *MspI* as compared to *BsuI*. Eight different restriction patterns were obtained with with *Alu*I and *MspI* whereas six with *BsuI*.

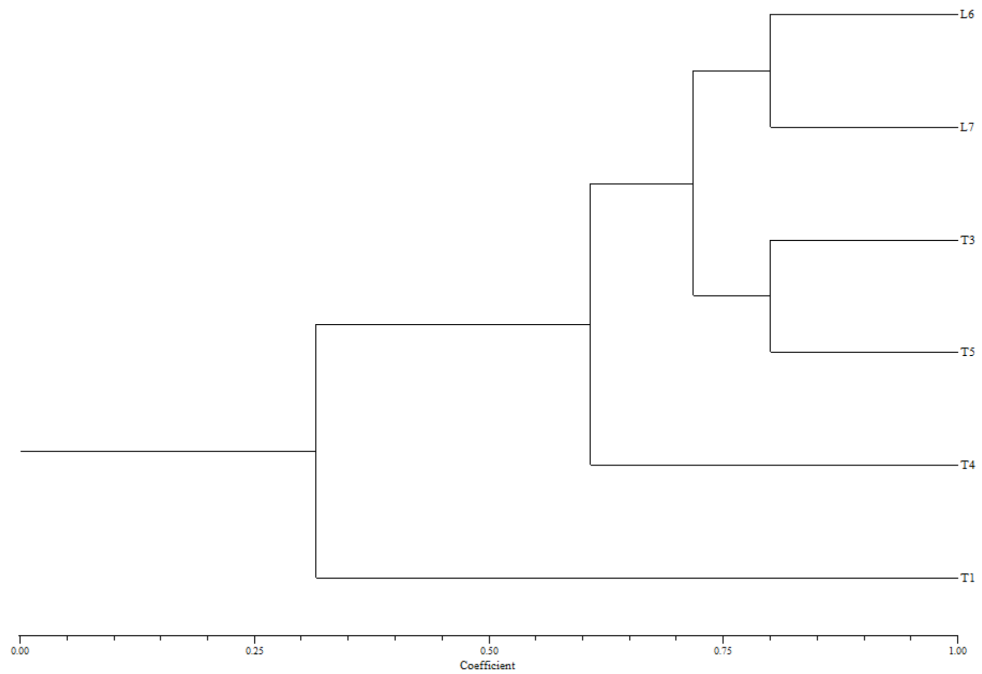
Phylogenetic relationship within gram negative and gram positive isolates were revealed in separate UPGMA clusters. In a UPGMA cluster based on RFLP with *Alu* I, *BsuI* and *Msp* I, all gram negative strains grouped into two major clusters A and B **(Fig. 4.22a)**. Cluster A included five isolates L1, L3, L5, P1 and P3. The cluster A was further divided into two subclusters. Subcluster I included L1, L3 and L5 and subcluster II grouped P1 and P3. L3 and L5 in subcluster I exhibited 100% similarity and was related to L1 at a distance of 0.80 on Jaccard's scale. Cluster B included the remaining strains P4 and P2 related at a distance of 0.60 Jaccard's scale.

For gram positive bacteria a separate dendogram was constructed **(Fig. 4.22b)**. Majority of gram positive isolates were placed in a single cluster which was further divided into two subclusters at a distance of 0.80 on Jaccard's scale. Subcluster I included two isolates L6 and L7 whereas subcluster II included T3 and T5. Isolate T4 was placed singly on an outlying branch at a distance of 0.60 on Jaccard's scale. Isolate T1 was distantly (0.35on Jaccard's scale) related to all the other strains.

The use of 16SrRNA gene alone is not sufficient for differentiating among closely related species. Therefore, analysis of one or more of housekeeping genes is carried out for genetic diversity studies **(Grimont, 2002; Rangel-Castro *et al*., 2002; Gomila *et al.,* 2007; Naik *et al*., 2008; Mehri *et al.,* 2011).**



(a)



(b)

Fig. 4.22: Combined UPGMA dendrogram of 16S rDNA region of isolates on the basis of ARDRA with *Alu*I, *BsuR*I and *Msp*I

4.11 Identification based on sequencing of 16S rRNA gene of PSB isolates from D.S. rhizosphere.

Bacterial isolates were identified by comparison of 16S rDNA sequences with reference strains using BLASTN programme. Out of eighteen isolates, seven were identified within genus *Pseudomonas*. Out of these seven, 3 isolates were from Lachhiwala (L1, L3 and L5) whereas four were from Pantnagar (P1, P2, P3 and P4). Out of remaining eleven, four isolates were identified as *Streptomyces sp.* (L6, L7, T3 and T5), two each as *Klebsiella sp.* (L4 and T2) and *Staphylococcus sp.* (L2 and T6), and one each as *Pantoea sp*. (L8), *Kitasatospora sp*. (T1) and *Micrococcus sp*. (T4).

Several members within these genera are identified as exhibiting plant growth promoting properties for example: *Pantoea, Pseudomonas* and *Streptomyces* **(Sturz *et al.,* 2000),** *Klebsiella* and *Micrococcus* **(Felici *et al.*, 2008; Forchetti *et al.,* 2007; Swain and Ray, 2009),** *Kitasatospora* **(Shrivastava *et al.*, 2008)**, *Staphylococcus* **(Tariq *et al.*, 2010 ;Berg, 2009; Soylu *et al.*, 2005).** Several isolates of these genera have been previously described as P solubilizers **(Seong *et al.*, 1996; de-Bashan and Bashan, 2004; Chung *et al.*, 2005; Pérez *et al.*, 2007)** depicting antagonistic properties **(Berg *et al.,* 2006).** The 16S rDNA sequences of all eighteen isolates are deposited in NCBI GenBank under accession numbers MG966339-MG966355 (**Table 4.15**).

4.12 Amplification of *pqq*C and *pqq*A gene from Bacterial Isolates

Pyroloquinoline quinone (PQQ), is a cofactor for enzyme glucose dehydrogenase **(Kaur *et al.,* 2006)**. Glucose dehydrogenase enzyme is involved in gluconic acid synthesis a major P solubilizing mechanism exhibited by rhizobacteria specially Pseudomonads. PQQ operon consists of six genes of which *pqq* C and *pqq* A are important. *Pqq* C gene encodes the pyrroloquinoline quinone synthase C, which catalyzes cyclization and oxidation of the intermediate 3a-(2-amino-2-carboxy-ethyl)-4,5-dioxo83 4,5,6,7,8,9-hexahydroquinoline-7,9-dicarboxylic acid to PQQ a final step of PQQ biosynthesis and *pqq*A gene encode precursors i.e. tyrosine and glutamate required for the synthesis of PQQ. In all sixteen bacterial isolates showed positive amplification for 82 bp *pqq* C gene whereas six for 72 bp *pqq* A gene. All six isolates with positive amplification for both *pqq* C and *pqq* A genes suggests that they possess two crucial genes of PQQ biosynthesis pathway. Therefore, isolates with positive *pqq* C and *pqq* A gene amplification might solubilise phosphorus via gluconic acid mediated mechanism **(Ge *et al.,* 2013) (Fig. 4.23a; Fig. 4.23b).**

Table 4.15: Molecular characterization of the phosphate solubilizing bacterial isolates based upon 16S rDNA sequences

Strain	Isolate	Percent Similarity	NCBI Gen Bank Accession no.
L1	*Pseudomonas simiae*	98.14%	MG966339
L2	*Staphylococcus petrasii*	97.98%	MG966340
L3	*Pseudomonas paralactis*	99.16%	MG966341
L4	*Klebsiella variicola*	99.51%	MG966342
L5	*Pseudomonas paralactis*	99.17%	MG966343
L6	*Streptomyces curacoi*	87.00%	MG966344
L7	*Streptomyces cellostaticus*	95.00%	MH031699
L8	*Pantoea conspicua*	96.83%	MG966345
P1	*Pseudomonas hunanensis*	98.89%	MG966346
P2	*Pseudomonas aeruginosa*	97.00%	MG966347
P3	*Pseudomonas putida*	97.00%	MG966348
P4	*Pseudomonas plecoglossicida*	98.42%	MG966349
T1	*Kitasatospora kifunensis*	93.86%	MG966350
T2	*Klebsiella singaporensis*	96.37%	MG966351
T3	*Streptomyces antibioticus*	94.22%	MG966352
T4	*Micrococcus yunnanensis*	98.00%	MG966353
T5	*Streptomyces griseoruber*	97.92%	MG966354
T6	*Staphylococcus pasteuri*	98.20%	MG966355

It is well established that P solubilisation in the genus *Pseudomonas* is via PQQ mediated synthesis of gluconic acid **(Meyer *et al.,* 2011; Azziz *et al.,* 2012; Oteino *et al.,* 2015)**. High PQQ-producing bacteria have been identified in bacteria of diverse genera, including *Acinetobacter, Ancylobacter, Gluconobacter, Hyphomicrobium, Klebsiella, Paracoccus, Polyporus, Pseudomonas, Methylobacillus, Methylophilus, Methylovorus, Methylobacterium, Mycobacterium, Thiobacillus, and Xanthobacter* **(Xiong *et al.,* 2011).** Insilico studies have shown that PQQ operon in *Klebsiella*

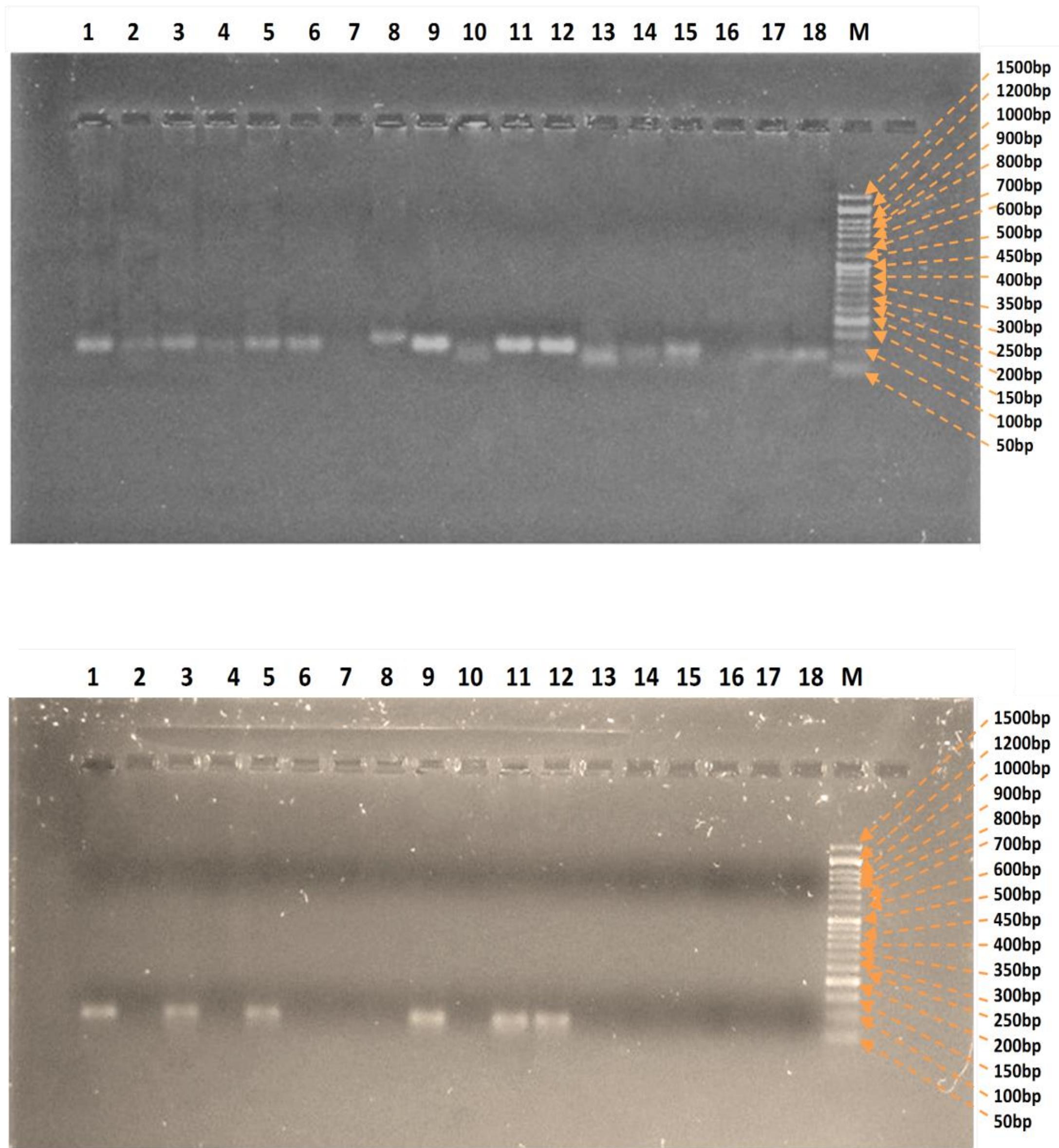


Fig 4.23 : Partial (a) *pqq* C and (b) *pqq* A amplification of the bacterial cultures. Lanes: M. 50 bp DNA ladder Lanes: 1. L1; 2. L2; 3. L3; 4. L4; 5. L5; 6. L6; 7. L7; 8. L8; 9. P1; 10.P2; 11.P3; 12.P413.T1; 14.T2; 15.T3; 16.T4; 17.T5; 18.T6 Lanes: M. 50 bp DNA ladder

pneumonia and *Pantoea ananatis* SC17(0) strain are structurally homologous (**Andreeva *et al.,* 2010; Castagno *et al.,* 2011; Meulenberg *et al.,* 1992**). The PQQ biosynthesis pathway is also present in *Klebsiella pneumoniae*, *Methylobacterium extorquens AM1*, *Gluconobacter oxydans 621H*, *Rahnella aquatilis* and *Streptomyces rochei* (**Shen *et al.,* 2012**). In present study, several strains did not show amplification of *pqq* C and *pqq* A genes. However, they were solubilizing phosphorus on pikovaskya medium. These strains might be solubilizing phosphate *via* secretion of organic acids other than gluconic acid such as lactic, isovaleric, isobutyric, acetic, glycolic, oxalic, malonic and succinic acid. Furthermore, acid production is not the only p solubilizing mechanism in bacteria **(Illmer and Shinnera, 1995).** For example, genetically manipulated *E. coli* JM109 shows MPS activity without alteration in pH of the medium (**Kim *et al.,* 1997**). A phosphoenol pyruvate carboxylase (*pcc*) gene from *Synechococcus* PCC 7942 **(Aditi *et al.,* 2009)** and (*gabY*) from *Pseudomonas cepacia* **(Khan *et al.,* 1995)** were involved in MPS**.** *Serratia marcescens* solubilizes phosphorus via gluconic acid synthesis but it has no genes homologous to PQQ or GDH genes **(Krishnaraj and Goldstein, 2001).**

4.13 Phylogenetic analysis of the 16S rRNA gene

All eighteen strains were identified as belonging to 7 genera distributed across three phyla; Proteobacteria, Actinobacteria and Firmicutes. The genera identified were; *Pseudomonas*, *Klebsiella, Streptomyces, Pantoea, Kitasatospora, Micrococcus* and *Staphylococcus* (**Fig. 4.24; Table 4.15**). Seven strains were identified as L1 (98.14% similarity to *Pseudomonas simiae* strain NR 042392.1), L3 and L6 (99.16% similarity to *Pseudomonas paralactis* strain KP756923), P1 (98.89% similarity to *Pseudomonas hunanensis* strain JX545210), P2(97% similarity to *Pseudomonas aeruginosa* strain NR 117678.1), P3 (98.14% similarity to *Pseudomonas putida* strain Z76667.1) and P4 (98.42% similarity to *Pseudomonas plecoglossicida* strain NR 114226.1). Strain L8 was identified as *Pantoea sp.* (96.83% similarity to *Pantoea conspicua* strain NR 116247.1). Two strains L4 and T2 were identified as *Klebsiella sp*. (99.51% similarity to *Klebsiella variicola* strain CP010523 and 96.37% similarity to *Klebsiella singaporensis* strain AF250285). Strain L2 was assigned to (97.98%) *Staphylococcus petrasii* (NR 118450.1) and T6 to *Staphylococcus pasteuri* (NR 114435.1). Isolates belonging to phylum Actinobacteria were clustered together which includes T4 (98.0%

similarity to *Micrococcus yunnanensis* strain NR 116578.1), T1 (93.86% similarity to *Kitasatospora kifunensis* strain NR 112085.2), L6 (87% similarity to *Streptomyces curacoi* strain KY585954.1), L7 (95% similarity to *Streptomyces cellostaticus* strain NR 112304.1), T3 (94.22% similarity to *Streptomyces antibioticus* strain NR 043348.1), T5 (97.92% similarity to *Streptomyces griseoruber* strain NR 041086.1).

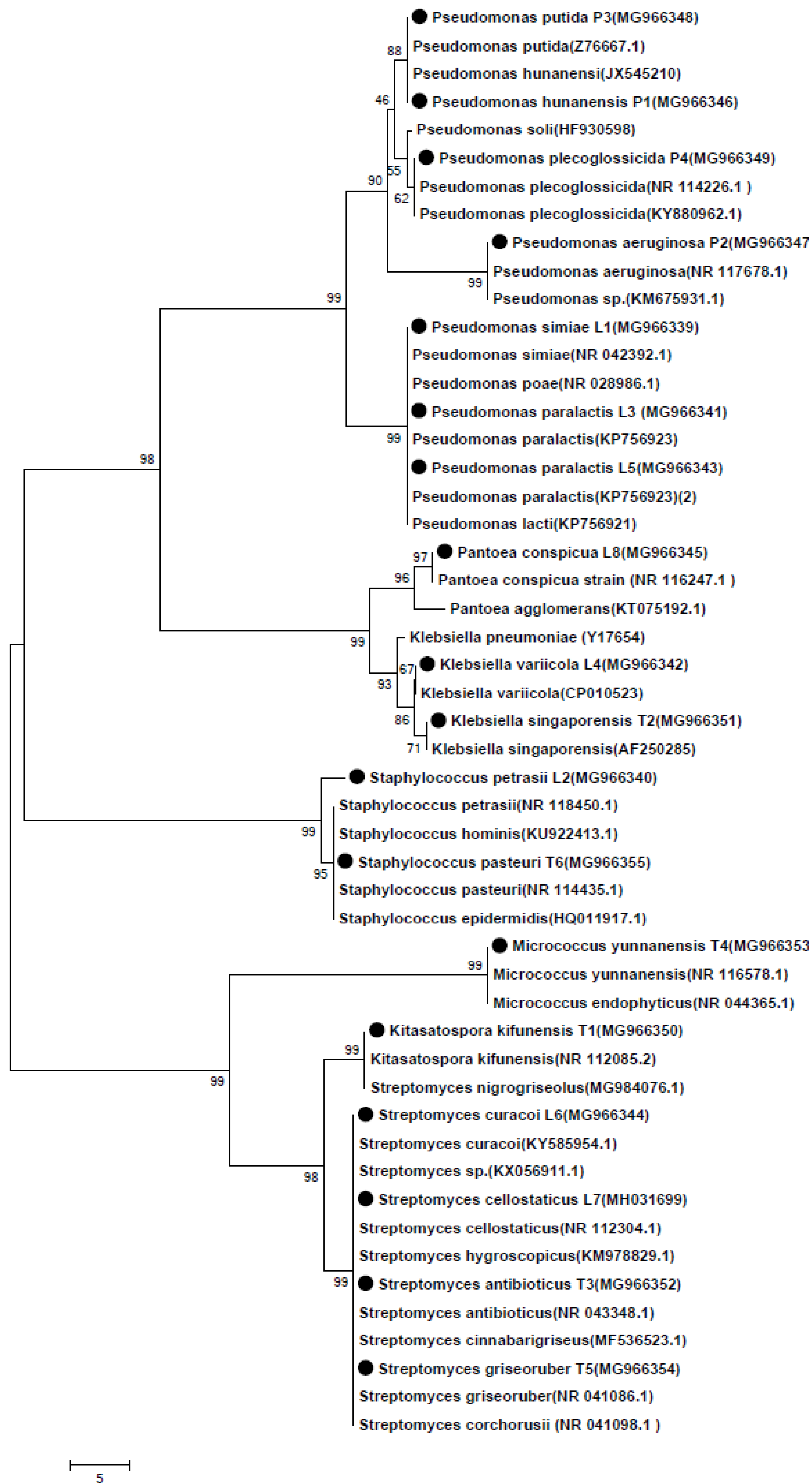
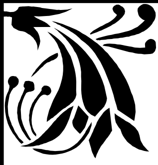
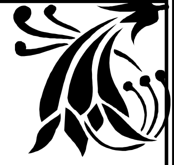


Fig 4.24: The neibhour joining tree based on 16 S rDNA sequences of bacterial isolates associated with *Dalbergia sissoo* rhizosphere from three provenances. Tree topology was obtained after 1000 runs. The bootstrap values are indicated at the nodes. Scale bar represents 5 nucleotide substitutions per site. The strains with bold circle are from this study

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

The present study was first to give an insight of the bacterial community structure in *Dalbergia sissoo* rhizosphere from natural forests. The diversity, composition and relative abundance of bacterial taxa in shisham rhizosphere were characterized across Lachhiwala, Tanakpur and Pantnagar provenances via Illumina high-throughput sequencing. The soil biological and chemical characteristics were also evaluated. Bacterial abundance diversity in shisham rhizosphere correlated to soil biotic and abiotic properties. In present work phosphate solubilizing and PQQ producing capabilities of the culturable rhizosphere dwelling bacteria, specifically targeted as they are important for regulation of soil and plant health and their abundance and diversity studied. Isolated PSBs were further characterized and identified through polyphasic approach. The PQQ gene was amplified to find whether PQQ is universally adopted mechanism by rhizospheric bacteria in mineral phosphate solubilization.

1. The physicochemical analysis of soils showed major differences between healthy and diseased soil samples. Disease conducive soil of Pantnagar was reported to be silty clay loam with high pH, high carbon, low phosphorus, and low micronutrients (Fe and Zn) content in comparison with other two samples (disease suppressive). Soil enzyme activities such as dehydrogenase and FDA hydrolysis were higher in DS rhizosphere from Tanakpur whereas acid phosphatase, alkaline phosphatase, and urease activities were higher in DS rhizosphere from Lachhiwala.
2. 16Sr RNA and *pqq* C gene copy number was highest for Pantnagar followed by Lachhiwala and Tanakpur. Copy number of *pqq* C gene was indirectly proportional to available phosphorus. Soils were also analysed through culturable approach on two different media (Angel's and Pikovaskya). Total mesophilic bacterial population count on Angel's medium was highest in Tanakpur soil whereas PSBs on Pikovaskya medium were highest in Lachhiwala soil.

3. Comparative assessment of bacterial communities associated with shisham rhizosphere was carried out using bar-coded Illumina sequencing of hypervariable V3-V4 region of 16S rRNA gene. Proteobacteria and Firmicutes were most abundant phyla at Lachhiwala and Tanakpur wheresas Acidobacteria was abundant at Pantnagar. Bacterial genera *Flavobacterium, Bradyrhizobium, Candidatus_ Solibacter, Phenylobacterium, Flavisolibacter* and *Rhodoplanes* were relatively more abundant in *D.sissoo* rhizosphere at Tanakpur and Lachhiwala, both representing healthy forest stands whereas *Williamsia, Blastocatella, Methylobacterium,* and *Brevibacterium* were relatively abundant at Pantnagar forest representing diseased stands.

4. The highest number of OTUs, chao I index and Shannon index were observed at Pantnagar whereas lowest at Tanakpur. β diversity analysis indicated that three samples aligned into two clusters. The disease conducive rhizospheric sample from Pantnagar formed independent cluster whereas from disease suppressive Tanakpur and Lacchiwala forest aligned in single cluster.

5. A total of eighteen PSBs (L1-L8, P1-P4 and T1-T6) were recovered from all three provenances. The solubilization index of strains ranged from 1.16 to 4.75. The highest solubilization index was recorded for L4 (4.75 ± 0.06) and lowest for T4 (1.16±0.01). The highest solubilized P concentrations in liquid medium was recorded for strain L4 (891.38 ± 18.55 μg ml^{-1}) and lowest for T4 (285.78±3.27 μg ml^{-1}). The solubilization index on agar plates positively correlated to the phosphorus solubilized in liquid medium.

6. All eighteen PSBs were screened for various functional traits. All the individual isolates exhibited one or more of the traits; amylase, urease, nitrate reductase, lipase, xylanase, protease, pectinase and catalase.

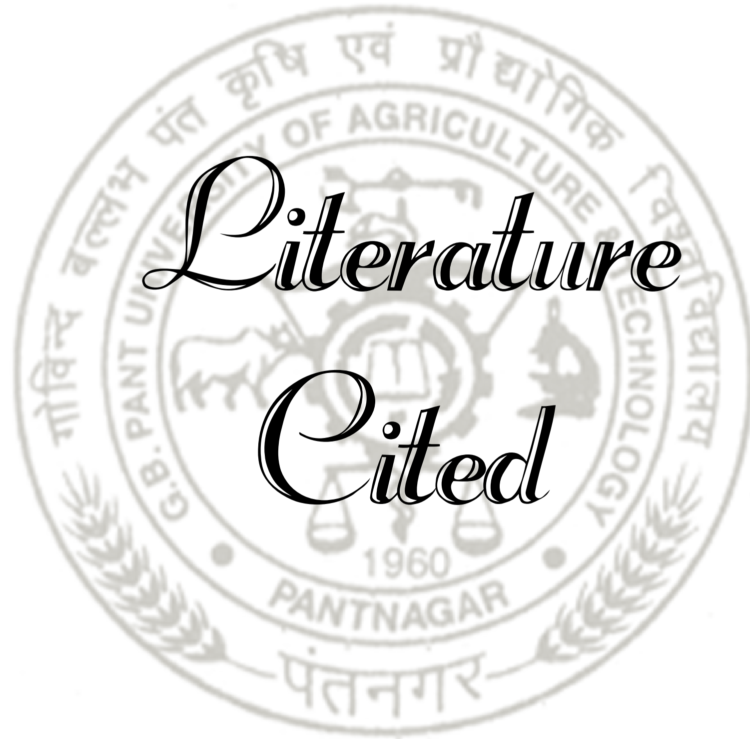
7. Among 18 PSBs seven bacterial isolates were positive for zinc solubilization, five were positive for siderophore production, fourteen for IAA production and fourteen for ammonia production. Only one isolate P2 was positive for HCN production.

8. Based on 16S rRNA sequence analysis and similarity searches all 18 PSBs were identified within genus *Pseudomonas*, *Klebsiella, Streptomyces, Pantoea, Kitasatospora, Micrococcus* and *Staphylococcus*. The 16S rRNA gene sequences of isolates were submitted under accession numbers MG966339-MG966355**.**

9. The PCR amplification of *pqq* C gene encoding catalyzation step was positive in all sixteen isolates whereas *pqq* A encoding the precursors tyrosine and glutamate was amplified only in six strains. All the six strains showing positive amplification for both the genes belonged to genera *Pseudomonas*.

This study leads to the conclusion that various factors contribute in shaping the community composition and numerical predominance of specific bacteria. Our study presents the first metagenomic characterisation of the diversity of rhizomicrobiome using a deep Illumina sequencing of 16S rRNA amplicons. The enrichment of some taxa in healthy rhizosphere supports the hypothesis of a functional specialization and an important ecological role of these taxa in soil functioning. However, many of the poorly characterized unculturable population, needs future research to obtain a comprehensive view of their role. Aside from this, our results suggest that the natural differences existing between soil parameters pH, nutrient availability and enzyme activities is responsible for differences in rhizospheric microbial communities of the same tree species. Still, we cannot yet link the entire microbiome to disease suppression in healthy soils and disease mortality in diseased stands. Thus isolation and identification of potential microbes seems more practicable. Studies based on 16S rRNA gene sequencing have extensively redefined and expanded our knowledge of soil microbial diversity. With the help of varied community analysis approaches, an initial framework of selecting disease-suppressive indicators was formed. Ecological knowledge on variations of PSBs among different environmental conditions might advance the development of more accurate and effective PGPR inoculants adaptive to different environmental conditions. This might be a prerequisite to develop more practical management strategies for sustainable agriculture. Future research with more extensive sampling and replication, greater depth of sequencing and direct assessment of metabolic rates is necessary to have a better knowledge of the functioning of these ecosystems.

Literature Cited



LITERATURE CITED

Adhikari, T. B., Joseph, C. M., Yang, . G, Phillips, D. A. and Nelson, L. M. (2001). Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. *Canadian Journal of Microbiology*. **47:** 916–924.

Aditi, D., Buch, G. and Archana. (2009). *Microbiology.* **155:** 2620-2629.

Afzal, A. and Bano, A. (2008). *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology.* **10:** 85-88.

Ahemad, M. and Khan, M. S. (2012). Effects of pesticides on plant growth promoting traits of *Mesorhizobium* strain MRC4. *Journal of the Saudi Society of Agricultural Sciences*. **11(1):** 63-71.

Ahemad, M. and Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University-Science*. **26(1):** 1-20.

Alef, K. and Nannipieri, P. (1998). Arylsulphatase activity. In: Alef K, Nannipieri P, editors. Methods in applied soil microbiology and biochemistry. London: Academic Press. Harcourt Brace and Company, Publishers. 362–363.

Alghazali, R., Kmuhammad, S. H. M. and Al-gzawl (1986). Some observations on P solubilization by aerobic microorganisms isolated d from sediments of Al Khair River Baghdad (Iraq). *Research Journal of Biological Sciences.* **47:** 157-72.

Alström, S. and Burns, R. G. (1989). Cyanide production by rhizobacteria as a possible mechanism of plant growth Inhibition. *Biology and Fertility of Soils.* **7:** 232–238.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. ad Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*. **215(3):** 403-410.

Amador, J. A., Glucksman, A. M., Lyons, J. B. and Gorres, J. H. (1997). Spatial distribution of soil phosphatise activity within a riparian forest. *Soil Science.* **162:** 808–825.

Amann, R. I., Ludwig, W. and Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological reviews.* **59(1):** 143-169.

An, R. and Luke, A. M. (2016). "Regulation of PQQ-dependent glucose dehydrogenase activity in the model rhizosphere dwelling bacterium *Pseudomonas putida* KT2440." *Applied and environmental microbiology*. AEM-00813.

Andreeva, I. G., Golubeva, L. I., Kuvaeva, T. M., Gak, E. R., Katashkina, J. I. and Mashko, S. V. (2011). Identification of Pantoea ananatis gene encoding membrane pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase and pqqABCDEF operon essential for PQQ biosynthesis. *FEMS Microbiology Letters.* **318(1):** 55-60.

Aneja, K.R. (2006). Experiments in Microbiology and Plant Pathology and Biotechnology 4th Edition New Delhi. 245-275.

Angle, J. S., McGrath, S. P. and Chaney, R. L. (1991). New culture medium containing ionic concentrations of nutrients similar to concentrations found in the soil solution. *Applied and Environmental Microbiology*. **57(12):** 3674-3676.

Antoun, H., Beauchamp, C. J., Goussard, N. and Chabot, R. (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumesı: effect on radishes (*Raphanus sativus* L.). *Plant Soil.* **204:** 57-67.

Aon, M. A. and Colaneri, A. C. (2001). Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology*. **18(3):** 255-270.

Armstrong, D. L. (1988). Role of phosphorus in plants: Better Crops with Plant Food., Potash and phosphate Institute, Atlanta, USA. 4-5.

Arun, K. S. (2007). Biofertilizers for sustainable agriculture. Agribios publishers, 6th Ed. Jodhpur, India.

Asea, P. E. A., Kucey, R. M. N. and Stewart, J. W`. B. (1988). Inorganic phosphate solubilization by two *Penicillium species* in solution culture and soil. *Soil Biology and Biochemistry.* **20(4):** 459-464.

Aslantas, R., Cakmakci, R. and Sahin, F. (2007). Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scietia Horticulturae.* **111:** 371-377.

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C. and Ranger, J. (2015) Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. *Biological Reviews.* **90:** 444–66.

Augusto, L., Ranger, J., Binkley, D. and Rothe, A. (2002). Impact of several common tree species of European temperate forests on soil fertility. *Annals of Forest Science.* **59:** 233-253.

Azad, M. A. K., Bae, J. H., Kim, J. S., Lim, J. K., Song, K. S., Shin, B. S. and Kim, H. R. (2009). Isolation and characterization of a novel thermostable α-amylase from Korean pine seeds. *New Biotechnology.* **26(3-4):** 143-149.

Azziz, G., Bajsa, N., Haghjou, T., Taulé, C., Valverde, Á., Igual, J. M. and Arias, A. (2012). Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Applied Soil Ecology.* **61:** 320–326.

Babalola, O. O. and Glick, B. R. (2012a). Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. *Scientific Research and Essays.* **7(28):** 2431–2439.

Babu-Khan, S., Yeo, T. C., Martin, W. L., Duron, M. R., Rogers, R. D. and Goldstein, A. H. (1995). Cloning of a mineral phosphate-solubilizing gene from Pseudomonas cepacia. *Applied and Environmental Microbiology.* **61:** 972–978.

Bäckman, J. S. K., Hermansson, A., Tebbe, C. C. and Lindgren, P. E. (2003). Liming induces growth of a diverse flora of ammoniaoxidising bacteria in acid spruce forest soil as determined by SSCP and DGGE. *Soil Biology and Biochemistry.* **35:** 1337-1347.

Bagyaraj, D. J., Sharma, M. P. and Maiti, D. (2015). Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. *Current Sciece.* **108(7):** 1288-1293.

Bajwa, R., Javaid, A. and Shah, M. B. M. (2003a). Extent of Shisham (*Dalbergia sissoo* Roxb.) Decline in Sialkot, Gujranwala, Lahore and Sargodha Districts. *Mycopathologia.* **1:** 1-5.

Bakker, A. W., and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth-stimulation. *Soil Biology and Biochemistry*. **19(4):** 451-457.

Bakshi, B. K. (1954). Wilt of shisham (*Dalbergia sissoo* Roxb.) due to *Fusarium solani* sensu Snyder and Hansen. *Nature*. **174 (4423):** 278.

Bakshi, B. K. (1957). Wilt disease of shisham *(Dalbergia sissoo* Roxb.). IV. The effect of soil moisture on the growth and survival of *Fusarium solani* in laboratory. *Indian Forester.* **83(8):** 505-512.

Baldrian, P., Merhautov´a V., Cajthaml, T. and Petránková, M. (2010). Small-scale distribution of extracellular enzymes, fungal, and bacterial biomass in *Quercus petraea* forest topsoil. *Biology and Fertility* of *Soils*. **46:** 717–26.

Bardin, S., Dan, S., Osteras, M. and Finan, T. M. (1996). A phosphate transport system is required for symbiotic nitrogen fixation by *Rhizobium meliloti*. *Journal of Bacteriology.* **178 (15):** 4540-4547.

Barford, C. and Lajtha, K. (1992). Nitrification and nitrate reductase activity along a secondary successional gradient. *Plant and Soil*. **145(1):** 1-10.

Bartholdy, B. A., Berreck, M. and Haselwandter, K. (2001). Hydroxamate siderophore synthesis by *Phialocephala fortinii*, a typical dark septate fungal root endophyte. *BioMetals.* 1**4:** 33-42.

Bartram, A. K., Lynch, M. D., Stearns, J. C., Moreno-Hagelsieb, G. and Neufeld, J. D. (2011). Generation of multi-million 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and environmental microbiology*.

Bar–Yosef, B., Rogers, R. D., Wolform, J. H. and Richman, E. (1999). *Pseudomonas cepacia* mediated rock phosphate solubilization in Kaolinite and montmorillonite suspensions. *Soil Science Society of America Journal.* **63:** 1703–8.

Bashan, Y. (1998). Inoculants of plant growth promoting bacteria for use in agriculture. *Biotechnology Advance*. **16:** 729-770.

Bayer, C., Martin-Neto, L., Mielniczuk, J., Pillon, C. N. and Sangoi, L. (2001). Changes in soil organic matter fractions under subtropical no-till cropping systems. *Soil Science Society of America Journal*. **65(5):** 1473-1478.

Bazzicalupo, M. and Fani, R. (1995). The use of RAPD for generating specific DNA probes for microorganisms, in Methods in Molecular Biology (Clapp, J. P., ed.), Humana, Totowa, NJ, pp. 155–175.

Bellenger, J. P., Xu, Y., Zhang, X., Morel, F. and Kraepiel, A. (2014). Possible contribution of alternative nitrogenases to nitrogen fixation by asymbiotic N2-fixing bacteria in soils. *Soil Biology and Biochemistry*. **69:** 413– 420.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*. **84(1):** 11-18.

Berg, G., Opelt, K., Zachow, C., Lottmann, J., Götz, M. and Costa, R. (2006). The rhizosphere effect on bacteria antagonistic toward the pathogenic fungus *Verticillium* differs depending on plant species and site. *FEMS Microbiology Ecology*. **56:** 250–261

Bergey, D. H. and Holt, J. G. (1994). *Bergey's manual of determinative bacteriology.*

Bergstrom, D. W., Monreal, C. M. and King, D. J. (1998). Sensitivity of soil enzyme activities to conservation practices. *Soil Science Society of America Journal.* **62:** 1286–1295.

Bettiol, W. (2014). Bacterial formulation which can be used for biologically controlling plant diseases and promoting plant growth. U.S. Patent Application 13/881, 936, filed.

Bhattacharya, M., Singh, A. and Ramrakhyani, C. (2014). *Dalbergia sissoo*-An Important Medical Plant. *Journal of Medicinal Plants*. **2(2).**

Bhattacharyya, P. N. and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology.* **28(4):** 1327-1350.

Bisht, R., Chaturvedi, S., Srivastava, R., Sharma, A. K. and Johri, B. N. (2009). Effect of arbuscular mycorrhizal fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient status of *Dalbergia sissoo* Roxb. *Tropical Ecology.* **50(2):** 231.

Boerner, R. E. J., Decker, K. L. M. and Sutherland, E. K. (2000). Prescribed burning effects on soil enzyme activity in a southern Ohio hardwood forest: a landscape-scale analysis. *Soil Biology and Biochemistry.* **32:** 899–908.

Bolton, J. H., Smith, J. L. and Link, S. O. (1993). Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. *Soil Biology and Biochemistry*. **25:** 545–552.

Buch, A., Archana, G. and Kumar, G. N. (2008). Metabolic channeling of glucose towards gluconate in phosphate-solubilizing *Pseudomonas aeruginosa* P4 under phosphorus deficiency. *Research in Microbiology*. **159(9-10):** 635-642.

Burger, J. A., Johnson, J. E., Andrews, J. A. and Torbert, J. L. (1994). Measuring mine soil productivity for forests. In: International Land Reclamation and Mine Drainage Conference on Reclamation and Revegetation, USDOI Bureau of Mines, Special Publication SP 06C-94. **3:** 48–56.

Burke, D. J., Weintraub, M. N., Hewins, C. R. and Kalisz, S. (2011). Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. *Soil Biology and Biochemistry.* **43:** 795–803.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N. and Zoppini, A. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*. **58**: 216–234.

Business Standard (2008). New Delhi Edition, India.

Cabello, M., Irrazabal, G., Bucsinszky, A. M., Saparrat, M. and Schalamuck, S. (2005). Effect of an arbuscular mycorrhizal fungus, *G. mosseae* and a rock-phosphate-solubilizing fungus, *P. thomii* in *Mentha piperita* growth in a soiless medium. *Journal of Basic Microbiology*. **45:** 182-189.

Cai, Y. and Sun, Y. (2011). ESPRIT-Tree: hierarchical clustering analysis of millions of 16S rRNA pyrosequences in quasilinear computational time. *Nucleic Acids Research.* **39(14):** 95.

Caldwell, B. A. (2005). Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia*. **49(6):** 637-644.

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B. and Shen, Q. (2011). *Bacillus subtilis* SQR 9 can control Fusarium wilt in cucumber by colonizing plant roots. *Biology and Fertility of Soils.* **47:** 495–506.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K. and Huttley, G. A. (2010). QIIME allows analysis of high throughput community sequencing data. *Nature Methods*. **7:** 335–336

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N. and Gormley, N. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *International Society for Microbial Ecology*. **6(8):** 1621.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J. and Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*. **108(1):** 4516-4522.

Cappuccino, J. C. and Sherman, N. (1992). In: Microbiology: A Laboratory Manual, third ed. Benjamin/cummings Pub. Co. New York, pp. 125-179.

Cappuccino, J. G. and Sherman, N. (2002). Microbiology: a laboratory manual (6th ed). Pearson.

Casida, L. Jr., Klein, D. and Santoro, T. (1964). Soil dehydrogenase activity. *Soil Science.* **98**: 371–377.

Castagno, L. N., Estrella, M. J., Sannazzaro, A. I., Grassano, A. E. and Ruiz, O. A. (2011). Phosphate solubilization mechanism and *in vitro* plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). *Journal of Applied Microbiology*. **110:** 1151-1165.

Chand, L. and Singh, H. (2006). Effect of phosphate solubilizers with different P-levels on yield and nutrient uptake of mung (*Vigna radiata*.) Research Council Meet Report, Division of Agronomy, SKUAST-K.

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A. and Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate abilities. *Applied Soil Ecology.* **34**: 33–41.

Chen, Z., Ma, S. and Liu, L. L. (2008). Studies on phosphorus solubilizing activity of a strain of phosphobacteria isolated from chestnut type soil in China. *Bioresource Technology*. **99(14):** 6702-6707.

Cheong, H., Park, S. Y., Ryu, C. M., Kim, J. F., Park, S. H. and Park, C. S. (2005). Diversity of root-associated *Paenibacillus* spp. in winter crops from the southern part of Korea. *Journal of Microbiology and Biotechnology.* **15(6):** 1286-1298.

Choi, O., Kim, J., Kim, J. G., Jeong, Y., Moon, J. S., Park, C. S. and Hwang, I. (2008). Pyrroloquinoline quinone is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. *Plant Physiology.* **146:** 657-668.

Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H. and Sa, T. (2005). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biology and Biochemistry*. **37(10):** 1970-1974.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T., Garrity, G. M. and Teidje, J. M. (2009). The ribosomal database project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research.* **37:** 141–145

Collignon, C., Calvaruso, C. and Turpault, M. P. (2011). Temporal dynamics of exchangeable K, Ca and Mg in acidic bulk soil and rhizosphere under Norway spruce (*Picea abies* Karst) and beech (*Fagus sylvatica* L) stands. *Plant Soil.* **349:** 355–366.

Collignon, C., Uroz, S., Turpault, M. P. and Frey-Klett, P. (2011). Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands. *Soil Biology and Biochemistry*. **43:** 2012-2022.

Collins, N. L. and Ford, M. B., (2010). Responding to the needs of others: The caregiving behavioral system in intimate relationships. *Journal of Social and Personal Relationships*. **27(2):** 235-244.

Cookson, P. (1999). Special variation in soil urease activity around irrigated date palms. *Arid Soil Research and Rehabilitation.* **13:** 155-169.

Curl, E. A. and Truelove, B. (1986). The Rhizosphere. Advanced Series in Agricultural Sciences, Vol. 15. Springer-Verlag, New York.

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M. and Kirkegaard, R. H. (2015). Complete nitrification by *Nitrospira bacteria*. *Nature*. **528(7583):** 504.

Dasila, H., Joshi, S., Sahgal, M. and Tiwari, S. (2018). *Talaromyces* sp. are associated with Shisham Nursery disease in Pantnagar, A Terai region of Western Himalayas. *Ecology*, *Environment and Conservation*. **24(3):** 366-370.

Dastager, S. G., Deepa, C. K. and Pandey, A. (2010). Potential plant growth promoting activity of *Serratia nematophila* NII- 0.928 on black pepper (*Piper nigrum* L.*). World Journal of Microbiology and Biotechnology*. **27:** 259–265.

Datta S. K. and Datta K. (1983). Auxin in induced regeneration of forest tree-*Dalbergia sissoo* Roxb. through tissue culture. *Current Science*. **52(9):** 434-436

De Filippo, C., Ramazzotti, M., Fontana, P. and Cavalieri, D. (2012). Bioinformatic approaches for functional annotation and pathway inference in metagenomics data. *Briefings in Bioinformatics*. **13:** 696–710.

De Smet, B., Van den Bossche, D., van de Werve, C., Mairesse, J., Schmidt-Chanasit, J., Michiels, J. and Cnops, L. (2016). Confirmed Zika virus infection in a Belgian traveler returning from Guatemala, and the diagnostic challenges of imported cases into Europe. *Journal of Clinical Virology*. **80:** 8-11.

De-Bashan, L. E. and Bashan, Y. (2004). Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). *Water Research*. **38(19):** 4222-4246.

Delmont, T. O., Prestat, E., Keegan, K. P., Faubladier, M., Robe, P., Clark, I. M., Pelletier, E., Hirsch, P. R., Meyer, F., Gilbert, J. A., Le Paslier, D., Simonet, P. and Vogel, T. M. (2012). Structure, fluctuation and magnitude of a natural grassland soil metagenome. *International Society for Microbial Ecology*. **6(9):** 1677–1687.

Delmont, T. O., Robe, P., Cecillon, S., Clark, I. M., Constancias, F. and Simonet, P. (2011). Accessing the soil metagenome for studies of microbial diversity. *Applied and Environmental Microbiology*. **77(4):** 1315-1324.

DeLong, E. F. (2002). Microbial population genomics and ecology. *Current Opinion in Microbiology*. **5(5):** 520-524.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P. and Andersen, G. L. (2006a). Greengenes: Chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*. **72(7)**: 5069-5072.

DeSantis, T. Z., P. Hugenholtz, K. Keller, E. L. Brodie, N. Larsen, Y. M. Piceno, R. Phan, and G. L. Andersen. (2006b). NAST*:* a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research.* **34**:W394-9.

Di Cello, F., Bevivino, A., Chiarini, L., Fani, R., Paffetti, D., Tabacchioni, S. and Dalmastri, C. (1997). Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages. *Applied and Environmental Microbiology.* **63:** 4485–4493.

Dick, R. P. (1997). Soil enzyme activities as integrative indictors of soil health**.** C. Pnkrst, B.M. Dube, V.V.S.R. Gupta (Eds.): Biological indicators of Soil Health, CAB International, Wallingford, Oxon, UK. pp. 121-157.

Dick, W. A., Cheng, L. and Wang, P. (2000). Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biology and Biochemistry.* **32**:1915–1919.

Dix, N. J. and Webster, J. (1995). Fungal Ecology. University Press, Cambrige, Great Britain.

Dojka, M. A., Harris, J. K. and Pace, N. R. (2000). Expanding the known diversity and environmental distribution of an uncultured phylogenetic division of bacteria. *Applied and Environmental Microbiology.* **66:** 1617–1621.

Doran, J. W., Sarrantonio, M. and Liebig, M. A. (1996). Soil health and sustainability. *Advances in Agronomy.* **56:** 2–54.

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development.* **28(1):** 33-46.

Dunfield, K. E. and Germida, J. J. (2001). Diversity of bacterial communities in the rhizosphere and root interior of the field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology*. **38:** 1–9.

Dwara S., Sharma, D. R. and Choudhury. J. B. (1984). Clonal propagation of *Dalbergia sissoo* Roxb. through tissue culture. *Current Science.* **53:** 807– 809.

Edi-Premono, Moawad, M. A. and Vleck, P. L. G. (1996). Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Agricultural Science.* **11:** 13-23.

Eichlerova, I., Homolka, L., Žifcˇakova, L., Lisa, L., Dobiašova, P. and Baldrian, P. (2015). Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecology.* **13:**10 –22.

Eivazi, F. and Tabatabai, M. A. (1977). Phosphatases in soils. *Soil Biology and Biochemistry.* **9**:167–172.

Elsas, V., J. D., Duarte, G. F., Rosado, A. S. and Smalla, K. (1997). Microbiological and molecular biological methods for monitoring microbial inoculants and their effects in the soil environment. *Journal of Microbiological Methods*. **32(2):** 133-154.

Enya, J., Shinohara, H., Yoshida, S., Tsukiboshi, T., Negishi, H., Suyama, K. and Tsushima, S. (2007). Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. *Microbial ecology*. **53(4):** 524-536.

F. A. O. (2010). Global Forest Resources Assessment (FRA). Main Report, FAO Forestry Paper 163, Food and Agriculture Organization (FAO) of United Nations, Rome.

Fakruddin, M. and Mannan, K. S. B. (2012). Next generation sequencing technologies-principles and prospects. *Research and Review in Biosciences*. **6(9):** 240-247.

Farhat, M. B., Farhat, A., Bezar, W., Kammoun, R., Bauchaala, K., Fourati, A., Antoun, H., Bejar, S. and Chouayekh, H. (2009). Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of Gafsa. *Archives of Microbiology.* **191(11):** 815–824.

Farkaš, V., Lišková, M. and Biely, P. (1985). Novel media for detection of microbial producers of cellulase and xylanase. *FEMS Microbiology Letters*. **28(2):** 137-140.

Fazekašová, D., Igaz, D., Klimovičová, M., Bobuľská, L., Angelovičová, L. and Michaeli, E. (2012). Activity of soil urease in selected soil types in the Nitra River basin. *J Int Sci Publ Ecol Saf.* **6:** 191-205.

Felici, C., Vettori, L., Giraldi, E., Forino, L. M. C., Toffanin, A., Tagliasacchi, A. M. and Nuti, M. (2008). Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. *Applied Soil Ecology*. **40(2):** 260-270.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution.* **39(4):** 783-791.

Fernandez, L. A., Zalba, P., Gomez, M. A. and Sagardoy, M. A. (2007). Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biology and Fertility of Soils.* **43(6):** 805-809**.**

Fernandez, M., Conde, S., Duque, E. and Ramos, J. L. (2013). In vivo gene expression of *Pseudomonas putida* KT2440 in the rhizosphere of different plants. *Microbial Biotechnology*. **6:** 307-313.

Fiske, C. H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry*. **66(2):** 375-400.

Forchetti, G., Masciarelli, O., Alemano, S., Alvarez, D. and Abdala, G. (2007). Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology*. **76(5):** 1145-1152.

Fouratt, M. A., Rhodes, J. S., Smithers, C. M., Love, N. G. and Stevens, A. M. (2003). Application of temperature gradient gel electrophoresis to the characterization of a nitrifying bioaugmentation product. *FEMS Microbiology and Ecology.* **43:** 277-286.

Franklin, R. B. and Mills, A. L. (2003). Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiology Ecology.* **44**: 335–346.

Garcia, C., Hernandez, T., Roldan, A. and Martin, A. (2002). Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate. *Soil Biology and Biochemistry*. **34(5):** 635-642.

García-Salamanca, A., Molina-Henares, M. A., van Dillewijn, P. and Solano, J. (2013). Bacterial diversity in the rhizosphere of maize and the surrounding carbonate-rich bulk soil. *Microbial Biotechnology.* **6:** 36-44.

Gauthier, S., Bernier, P. and Kuuluvainen, T. (2015). Boreal forest health and global change. *Science.* **349:** 819–22.

Ge, X., Zhao, Y., Hou, W., Zhang, W., Chen, W., Wang, J. and Wang, Q. (2013). Complete genome sequence of the industrial strain *Gluconobacter oxydans* H24. *Genome Announcements*. **1(1):** 3-13.

Ghosh, A., Dey, K., Bhowmick, N., Medda, P. S. and Dey, A. N. (2017). Reproductive behaviour of lemon (*Citrus limon* Burm.) affected by different pruning intensities and integrated nutrient management under various growing season. *International Journal of Current Microbiology and Appled Science*. **6(4):** 606-614.

Gianfreda, L., Rao, A. M., Piotrowska, A., Palumbo, G. and Colombo, C. (2005). Soil enzyme activities as affected by anthropogenic alterations: Intensive agricultural practices and organic pollution. *Science of the Total Environment*. **341:** 265-279.

Gill, M. A., Ahmad, I., Khan, A. U., Aslam, M., Ali, S., Rafique, R. M. and Khan, M. (2001). *Phytophthora cinnamomi*–a cause of Shisham decline in Punjab, Pakistan. In Proceedings of the 3rd National Conference on Plant Pathology (Oct) (pp. 1-3).

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications Hindawi Publishing Corporation. *Scientifica*.

Gliese, N., Khodaverdi, V. and Gorisch, H. (2010). The PQQ biosynthetic operons and their transcriptional regulation in *Pseudomonas aeruginosa*. *Archives of Microbiology*. **192:** 1-14.

Glöckner, J., Kube, M., Shrestha, P. M., Weber, M., Glöckner, F. O., Reinhardt, R. and Liesack, W. (2010). Phylogenetic diversity and metagenomics of candidate divi- sion OP3. *Environmental Microbiology*. **12:** 1218–1229.

Goenadi, D. H. Siswanto and Sugiarto, Y. (2000). Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. *Soil Science Society of America Journal*. **64:** 927-932.

Goldstein, A. H. (1995). Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. *Biological Agriculture and Horticulture*. **12:** 185-193.

Gomila, M., Ramirez, A. and Lalucat, J. (2007). Diversity of environmental Mycobacterium isolates from hemodialysis water as shown by a multigene sequencing approach. *Applied and Environmental Microbiology*. **73:** 3787–3797.

Goodwin, P. M. and Anthony, C. (1998). The biochemistry, physiology and genetics of PQQ and PQQ-containing enzymes. *Advances in Microbial Physiology*. **40:** 1-180

Goosen, N., Horsman, H. P., Huinen, R. G. and van de Putte, P. (1989). *Acinetobacter calcoaceticus* genes involved in biosynthesis of the coenzyme pyrroloquinoline-quinone: nucleotide sequence and expression in *Escherichia coli* K-12. *Journal of Bacteriology.* **171:** 447-455

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J. C., Glanville, H. C., Jones, D. L. and Angel, R. (2016). Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes. *Frontiers in Microbiology.* **7:** 214.

Griffiths, B. S., Ritz, K., Ebblewhite, N. and Dobson, G. (1999). Soil microbial community structure: effects of substrate loading rates. *Soil Biology and Biochemistry*. **31(1):** 145-153.

Grimont, P. A. D. (2002). Risque bactériologique: repérage des souches. *Comptes Rendus Biologies.* **325:** 901–906.

Grundmann, G. L. and Gourbiere, F. (1999). A micro-sampling approach to improve the inventory of bacterial diversity in soil. *Applied Soil Ecology.* **13**: 123-126

Gururani, M. A., Upadhyaya, C. P., Baskar, V., Venkatesh, J., Nookaraju, A. and Park, S. W. (2012). Plant growth -promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *Journal of Plant Growth Regulation.*

Hajare, S. W., Chandra, S., Sharma, J., Tandan, S.K., Lal J. and Telang, A. G. (2001). Anti-inflammatory activity of *Dalbergia sissoo* leaves. *Fitoterapia*. **72(2):** 131-139.

Hajare, S. W., Chandra, S., Tandan, S. K., Sarma, J., Lal J. and Telang, A. G. (2000). Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. *Indian Journal of Pharmacology.* **32(6):** 357-360.

Halajnia, A., Haghnia, G. H., Fotovat, A. and Khorasani, R. (2009). Phosphorus fractions in calcareous soils amended with P fertilizer and cattle manure. *Geoderma.* **150(1):** 209-213.

Hamdali, H., Bouizgarne, B., Hafidi, M., Lebrihi, A., Virolle, M. J. and Ouhdouch, Y. (2008). Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. *Applied Soil Ecology*. **38(1):** 12-19.

Hameeda, B., Harini, G., Rupela, O. P., Wani, S. P. and Reddy, G. (2008). Growth promotion of maize byphosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiology Research.* **163:** 234-242.

Han, S. H., Kim, C. H., Lee, J. H., Park, J. Y., Cho, S. M., Park, S. K. and Kim, Y. C. (2008). Inactivation of pqq genes of *Enterobacter intermedium* 60-2G reduces antifungal activity and induction of systemic resistance. *FEMS Microbiology Letters*. **282(1):** 140-146.

Hankin, L. and Anagnostakis, S. L. (1975). The use of solid media for detection of enzyme production by fungi. *Mycology*. **67:** 597-607.

Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*. **60(4):** 579-598.

Helgason, B. L., Walley, F. L. and Germida, J. J. (2010). Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosystems. *Soil Biology and Biochemistry.* **42:** 2192–2202.

Hong, C., Si, Y. X., Xing, Y. and Li, Y. (2015). Illumina MiSeq sequencing investigation on the contrasting soil bacterial community structures in different iron mining areas. *Environmental Science and Pollution Research.* **22:** 10788–10799.

Huang, L. N., Zhou, H., Zhu, S. and Qu, L. H. (2006). Phylogenetic diversity of bacteria in the leachate of a full-scale recirculating landfill. *FEMS Microbiology and Ecology.* **50:** 175-183.

Hugenholtz, P. (1998). Exploring prokaryotic diversity in the genomic era. *Genome Biology.* **3**: 0003-1.

Hyland, C., Ketterings, Q., Dewing, D., Stockin, K., Czymmek, K., Albrecht, G. and Geohring, L. (2005). Nutrient management spear program. Agronomy Fact Sheet Series, Fact Sheet 12.

Illmer, P. and Shinner, F. (1995). Phosphate solubilizing microorganisms under non-sterile conditions. *Bodenkultur.* **46:** 197-204.

Inbar, Y., Boehm, M. J. and Hoitink, H. A. (1991). Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. *Soil Biology and Biochemistry.* **23(5):** 479-483.

Islam, M. M., Karim, A. J. M. S., Jahiruddin, M., Majid, N. M., Miah, M. G., Ahmed, M. M. and Hakim, M. A. (2011). Effects of organic manure and chemical fertilizers on crops in the radish-stem amaranth-Indian spinach cropping pattern in homestead area. *Australian Journal of Crop Science.* **5**: 1370-1378.

Isobe, K. and Ohte, N. (2014). Ecological Perspectives on Microbes Involved in N-Cycling. *Microbes and Environments*. **29(1):** 4–16.

Janssen, P. H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology*. **72(3):** 1719-1728.

Javaid, A. (2008). Research on shisham (*Dalbergia sissoo* Roxb.) decline in Pakistan–a review. *Pakistan Journal of Phytopathology*. **20(1):** 134-142.

Jeon, J. S., Lee, S. S., Kim, H. Y., Ahn, T. S. and Song, H. G. (2003). Plant growth promotion in soil by some inoculated microorganisms. *Journal of Microbiology.* **41:** 271–276.

Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R. and Fierer, N. (2009). A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *International Society for Microbial Ecology.* **3:** 442–453.

Kabir, S., Rajendran, N., Amemiya, T. and Itoh, K. (2003). Quantitative measurement of fungal DNA extracted by three different methods using real-time polymerase chain reaction. *Journal of Bioscience and Bioengineering.* **96(4):** 337–34.

Kandeler, E. and Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils.* **6(1):** 68- 72.

Karthikeyan, A. S., Varadarajan, D. K., Mukatira, U. T., D'Urzo, M. P., Damaz, B. and Raghothama, K. G. (2002). Regulated expression of Arabidopsis phosphate transporters. *Plant Physiology.* **130:** 221–233.

Kasana, R. C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram s iodine. *Current Microbiology*. **57:** 503-507.

Kaur, R., Macleod, J., Foley, W. and Nayudu, M. (2006). Gluconic acid: An antifungal agent produced by *Pseudomonas species* in biological control of take-all. *Phytochemistry*. **67(6):** 595-604.

Kavamura, V. N., Santos, S. N., da Silva, J. L., Parma, M. M., Ávila, L. A., Visconti, A., Zucchi, T. D., Taketani, R. G., Andreote, F. D. and de Melo, I. S. (2013). Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiological research*. **168(4):** 183-191.

Keenan, R. J., Reams, G. A., Achard, F., de Freitas, J. V., Grainger, A. and Lindquist, E. (2015). Dynamics of global forest area: results from the FAO Global Forest Resources Assessment. *Forest Ecology Management.* **352**: 9 –20.

Khan M. M., Mahmood, T. and Rafique, R. M. (1999). Diagnostic study of shisham dieback in Punjab. Forestry Research Institute, Faisalabad. Proc. of 2nd Natl. Conference of Plant Pathology. pp-27-29.

Khan, A. G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology.* **18:** 355–364

Khan, B. S., Yeo, T. C., Martin, W. L., Duron, M. R., Rogers, R. D. and Goldstein, A. H. (1995). Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia. Applied and Environmental Microbiology*. **61:** 972–978.

Khan, S. H., Idress M. F., Muhammad, A., Mahmood and Zaidi, S. H. (2004). Incidence of shisham (*Dalbergia sissoo* Roxb.) decline and In vitro response of isolated fungus spp., to various fungicides. *International Journal of Agriculture and Biology*. **6:** 611-614.

Kim, B. S., Jeon, Y. S. and Chun, J. (2013). Current status and future promise of the human microbiome. *Pediatric Gastroenterology*, *Hepatology and Nutrition*. **16:** 71–79.

Kim, C. H., Han, S. H., Kim, K. Y., Cho, B. H., Kim, Y. H., Koo, B. S. and Kim Y. C. (2003). Cloning and expression of pyrroloquinoline quinine (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. *Current Microbiology.* **47(6):** 457–461.

Kim, K. Y., Jordan, D. and Krishnan, H. B. (1997). *Rahnella aquatilis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. *FEMS Microbiology Letters.* **153(2):** 273–277.

Kim, K. Y., Jordan, D. and McDonald, G. A. (1998). Enterobacter agglomerans, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. *Soil Biology and Biochemistry.* **30:** 995–1003.

Kim, K., Jordan, D. and Krishnan, H. B. (1997). Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*. *FEMS Microbiology Letters.* **159:** 121–127.

Kinsella, K., Schulthess, C. P., Morris, T. F. and Stuart, J. D. (2009). Rapid quantification of *Bacillus subtilis* antibiotics in the rhizosphere. *Soil Biology and Biochemistry*. **41(2):** 374-379.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H. and Trevors, J.T. (2004). Methods of studying soil microbial diversity. *Journal of Microbiological Methods*. **58**: 169–188.

Kleyer, H., Tecon, R. and Or, D. (2017). Resolving species level changes in a representative soil bacterial community using microfluidic quantitative PCR. *Frontiers in Microbiology*. **8**.

Klinman, J. P. and Bonnot, F. (2014). Intrigues and intricacies of the biosynthetic pathways for the enzymatic quinocofactors: PQQ, TTQ, CTQ, TPQ, and LTQ. *Chemical Reviews.* **114:** 4343-4365.

Knietch, A., Waschkowitz, T., Bowien, S., Henne, A. and Daniel, R. (2003). Metagenome of complex microbial consortia derived from different soils as sources for novel genes conferring formation of carbonyls from short-chain polyols on *Escherichia coli*. *Journal of Microbiology and Biotechnology.* **5:** 46–56.

Knight, R., Maxwell, P., Birmingham, A., Carnes, J., Caporaso, J. G., Easton, B. C. and Lozupone, C. (2007). PyCogent: a toolkit for making sense from sequence. *Genome Biolology.* **8(8)**: R171.

Kodzius, R. and Gojobori, T. (2015). Marine metagenomics as a source for bioprospecting. *Marine genomics*. **24:** 21-30

Kolte, A. P., Dhali, A., Malik, P. K., Samanta, A. K. and Bhatta, R. (2017). Rumen Metagenomics and Its Implications in Animal Nutrition: A Review. *Indian Journal of Animal Nutrition*. **34(2):** 124-134

Korboulewsky, N., Perez, G. and Chauvat, M. (2016). How tree diversity affects soil fauna diversity: a review. *Soil Biology and Biochemistry.* **94:** 94-106.

Kourtev, P. S., Ehrenfeld, J. G. and Haggblom, M. (2002). Exotic plant species alter the microbial community structure and function in the soil. *Ecology*. **83:** 3152–3166.

Krishnaraj, P. U. and Dahale, S. (2014). Mineral phosphate solubilization: concepts and prospects in sustainable agriculture. *Proceedings of the Indian National Science Academy*. **80(2):** 389-405.

Krishnaraj, P. U. and Goldstein, A. H. (2001). Cloning of a *Serratia marcescens* DNA fragment that induces quinoprotein glucose dehydrogenase-mediated gluconic acid production in *Escherichia coli* in the presence of stationary phase *Serratia marcescens*. *FEMS Microbiology Letters*. **205(2):** 215-220.

Kucey, R. M. N. (1983). Phosphate solubilizing bacteria and fungi in various cultivated and fungi in various cultivated and virgin Alberta soils. *Canadian Journal of Soil Science*. **63:** 671-678.

Kucey, R. M. N., Janzen, H. H. and Legett, M. E. (1989). Microbially mediated increases in plant available phosphorus. *Advance in Agronomy*. **42:** 199-228.

Kumar, A., Maurya, B. R. and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and the effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatalysis and Agricultural Biotechnology*. **3:** 121–128.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. **33(7):** 1870-1874.

Kumar, S., Suyal, D. C., Dhauni, N., Bhoriyal, M. and Goel, R. (2014). Relative Plant Growth Promoting Potential of Himalayan Psychrotolerant *Pseudomonas jesenii* Strain MP1 against Native *Cicer arietinum* L., *Vigna mungo* (L.) Hepper; *Vigna radiata* (L.) Wilczek., *Cajanus cajan* (L.) Millsp. and *Eleusine coracana* (L.) Gaertn. *African Journal of. Microbiology*. **8(50):** 3931-3943

Kumari, A. and Kakkar, P. (2008). Screening of antioxidant potential of selected barks of Indian medicinal plants by multiple *in vitro* assays. *Biomedical and Environmental Sciences*. **21(1):** 24-29.

Kuzyakov, Y. and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: concept & review. *Soil Biology and Biochemisty*. **83:** 184–199.

Kyselková, M., Kopecký, J., Frapolli, M., Défago, G., Ságová-Marecková, M. and Grundmann, G. L. (2009). Comparison of rhizobacterial community composition in soil suppressive or conducive to tobacco black root rot disease. *International Society for Microbial Ecology.* **3:** 1127–1138.

Labrenz, M., Brettar, I., Christen, R., Flavier, S., Bötel, J. and Hofle, M. G. (2004). Development and application of a real-time PCR approach for quantification of uncultured bacteria in the central Baltic Sea. *Applied and Environmental Microbiology*. **70(8):** 4971-4979.

Ladd, J. N. (1978). Origin and range of enzymes in soil. In Soil Enzymes (R. G. Burns, Ed.). Academic Press, New York. pp. 51-96.

Lakay, F. M., Botha, A. and Prior, B. A. (2007). Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *Journal of Applied Microbiology*. **102:** 265–273

Lauber, C. L., Hamady, M., Knight, R. and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology.* **75:** 5111–5120.

Li, S. M., Li, L., Zhang, F. S. and Tang, C. (2004). Acid phosphatase role in chickpea/maize intercropping. *Annals of Botany*. **94(2):** 297-303.

Li, Z. G., Zeng, H., Nie, X. X., Liu, Y., Zhang, G. S. and Pan, J. F. (2015). Effect of different N, P, K and organic fertilization rates on growth and yield of *Actinidia chinensis* 'Hongyang'. *Plant Science- Journal*. **33:** 98–108.

Liang, W., Wu, Z. B., Cheng, S. P., Zhou, Q. H. and Hu, H. Y. (2003). Roles of substrate microorganisms and urease activities in wastewater purification in a constructed wetland system. *Ecological Engineering*. **21(2-3):** 191-195.

Lodhiyal, L. S., Singh, R. P. and Singh, S. P. (1994). Productivity and nutrient cycling in poplar stands in central Himalaya, India. *Canadian Journal of Forest Research.* **24:** 1199–1209.

Lopez-Monejar, R., Voriskov, J., Vetrovský, T. and Baldrian, P. (2015). The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biology and Biochemistry.* **87:** 43-50.

Lucy, M., Reed, E. and Glick, B. R. (2004). Application of free living plant-promoting rhizobacteria. *Antonie van Leeuwenhoek.* **86:** 1–25.

Lugtenberg, B. and Kamilova, F. (2009). Plant-growth-promoting rhizobacte- ria. *Annual Review of Microbiology*. **63:** 541–556.

Lugtenberg, B. J., Chin-A-Woeng, T. F. and Bloemberg, G. V. (2002). Microbe–plant interactions: principles and mechanisms. *Antonie Van Leeuwenhoek*. **81(1-4):** 373-383.

Magnusson, O. T., Toyama, H., Saeki, M., Rojas, A., Reed, J. C., Liddington, R. C., Klinman, J. P. and Schwarzenbacher, R. (2004). Quinone biogenesis: Structure and mechanism of PqqC, the final catalyst in the production of pyrroloquinoline quinone. *Proceedings of the National Academy of Sciences of the United State of America*. **101:** 7913-7918.

Makino, S. I. and Cheun, H. I. (2003). Application of the real-time PCR for the detection of airborne microbial pathogens in reference to the anthrax spores. *Journal of Microbiological Methods*. **53(2):** 141-147.

Manion, P. D. (1981). *Tree disease concepts*. Prentice-Hall, Inc.

Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S. and Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial iso- lates using *Zea mays* as indicator plant. *Soil Biology and Biochemistry.* **42:** 1229–1235.

Masto, R. E., Chhonkar, P. K., Singh, D. and Patra, A. K. (2009). Changes in soil quality indicators under long-term sewage irrigation in a sub-tropical environment. *Environmental Geology*. **56(6):** 1237-1243.

Matsushita, K., Arents, J. C., Bader, R., Yamada, M., Adachi, O. and Postma, P. W. (1997). *Escherichia coli* is unable to produce pyrroloquinoline quinone (PQQ). *Microbiology.* **143(10):** 3149-3156.

Matushita, K. and Adachi, O. (1993). Bacterial quinoprotein dehydrogenases, Glucose dehydrogenase. *In Principles and Applications of Quinoproteins.* 47-64.

McBride, M. B. (1994). *Environment Chemistry Soils*. New York, NY: Oxford University Press.

Mehnaz, S. and Lazarovits, G. (2006). Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. *Microbial Ecology*. **51(3):** 326-335.

Mehri, I., Turki, Y., Chair, M., Chérif, H., Hassen, A. and Meyer, J. M. (2011). Genetic and functional heterogeneities among fluorescent Pseudomonas isolated from environmental samples. *Journal of General and Applied Microbiology.* **57:** 101–114.

Mehrvarz, S., Chaichi, M. R. and Alikhani, H. A. (2008). Effects of phosphate solubilising microorganisms and phosphorus chemical fertilizer on yield and yield components of Barely (*Hordeum vulgare* L.). *Journal of Agricultture and Environtal Sciences.* **3:** 822-828.

Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A. and Raaijmakers, J.M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science.* **332:** 1097–1100.

Meulenberg, J. J. M., Sellink, E., Riegman, N. H. and Postma, P. W. (1992). Nucleotide sequence and structure of the *Klebsiella pneumoniae* pqq operon. *Molecular and General Genetics*. **232:** 284-294.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M. and Wilkening, J. (2008). The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics*. **9(1):** 386.

Meyer, J. B., Frapolli, M., Keel, C. and Maurhofer, M. (2011). Pyrroloquinoline quinine biosynthesis gene *pqqC*, a novel molecular marker for studying the phylogeny and diversity of phosphate-solublizing pseudomonads. *Applied and Environmental Microbiology.* **77:** 7345-7354.

Miller, W. R. and Donachue, R. L. (1992). Soils: An introduction to soils and plant growth. India, New: Prentice Hall.

Mills, M. M., Ridame, C., Davey, M., La Roche, J. and Geider, R. J. (2004). Iron andphosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature.* **429(6989):** 292–294.

Minaxi, L. N., Yadav, R. C. and Saxena, J. (2012). Characterization of multifaceted *Bacillus* sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown insemi arid deserts. *Applied Soil Ecology*. **59:** 124–135.

Mintie, A. T., Heichen, R. S., Cromack Jr, K., Myrold, D. D. and Bottomley, P. J. (2003). Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains. *Applied and Environmental Microbiology*. **69(6):** 3129-3136.

Mittal, V., Singh, O., Nayyar, H., Kaur, J. and Tewari, R. (2008). Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biology and Biochemistry.* **40:** 718-727.

Moeinzadeh, A., Sharif-Zadeh, F., Ahmadzadeh, M. and Tajabadi, F. (2010). Biopriming of Sunflower (*'Helianthus annuus'* L.) Seed with *Pseudomonas fluorescens* for Improvement of Seed Invigoration and Seedling Growth. *Australian Journal of Crop Science*. **4(7):** 564.

Monreal, C. M. and Bergstrom, D. W. (2000). Soil enzymatic factors expressing the influence of land use, tillage system and texture on soil biochemical quality. *Canadian Journal of Soil Science*. **80(3):** 419-428.

Mudge, S. R., Rae, A. L., Diatloff, E. and Smith, F. W. (2002). Expression analysis suggests novel roles for members of Pht1 family of phosphate transporters in *Arabidopsis*. *Plant Journal.* **31:** 341–353.

Muyzer, G., De Waal, E.C. and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiol*ogy. **59(3):** 695– 700.

Muyzer, G., Teske, A., Wirsen, C. O. and Jannasch, H. W. (1995). Phylogenetic relationships ofThiomicrospira species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Archives of Microbiology*. **164(3):** 165-172.

Nacke, H., Engelhaupt, M., Brady, S., Fischer, C., Tautzt, J. and Daniel, R. (2012). Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. *Biotechnology Letters.* **34:** 663–675.

Naik, P. R., Raman, G., Narayanan, K. B. and Sakthivel, N. (2008). Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. *BMC Microbiology.* **8:** 230.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. *European journal of soil science.* **54(4):** 655–670.

Naseby, D. C. and Lynch, L. M. (1998). Impact of wild type and genetically modified Pseudomonas fluorescens on soil enzyme activities and microbial population structure in the rhizosphere of pea. *Molecular Ecology*. **7:** 617–625.

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters.* **170:** 265–270.

Navarrete, A. A., Kuramae, E. E., deHollander, M., Pijl, A. S., vanVeen, J. A. and Tsai, S. M.(2013b). Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiology Ecology*. **83:** 607–621.

Navas-Molina, J. A., Peralta-S´anchez, J. M., Gonzalez, A., McMurdie, P. J., Vázquez-Baeza, Y., Xu, Z. and Huntley, J. (2013). Advancing our understanding of the human microbiome using QIIME. *Methods in Enzymology*. **531:** 371–444.

Naz, S. I. (2002). The vanishing shisham tree. The Daily Dawn. Lahore, Pakistan.

Negi, J. D. S., Pokhriyal, T. C., Sharma, S. D. and Bhandari, R. S. (1999). Shisham mortality in India – A case study. Unpublished report.

Nelson, D. R. and Mele, P. M. (2007). Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. *Soil Biology and Biochemistry*. **39(1):** 340-351.

Nguyen, C. (2003). Rhizodeposition of organic C by plants: Mechanisms and controls. *Agronomie.* **23:** 375–396.

Nielson, M. N. and Sorensen, J. (1999). Chitinolytic activity of ***Pseudomonas fluorescens*** **isolates from barley and sugar beet rhizosphere.** *FEMS Microbiology Ecology.* **30:** 217-227.

Ofek, M., Hadar, Y. and Minz, D. (2012). Ecology of root colonizing *Massilia* sp. (Oxalobacteraceae). *PloS one*. **7(7):** 40117.

Olsen S. R. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture; Washington.

Olsen, G. J., Lane, D. J., Giovannoni, S. J., Pace, N. R. and Stahl, D. A. (1986). Microbial ecology and evolution: a ribosomal RNA approach. *Annual Reviews in Microbiology.* **40:** 337-365.

Orlando, J., Ch avez, M., Bravo, L., Guevara, R. and Car u, M. (2007). Effect of Colletia hystrix (Clos), a pioneer actinorhizal plant from the Chilean matorral, on the genetic and potential metabolic diversity of the soil bacterial community. *Soil Biology and Biochemistry.* **39:** 2769–2776.

Otieno, N., Lally, R. D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K. J. and Dowling, D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. *Frontiers in Microbiology*. **6:** 745.

Pal, S. S. (1998). Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant Soil.* **198:** 169-177.

Patel, D. K., Archana, G. and Kumar, G. N. (2008). Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter sp*. DHRSS in the presence of different sugars. *Current Microbiology.* **56:** 168-174.

Pathan, M. A., Rajput N. A, Jiskani M. M and Wagan K. H. (2007). Studies on intensity of shisham dieback in Sindh and impact of seedborne fungi on seed germination. *Pakistan Journal of Agriculture Agricultural Engineering and Veterinary Sciences.* **23(2):** 12-17.

Patten, C. L. and Glick, B. R. (2002). Bacterial biosynthesis of indole3-acetic acid. *Canadian Journal of Microbiology.* **42:** 207–220.

Peix, A., Rivas-Boyero, A. A., Mateos, P. F., Rodriguez-barrueco, C., Martinez-Molina, E. and Velaqez, E. (2001). Growth promotion of chickpea and barley by a phosphate solubilising strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biology and Biochemistry.* **33:** 103-110.

Perez, E., Sulbaran, M., Ball, M. M. and Yarzabal, L. A. (2007). Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry*. **39(11):** 2905-2914.

Pietr, S. J., Koran, B. and Stankiewcz, M. (1990). "Influence of rock phosphate-dissolving rhizobacteria on the growth and the P-uptake by oat-preliminary results" in 2nd International Workshop on Plant Growth Promoting *Rhizobacteria* (Interlaken), 14–19.

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

Ponmurugan, P. and Gopi, C. (2006). Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *Journal of Agronomy*. **5:** 600-604.

Ponmurugan, P. and Gopi, C. (2006). *In vitro* production of growth regulators of phosphatase activity by phosphate solubilizing bacteria. *African Journal of Biotechnology*. **5:** 348-350.

Poupin, M. J., Timmermann, T., Vega, A., Zuniga, A. and Gonzalez, B. (2013). Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. *PLoS One*. **8(7):** 69435.

Puehringer, S., Metlitzky, M. and Schwarzenbacher, R. (2008). The pryrroloquinoline quinone biosynthesis pathway revisited: A structural approach. *BMC Biochemistry*. **9:** 8.

Puente, M. E., Bashan, Y., Li, C. Y. and Lebsky, V. K. (2004). Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biology*. **6:** 629–642

Raaijmakers, J. M. and Weller, D. M. (1998). Natural plant protection by 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Molecular Plant-Microbe Interactions*. **11(2):** 144-152.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and soil*. **321(1-2):** 341-361.

Rajan, S. S. S., Watkinson, J. H. and Sinclair, A. G. (1996). Phosphate rocks for direct application to soils. *Advances in agronomy*. **57:** 77-159.

Ramette, A., Moënne, L. Y. and Défago, G. (2003). Prevalence of fluorescent Pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanidein soils naturally suppressive or conducive to tobacco black root rot. *FEMS Microbiology Ecology*. **44:** 35–43.

Rampelotto, P. H., de Siqueira Ferreira, A., Barboza, A. D. M. and Roesch, L. F. W. (2013). Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian Savanna under different land use systems. *Microbial Ecology*. **66:** 593–607.

Rangel-Castro, J. I., Levenfors, J. J. and Danell, E. (2002). Physiological and genetic characterization of fluorescent *Pseudomonas* associated with *Cantharellus cibarius*. *Canadian Journal of Microbiology.* **48:** 739–748.

Rani, A., Souche, Y. and Goel, R. (2013). Comparative in situ remediation potential of *Pseudomonas putida* 710A and *Commamonas aquatica* 710B using plant (*Vigna radiata* (L.) wilczek) assay. *Annals of Microbiology.* **63(3):** 923-928.

Reeves, D. W. (1997). The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil and Tillage Research.* **43(1-2):** 131-167.

Renella, G., Egamberdiyeva, D., Landi, L., Mench, M. and Nannipieri, P. (2006). Microbial activity and hydrolase activities during decomposition of root exudates released by an artificial root surface in Cd-contaminated soils. *Soil Biology and Biochemistry.* **38(4):** 702–708.

Revathi R., Mohan V. and Jha M. N. (2013). Integrated nutrient management on the growth enhancement of *Dalbergia sissoo* Roxb. seedlings**.** *Journal of Academia and Industrial Research.***1(9):** 550-557.

Ribeiro, C. M. and Cardoso, E. J. B. N. (2012). Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (Araucaria *angustifolia*). *Microbiological Research.* **167(2):** 69-78.

Richardson, A. E., Baréa, J. M., McNeill, A. M. and Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil.* **321:** 305–339.

Riesenfeld, C. S., Schloss, P. D. and Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. *Annual Review of Genetics.* ***38*****:** 525-552.

Rodríguez, H. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*. **17(4-5):** 319-339.

Rodriguez, H., Gonzalez, T. and Selman, G. (2001). Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. *Journal of Biotechnology.* **84:** 155-161.

Rotini, O. T. (1935). La trasformazioneen zimaticadell'ureanelterreno. *Ann. Labor. Ric. Ferm. Spallanrani*. **3**: 143-154.

Rumberger, A., Yao, S., Merwin, I. A., Nelson, E. B. and Thies, J. E. (2004). Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil. *Plant Soil.* **264:** 247–260.

Russo, A., Vettori, L., Felici, C., Fiaschi, G., Morini, S. and Toffanin, A. (2008). Enhanced micropropagation response and biocontrol effect of *Azospirillum brasilense* Sp245 on *Prunus cerasifera* L. clone Mr. S 2/5 plants. *Journal of biotechnology*. **134(3):** 312-319.

Sah, S. P., Upadhyay, S. and Pandit, P. (1999). Assessing the effects of physical properties of soil on sissoo (*Dalbergia sissoo*, Roxb.) growth in a plantation forest. *Final report submitted to DANIDA/HMG*, *Nepal*. *51*.

Şahin, F., Çakmakçi, R. and Kantar, F. (2004). Sugar beet and barley yields in relation to inoculation with N 2-fixing and phosphate solubilizing bacteria. *Plant and Soil*. **265(1-2):** 123-129.

Sahrawat, K. L. (1983). Relationships between soil urease activity and other properties of some tropical wetland rice soils. *Nutrient Cycling in Agroecosystems.* **4:** 145–150.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. **4(4):** 406-425.

Sanikidze, G. S., Gogorikidze, N. I. and Shoniya, N. K. (1973). Enzymatic activity of basic soil types of the subtropical zones of the western Georgian. *Subtropicheskie kul'tury.*

Saravanan, V. S., Madhaiyan, M. and Thangaraju, M. (2007). Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere*. **66(9):** 1794-1798.

Saravanan, V. S., Subramoniam, S. R. and Raj, S. A. (2003). Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian Journal of Microbiology*. **35(1-2):** 121-125.

Schimel, J.P. and Weintraub, M.N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry.* **35:** 549–563.

Schjonning, P., Elmholt, S. and Christensen, B. T. (2004). Soil quality management concepts and terms. *Managing soil quality: Challenges in modern Agriculture*. 1-15.

Schloss, P. D. and Handelsman, J. (2003). Biotechnological prospects from metagenomics. *Current Opinion in Biotechnology*. **14:**303–310.

Schnuerer, J. and Rosswall, T. (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and Environmental Microbiology*. **43 (1982):** 1256-1261.

Schwyn, B. and Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*. **160(1):** 47-56.

Sebiomo, A., Ogundero, V. W. and Bankole, S. A. (2011). Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal Biotechnology*. **10**: 770-778.

Selvi, K., John, P. B., Ravindran, J.A. and Vijaya, V. (2011). Quantitative estimation of insoluble inorganic phosphate solubilization. *International Journal of Security and Network.* **2(2):** 292-295.

Serkebaeva, Y. M., Kim, Y., Liesack, W. and Dedysh S. N. (2013). Pyrosequencing-Based Assessment of the Bacteria Diversity in Surface and Subsurface Peat Layers of a Northern Wetland, with Focus on Poorly Studied Phyla and Candidate Divisions. *PLoS One.* **8(5)**: e63994

Shah, P., Kakar, K. M. and Zada, K. (2001). Phosphorus use efficiency of soybean by phosphorus application and inoculation. In: *Plant nutrition- food security and sustainability of agroecosystems.* Ed. Horst, W. J. 670-671.

Shaheen, M., Shah, A., Hameed, A. and Hasan, F. (2008). Influence of culture conditions on production of protease enzyme in *Bacillus.* **8:** 240-246.

Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M. and Sharma, R. (2007). Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. *Journal of Herbal Medicine and Toxicology*. **1:** 61-63.

Sharma, M. K., Singaland, R. M. and Pokhriyal, T. C. (2000). *Dalbergia sissoo* Roxb. in India. In: Proc. of the Sub-Regional seminar on dieback of sissoo (*Dalbergia sissoo* Roxb.). 5-16.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus*. **2(1):** 587.

Shen, Y. Q., Bonnot, F., Imsand, E. M., RoseFigura, J. M., Sjölander, K. and Klinman J. P. (2012). Distribution and properties of the genes encoding the biosynthesis of the bacterial cofactor, pyrroloquinoline quinone. *Biochemistry.* **51:** 2265-2275.

Shen, Z., Wang, D., Ruan, Y., Xue, C., Zhang, J., Li, R. and Shen, Q. (2014). Deep 16S rRNA pyrosequencing reveals a bacterial community associated with banana Fusarium wilt disease suppression induced by bio-organic fertilizer application. *PLoS One*. **9(5):** 98420.

Shrivastava, S, D'souza, S. F, and Desai, P. D. (2008). Production of indole-3-acetic acid by immobilized actinomycete (*Kitasatospora* sp.) for soil applications. *Current Science*. **94:** 1595–1604.

Si, Z., Zhu, J., Wang, W., Huang, L., Wei, P., Cai, J. and Xu, Z. (2016). Novel and efficient screening of PQQ high-yielding strains and subsequent cultivation optimization. *Applied Microbiology and Biotechnology*. 1-10.

Siciliano, S. D., Theoret, C. M., de Freitas, J. R., Hucl, P. J. and Germida, J. J. (1998). Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. *Canadian Journal of Microbiology*. **44:** 844–851.

Singh, A. V., Chandra, R. and Goel, R. (2012). Phosphate solubilization by *Chryseobacterium* sp. and their combined effect with N and P fertilizers on plant growth promotion. *Archives of Agronomy and Soil Science*. **59(5):** 641-651.

Singh, D. K., Sale, P. W. G. and Routley, R. R. (2005). Increasing phosphorus supply in subsurface soil in northern Australia: Rationale for deep placement and the effects with various crops. *Plant Soil*. **269:** 35-44.

Sinsabaugh, R. L., Carreiro, M. M. and Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry*. **60:** 1–24.

Smit, E., Leeflang, P., Gommans, S., vandenBroek, J., vanMil, S. and Wernars, K. (2001). Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Applied and Environmental Microbiology*. **67:** 2284–2291.

Soltis, D. E., Soltis, P. S., Morgan, D. R., Swensen, S. M., Mullin, B. C., Dowd, J. M. and Martin, P. G. (1995). Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proceedings of the National Academy of Sciences*. **92(7):** 2647-2651.

Soylu, S., Soylu, E. M., Kurt, S. and Ekici, O. K. (2005). Antagonistic potentials of rhizosphere-associated bacterial isolates against soil-borne diseases of tomato and pepper caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. *Pakistan Journal of Biological Sciences*. **8(1):** 43-48.

Spain, A. and Alm, E. (2003). Implications of microbial heavy metal tolerance in the environment. *Reviews in Undergraduate Research.* **2:** 1-6.

Sparling, G. P. (1997). Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health. CAB International, Wallingford. pp. 97–119.

Spier, T. W. and Ross, R. J. (1978). Soil phosphatase and sulphatase, in: R.G. Burns (Ed.), soil enzymes, Academic Press, London. 198-250.

Sridevi, M. and Veera Mallaiah, K. (2007). Bioproduction of indole acetic acid by Rhizobium strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.) Merr. *Iranian Journal of biotechnology*. **5(3):** 178-182.

Stephan, A., Meyer, A. H. and Schmid, B. (2000). Plant diversity affects culturable soil bacteria in experimental grassland communities. *Journal of Ecology.* **88:** 988–998.

Stubberfield, L. C. F. and Shaw, P. J. A. (1990). A comparison of tetrazolium reduction and FDA hydrolysis with other measurements of microbial activity. *Journal of Microbiological Methods.* **12**: 151-162.

Sturz, A. V., Christie, B. R. and Nowak, J. (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Sciences*. **19(1):** 1-30.

Sugihara, S., Funakawa, S., Kilasara, M. and Kosaki, T. (2010). Dynamics of microbial biomass nitrogen in relation to plant nitrogen uptake during the crop growth period in a dry tropical cropland in Tanzania. *Soil Science and Plant Nutrition*. **56(1):** 105-114.

Swain, M. R. and Ray, R. C. (2009). Biocontrol and other beneficial activities of *Bacillus subtilis* isolated from cowdung microflora. *Microbiological Research*. **164(2):** 121-130.

Swensen, B. and Bakken, L. R. (1998). Nitrification potential and urease activity in a mineral subsoil. *Soil Biology and Biochemistry*. **30(10-11):** 1333-1341.

Swisher, R., and G. C. Carroll. (1980). Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. *Microbial Ecology*. **6**: 217-226.

Tabacchioni, S., Chiarini, L., Bevivino, A., Cantale, C. and Dalmastri, C. (2000). Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. *Microbial Ecology*. **40**: 169–176.

Tabatabai, M. A. (1982). Soil enzyme. In: Page AL (ed) Methods of soil analysis, Part 2. American Society of Agronomy, Madison, WI. 903–948.

Tabatabai, M. A. and Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry.* **1(4):** 301-307.

Tantau, H., Hoque, M. I., Sarker, R. H. and Muhlbach, H. P. (2005). 16S rDNA sequence analysis of bacterial isolates from dieback-affected sissoo trees (*Dalbergia sissoo* Roxb.) in Bangladesh. *Journal of Phytopathology.* **153:** 517-521.

Tariq, M., Yasmin, S. and Hafeez, F.Y. (2010). Biological control of potato black scurf by rhizosphere associated bacteria. *Brazilian Journal of Microbiology*: **41(2):** 439-451.

Taurian, T., Anzuay, M. S., Angelini, J. G., Tonelli, M. L., Luduena, L., Pena, D., Inanez, F. and Fabra, A. (2010). Phosphate-solubilizing peanut associated bacteria: screening for plant growth promoting activities. *Plant Soil.* **329:** 421-431.

Tedersoo, L., Bahram, M. and Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., Harend, H., Buegger, F., Pritsch, K., Koricheva, J. and Abarenkov, K. (2016). Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *International Society for Microbial Ecology Journal.* **10:** 346–62.

Thalmann, A. (1968). Zur Methodik der Bestimmung der Dehydrogenase aktivit At im Boden mittels triphenyl tetrazoliumchlorid (TTC). *Landwirtsch Forsch.* **21:** 249-258.

Theodorou, M.E. and Panxton, W.C. (1993). Metabolic adaptations to plant respiration to nutritional phosphate deprivation. *Plant Physiology*. **101:** 339-344.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*. **25(24):** 4876-4882.

Thomson, B. C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R. I., Hannula, S. E., Buee, M., Mougel, C., Ranjard, L., Van Veen, J. A., Martin, F., Bailey, M. J. and Lemanceau, P. (2015). Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. *Soil Biology and Biochemistry.* **88:** 403-413.

Tilman, D., Fargione, J., Wolff, B., D'antonio, C., Dobson, A., Howarth, R. and Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*. **292(5515):** 281-284.

Tomar, R. K. S., Namdeo, K. N. and Ranghu, J. S. (1996). Efficacy of phosphate solubilizing bacteria biofertilizers with phosphorus on growth and yield of gram (*Cicer arietinum*). *Indian Journal of Agronomy.* **41:** 412-415.

Torsvik, V. and Overeas, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*. **5**: 240–245.

Trevors, F.D. (1998). Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Applied and Environmental Microbiology*. **64**: 4950 4957.

Trevors, J. T. (1984). Effect of substrate concentration, inorganic nitrogen, O_2 concentration, temperature, pH on dehydrogenase activity in soil. *Plant Soil*. **77**: 285.

Trivedi, P., Anderson, I. C. and Singh, B. K. (2013). Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiology*. **21:** 641– 651.

Troup, R.S. (1921). The Silviculture of Indian Trees, Review Edition, Vol. 1, Clarendon Press, Oxford, 1980.

Tscherko, D., Rustemeier, J., Richter, A., Wanek, W. and Kandeler, E. (2003). Functional diversity of the soil microflora in the primary successon across two glacier forelands in the Central Alps. *European Journal of Soil Science.* **54:** 685–696.

Upchurch, R., Chiu, C., Everett, K., Dyszynski, G., Coleman, D. C. and Whitman, W. B. (2008). Differences in the composition and diversity of bacterial communities from agricultural and forest soils. *Soil Biology and Biochemistry.* **6(40):** 1294–1305.

Uroz, S, Calvaruso, C. and Turpault, M. P. (2009). Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology*. **17:** 378–87.

Uroz, S., Buee, M., Deveau, A., Mieszkin, S. and Martin, F. (2016). Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *Soil Biology and Biochemistry*. **103:** 471-488.

Uroz, S., Oger, P., Lepleux, C., Collignon, C., Frey-Klett, P. and Turpault, M. P. (2011). Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. *Research in Microbiology*. **162:** 820-831.

Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënne-Loccoz, Y., Muller, D. and Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in plant science*. **4:** 356.

van der Heijden, M. G. A., Bardgett, R.D. and van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters.* **11:** 296–310.

van der Heijden, M. G., Martin, F. M., Selosse, M. A. and Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*. **205:** 1406–1423.

Velterop, J. S., Sellink, E., Meulenberg, J. J., David, S., Bulder, I. and Postma, P. W. (1995). Synthesis of pyrroloquinoline quinone in vivo and in vitro and detection of an intermediate in the biosynthetic pathway. *Journal of Bacteriology.* **177:** 5088-509

Verma, J. P., Yadav, J., Tiwari, K. N. and Kumar, A. (2013). Effect of indigenous *Mesorhizobium* sp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. *Ecological Engineering.* **51:** 282-286.

Versaw, W. K. and Harrison, M. J. (2002). A chloroplast phosphate transporter, PHT2; 1, influences allocation of phosphate within the plant and phosphate-starvation responses. *Plant Cell.* **14:**1751–1766.

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

Vyas, P. and Gulati, A. (2009). Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology*. **9:** 174.

Wachowska, U., Kucharska, K., Jędryczka, M. and Łobik, N. (2013). Microorganisms as biological control agents against Fusarium pathogens in winter wheat. *Polish Journal of Environmental Studies*. ***22*(2).**

Wahyudi, A. T., Astuti, R. P., Widyawati, A., Meryandini, A. and Nawangsih, A. A. (2011). Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybeanplants for their use as potential plant growth for promoting rhizobacteria. *Journal of Microbiology and Antimicrobials*. **3:** 34–40.

Waldrop, M. P., Balser, T. C. and Firestone, M. K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry*. **32:** 1837–1846.

Walpola, B. C. and Yoon, M. (2013). Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. *African Journal of Microbiology Research.* **7(3):** 266-275.

Wani, P. A., Khan, M. S. and Zaidi, A. (2007). Co-inoculation of nitrogen-fixing and phosphate- solubilising bacteria to promote growth, yield and nutrient uptake in chickpea. *Acta Agronomica Hungarica*. **55:** 315-323.

Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T. and Sorek, R. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*. **450(7169):** 560.

Wellinghausen, N., Frost, C. and Marre, R. (2001). Detection of legionellae in hospital water samples by quantitative real-time Light Cycler PCR. *Applied and Environmental Microbiology*. **67(9):** 3985-3993.

Westerman, R. L., Case, V. W. and Jones, J. R. (1990). Sampling handling and analyzing plant tissue samples. *Soil testing and plant analysis.* **3:** 389-427.

Wolinska, A. and Ste, Z. (2012). Dehydrogenase activity in the soil environment.

Xiao, X., Fan, M., Wang, E., Chen, W. and Wei, G. (2017). Interactions of plant growth-promoting rhizobacteria and soil factors in two leguminous plants. *Applied Microbiology and Biotechnology*. **101(23-24):** 8485-8497.

Xiao, Y., Wang, X., Chen, W. and Huang, Q. (2017). Isolation and identification of three potassium-solubilizing bacteria from rape rhizospheric soil and their effects on ryegrass. *Geomicrobiology Journal*. **34(10):** 873-880.

Xiong, X. H., Zhao, Y., Ge, X., Yuan, S. J., Wang, J. H., Zhi, J. J., Yang, Y. X., Du, B. H., Guo, W. J., Wang, S. S., Yang, D. X. and Zhang, W. C. (2011). Production and radioprotective effects of pyrroloquinoline quinone. *Internattional Journal of Molecular Sciences.* **12:** 8913-8929.

Xiong, X. H., Zhi, J. J., Yang, L., Wang, J. H., Zhao, Y., Wang, X. and Wei, N. (2011). Complete genome sequence of the bacterium *Methylovorus* sp. strain MP688, a high-level producer of pyrroloquinolone quinone. *Journal of Bacteriology*. **193(4):** 1012-1013.

Xu, P. and Gunsolley, J. (2014). Application of metagenomics in understanding oral health and disease. *Virulence*. **5(3):** 424-432.

Xuan, Y. X., Liu., T. H., Guang, H. L. and Cui, M. (2011). Isolation and characterization of phosphate solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biology and Fertility of Soils.* **47:** 437-446.

Yadav, K. and T. Singh. (1991). Phosphorus solubilization by microbial isolate from *Caci fluvent*. *Journal of Indian Society for Sciences.* **39:** 89-93.

Yadav, R. and Tarafdar, J. (2001). Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biology and Fertility of Soils*. **34(3):** 140-143.

Yandigeri, M. S., Meena, K. K., Srinivasan and Pabbi, S. (2011). Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. *Indian Journal of Microbiology.* **51(1):** 48-53.

Yang, C. H. and Crowle, D. E. (2000). Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Applied and Environmental Microbiology.* **63:** 345–351.

Yang, X. P., Zhong, G.F., Lin, J. P., Mao, D. B. and Wei, D. Z. (2010). Pyrroloquinoline quinone biosynthesis in *Escherichia coli* through expression of the *Gluconobacter oxydans pqqABCDE* gene cluster. *Journal of Industrial Microbiology and Biotechnology.* **37:** 575-580.

Yang, Y., Wang, N., Guo, X., Zhang, Y. and Ye, B. (2017). Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. *PloS One*. **12(5):** 0178425.

Yao, H. C. (2002). Veterinary microbiology experiment manual. 2nd ed. Beijing: China Agricultural Press. pp. 43-55.

Yao, H., Xu, J. and Huang, C. (2003). Substrate utilization pattern, biomass and activity of microbial communities in a sequence of heavy metal-polluted paddy soils. *Geoderma*. **115:** 139–148.

Yazdani, M., Bahmanyar, M. A., Pirdashti, H. and Esmaili, M. (2009). Effect of Phosphate solubilisation microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays* L.). *Proceedings of World Academy of Sciece*, *Engineering and Technology*. **37:** 90-92.

Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*. **67(5):** 1613-1617.

Yuan, K. N. (1983). Chemical analysis of plant nutrients in soil (In Chinese). Science Press, Beijing.

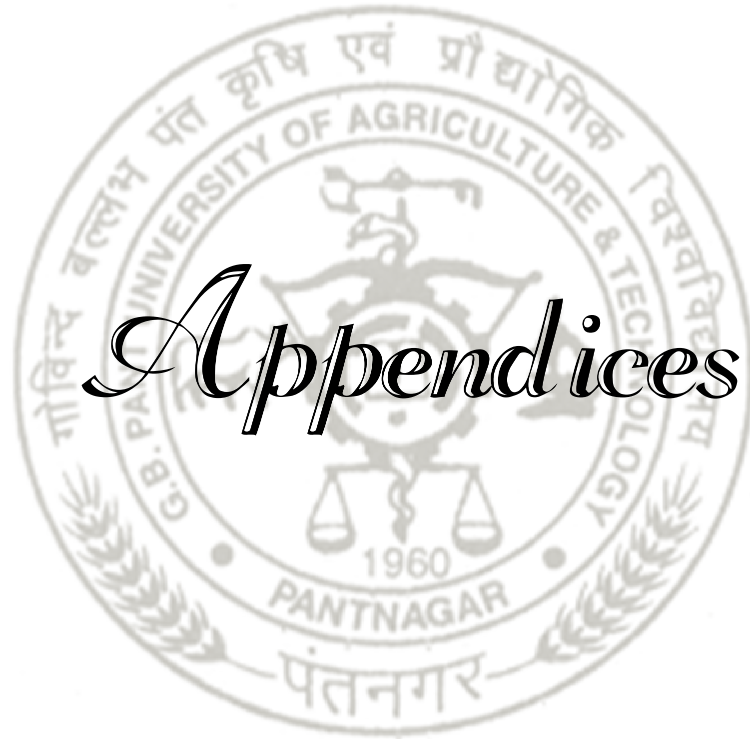
Zayed, G. and Motaal, H. A. (2005). Bioactive composts from rice straw enriched with rock phosphate and their effect on the phosphorus nutrition and microbial community in rhizosphere of cowpea. *Bioresource Technology.* **96:** 929-935.

Zhang, M. and Lidstrom, M. E. (2003). Promoters and transcripts for genes involved in methanol oxidation in *Methylobacterium extorquens* AM1. *Microbiology*. **149:** 1033-1040.

Zhang, X., Zhang, R., Gao, J., Wang, X., Fan, F., Ma, X. and Deng, Y. (2017). Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. *Soil Biology and Biochemistry.* **104:** 208–217.

Zhu, F., Qu, L., Hong, X. and Sun, X. (2011). Isolation and Characterization of a Phosphate- Solubilizing Halophilic Bacterium *Kushneria sp.* YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine*.

Appendices



Appendix I

Media Used

Nutrient Agar	**gl^{-1}**
Beef extract	3
Peptone	5
Agar	20
pH	7.2±0.2

Nutrient Broth	**gl^{-1}**
Peptone	5.0
Beef extract	3.0
pH	±7.0

NBRIP Media	**gl^{-1}**
Glucose	10
$Ca_3(PO_4)_2$	5
$MgCl_2.6H_2O$	5
KCl	0.2
$(NH4)_2SO_4$	0.1
BPB	0.25
Agar	15
pH	7.0±0.2

Pikovaskaya's Agar	**gl^{-1}**
Yeast extract	0.5
Dextrose	10
$CaPO_4$	5.0
$(NH_4)_2SO_4$	0.5
KCl	0.2
$MgSO_4.7H_2O$	0.1
$MnSO_4$	0.0001
$FeSO_4$	0.001
Agar	20

Angle's Media

Solution Fe/EDTA

Na_2 EDTA	14 mg
$FeSO_4.7H_2O$	50 mg
H_2SO_4(conc.)	5 µl
Distilled water	10 ml

Autoclave the solution for 15 min at 121˚C

Solution of D-oligo elements

$ZnSO_4.7H_2O$	100 mg
$MnCl_2.4H_2O$	30 mg
H_3BO_3	300 mg
$CaCl_2.6H_2O$	200 mg
$NiCl_2.6H_2O$	20 mg
$Na_2MoO_4.2H_2O$	30 mg
Distilled water	

Autoclave the solution for 15 min at 121˚C

Composition

Tris HCl 0.5M pH 7.0	40ml
Sol. Fe/EDTA	110 µl
Sol. D oligo- elements	1ml
Yeast extract	2gm
KH_2PO_4 0.1% w/v	680 µl
1M K	500 µl
NH_4NO_3	0.2g
$CaSO_4$	0.69g
$MgCl_2$	0.406g
Distilled water	1000 ml
Agar	20g

Adjust the pH at 7.0 with 1 M NaOH

Autoclave the solution for 15 min at 121°C. At the working add 1 ml of glucose solution (1mg/ml) in 1 litre of media

Glucose solution

Glucose	1mg
Distilled water	1ml

Filter sterilize the solution with 0.2 m filter

King's B Agar	**gl^{-1}**
Proteose peptone	20
K_2HPO_4	1
$MgSO_4.7H_2O$	0.4
Glycerol	8ml
Agar	20
pH	7.0±0.2

APPENDIX II

Reagents

TRIS –EDTA (TE) Buffer

TrisHCl	10 mM
EDTA	1 mM
pH	8
Sodium Dodecyl Sulphate	10 %(w/v)
Proteinase K	20 mg ml^{-1} (w/v)
CTAB	10 % (w/v)
Phenol: Chloroform	1:1(v/v)
Chloroform: Isoamyl alcohol	24:1(v/v)
RNAase	10 mg ml^{1}
Chilled ethanol (absolute)	70%
TRIS Acetate EDTA (TAE)	
TRIS Acetate	40mM
Na_2 EDTA	1mM

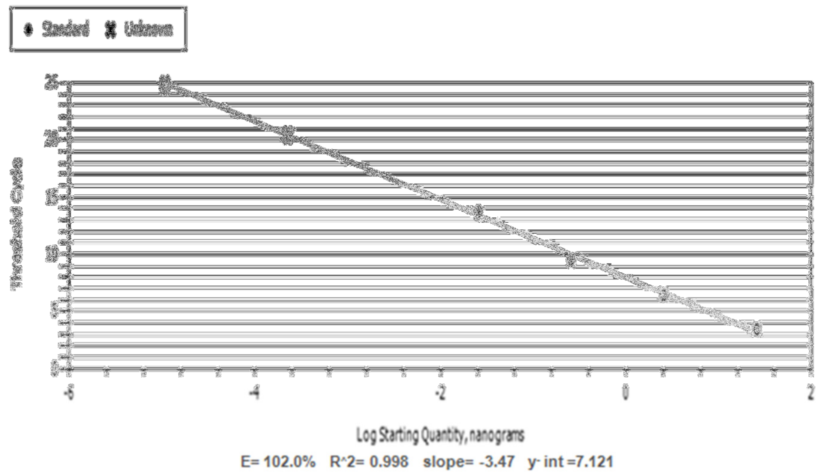
Loading dye (6X)

Sucrose	40% (w/v)
Bromophenol blue	0.25% (w/v)
Xylene Cyanol	0.25% (w/v)
Ethedium Bromide	
Stock	10.00mg ml^{1}(w/v)
Working Solution	0.5µg ml^{1}

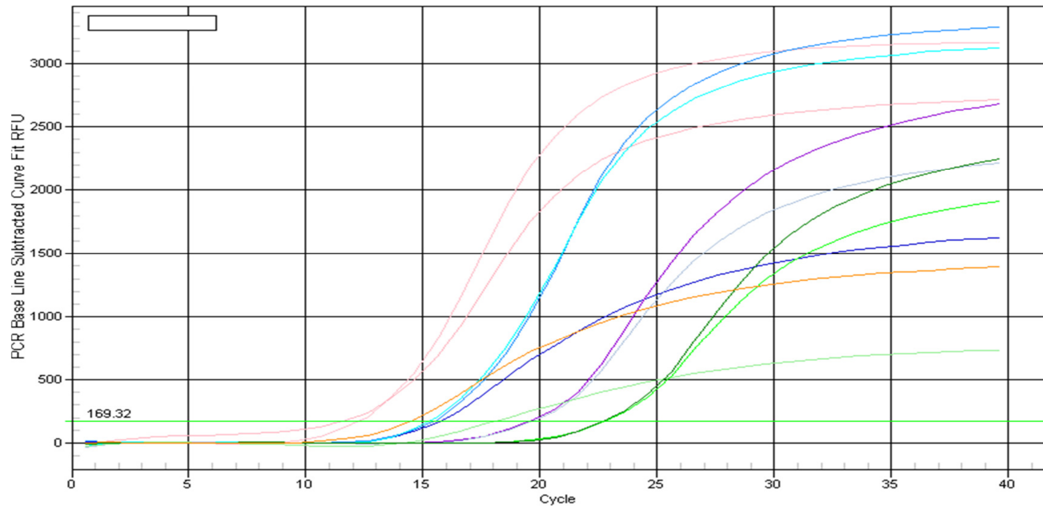
EDTA solution (0.1 M)

Add 3.724g EDTA (disodium salt) in 60 ml of distilled water and adjust the pH to 8 (EDTA will not dissolve until the pH is adjusted to 8.0).Make the final volume 100 ml.

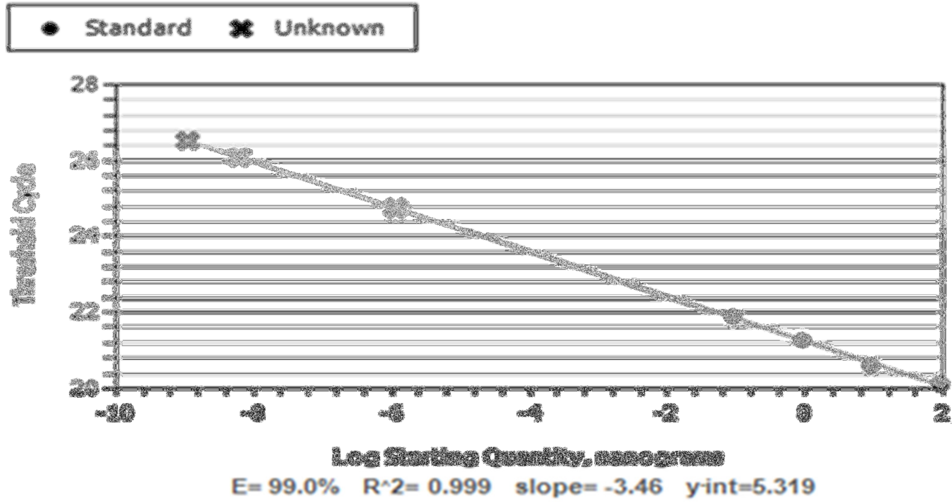
APPENDIX III



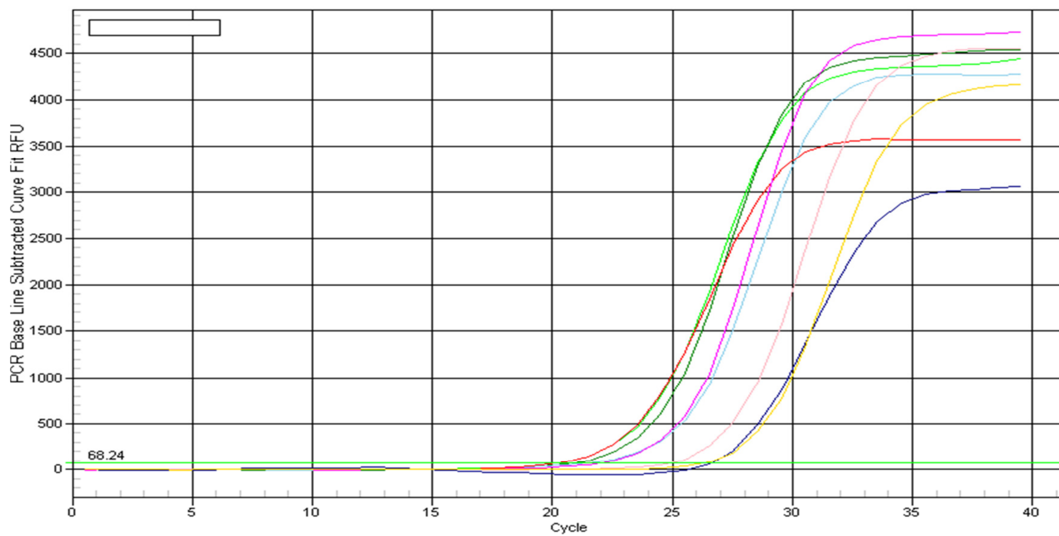
Standard curve for the 16 S rRNA gene during the qPCR experiment



Amplification curve of the 16S rRNA gene amplicons during q-PCR soil



Standard curve for the *pqq* gene during the qPCR experiment



Amplification curve of the *pqq* amplicons during q-PCR of *pqq* gene in soil DNA

APPENDIX IV

Reagents for Gram's staining

1.**Crystal violet**

Solution A	Crystal violet	2g
	Ethyl alcohol (95%)	20ml
Solution B	Ammonium oxalate	0.8g
	Distilled water	80ml

2.**Gram's iodine**

	Iodine	1g
	Potassium iodide	2g
	Distilled water	300ml
	Sodium bicarbonate	3g

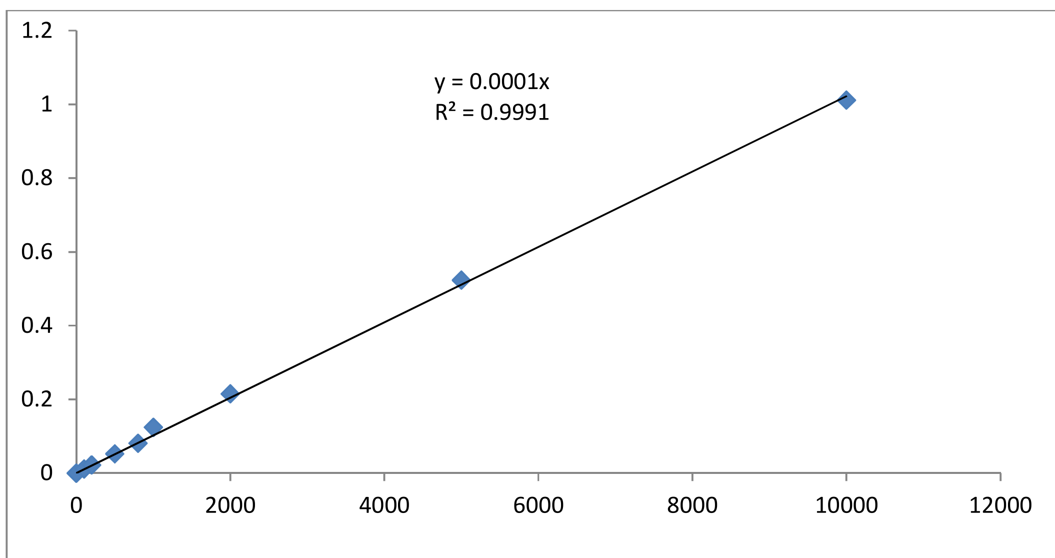
3.**Destaining solution**

	Ethyl alcohol	95ml
	Gram's iodine/Distilled water	5ml

4.**Saffaranin**

	Saffranin	10ml
	(2.5% solution in 100ml ethyl alcohal)	
	Distilled water	90ml

APPENDIX V



Standard curve of KH_2PO_4 for estimation of phosphorus

***In vitro* qualitative and quantitative estimation of tricalcium phosphate solubilization by bacterial isolates**

S.NO.	Strain id	P solubilization index* (mm)	Psolubilization (µg/ml)
1	L1	2.75±0.02	590.63±12.57
2	L2	2.50±0.03	506.25±0.79
3	L3	2.75±0.05	559.32±10.76
4	L4	4.75±0.06	891.38±18.55
5	L5	3.50±0.03	721.27±10.51
6	L6	3.75±0.06	762.10±7.5
7	L7	2.60±0.04	530.75±12.15
8	L8	2.80±0.07	616.48±7.05
9	P1	2.50±0.005	510.33±7.17
10	P2	4.00±0.07	850.56±14.16
11	P3	2.85±0.06	639.62±4.66
12	P4	1.85±0.01	476.31±4.46
13	T1	2.60±0.01	537.55±6.01
14	T2	2.60±0.03	523.94±2.99
15	T3	1.71±0.04	340.22±1.39
16	T4	1.16±0.01	285.78±3.27
17	T5	2.00±0.01	421.87±6.36
18	T6	2.50±0.04	559.32±6.98

*Values are the mean of triplicates with standard error of mean

$$* \, SolubilizationIndex \; (SI) = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

APPENDIX VI

Sequences of 16S rDNA region of-

>L1 *Pseudomonas simiae* strain MG966339

CTAGCTAAAGGTGKGGGAKGRAGRWMTCYMYKGCWTCTCTTGAGAGCGG
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AGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GGGGAATATTGRACAATGGGCRAAAGCCTGATCCAGCCATGCCGCGTGTGT
GAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTA
GATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAAC
TCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATT
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GTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACT
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>L2 *Staphylococcus petrasii* strain MG966340

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GAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTT
CCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCG
AAGCCGGTGGAGTAACCATTTGAGCAGCCTCAAGGTGCGATSWCMRAGAA
ATCACCTTCG

>L3 *Pseudomonas paralactis* strain MG966341
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>L4 *Klebsiella variicola* strain MG966342

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>L5 *Pseudomonas paralactis* strain MG966343

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AGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG
CGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGARA
CACGGTCCAGACTCCTACGGGAGGCAGCAGKGGGGAATATTGGACAATGG
GCRAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTG
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ATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAA
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>L6 *Streptomyces curacoi* strain MG966344

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GAGTGTGKTARGGGAGATCGKAATTCCTGGTGTARCGGTGAGATGCRCWK
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CGTCGGSTGCCRCAGCTRAYGCATTAAGTTCCCCGCCWGKGMGTACTGCKC
ACGCCTARRSKCAAASGACTGACGTGACCCCWMCAAKCAGYGGAKSAKCG
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>L7 *Streptomyces cellostaticus* strain MH031699

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TKCTGCATCCSATACSGGCTASCTAGAGTGTGRTACGAGAGATCGGAATTCC
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CGAYTGACCTGTGCTATCGASGCGASCKATGATCTTATCAGTCTAGCCTAAC
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>L8 *Pantoea conspicua* strain MG966345

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CGGTAYTCCTCCAGATCTCTACGCATTTCACCGCTACACTGGGAATTCTACC
CCCCCTCTACAGACTCAAGCCTGCCAGTTTCAAATGCAGTTCCGAGTTAAG
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ARGTTAACTTCACGATWTATRAACSGC

>P1 *Pseudomonas hunanensis* strain MG966346

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CGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAG
CCTAGGTCGGATTAGCTAGTTGGKGGGGTAATGGCTCACCAAGGCRACGAT
CCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTC
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GGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGAS
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>P2 *Pseudomonas aeruginosa* strain MG966347

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GGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACC
GCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
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>P3 *Pseudomonas putida* strain MG966348

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GCTTGGCAACCCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCG
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AGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTAC
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GCCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACT
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CACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCT
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TAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGA
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GGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTA
GGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGA
CGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAG
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GTCATCCGCTCGACTTGCAKTKTAGGCTKCGCCTKGSSSMAYCWMKTKRYT
TTTTA

>P4 *Pseudomonas plecoglossicida* strain MG966349

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GTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGC
CTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCRACGATC
CGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
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TCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAA
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>T1 *Kitasatospora kifunensis* strain MG966350

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TAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCRGTGGGGAATATTGCACAATGGGCGAAAGCC
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TTCACTWKGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCMCCGGS
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AATTATTGGGCGTAAAGAKCTCKTAGGCGGCCTGTCSCGTCRGATGTGAAA
GCCCGGGGCTTAACCCCGGGTCTGCATTCSATRCSGGCAGGCTGGAGTGTG
GTAGGGGAGATCRGAATTCCTGGTGTARCGGGGAAATGCGCTTATATCAGG
AGGAACACCGGTGGYRAAGGTSGATCTCTGGRCCATTACTGACGCTGASGA
GCKAAACCRTGGGKARCGMTCAGGATTARATACCCKGGGTAGTMCACRCS
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AGCTMATGCRKYRAGGTTTCCCGGCMTGGAGSAGTAYGRCKCAACGGYWA
ARCTMRWACGAMTGACGKGRACCCSTCACAAAKCARYTGAASSAKGYGCS
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>T2 *Klebsiella singaporensis* strain MG966351

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AGTCGAGTTGCAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGC
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CTCCRGAAGCCACSCCTCARGGGCACAACCTCCAAATCGACATCGTTTACG
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GAGCGTCAGTCTTTGTCCRGGGGGGCCGYCTTCGCCACCGGTATTCCTCCA
GATCTCTACGCATTTCACCGCTACACCTGRGAAYTCTACCCCCTCTACARGA
CTCTAGCSCTGCCAGTTTMGGATGCRGTTCCCGAGGTWGGAGCSCSGGGAG
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>T3 *Streptomyces antibioticus* strain MG966352

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CGCGTGAGGGATGACGGCCTTCSGGTTGTAAACCTCTTTCARCAGGGAAGA
AGCGAAAGTGACGGTACCTGCAGAAGAATCGCCGGCTAACTACKTGCCAR
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>T4 *Micrococcus yunnanensis* strain MG966353

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CGGATAGGAGCGTCCACCGCATGGKGGGTGTTGGAAAGATTTATCGGTTTT
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>T5 *Streptomyces griseoruber* strain MG966354

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CCTCTTTCASCAGGGAAGAAGCGAAAGTGACGGTACCTGCARAAGAAGCGC
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MCGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGAGGGR
RGCTWSMMASMGGKMG

>T6 *Staphylococcus pasteuri* strain MG966355

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CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCYTAAGCTTAGTT
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AGCCGGTGGAGAACCCATTTGGAGCASCCTARAAGGACAAASRMGCCGCC
AATCGTCTAA

VITA

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Thesis title	:	**Rhizospheric bacterial diversity in different *Dalbergia sissoo* Roxb. provenances**			
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ABSTRACT

Dalbergia sissoo Roxb., common name shisham is a leguminous tree known internationally for its priced timber. Therefore, there is an urgent need to understand the cause and ways to control large scale mortality of mature trees in natural forests. Soil microbes play an important role in tree growth and health. Hence, a comparative study of bacterial communities in shisham rhizosphere at three provenances Lachhiwala, Tanakpur and Pantnagar was carried out through illumina-MiSeq sequencing. The bacterial community composition and diversity based on V3-V4 16S rDNA region was variable and distinct for each provenance. The bacterial diversity index values indicated by species richness (Chao metric), species richness and evenness (Shannon metric) and count of unique OTUs (observed species metric) were significantly higher at Pantnagar than at Lachhiwala and Tanakpur with highest coverage for Lachhiwala (88.86%) and least for Tanakpur (67.19%). β diversity analysis depicted closer relationship between rhizospheric bacterial diversity at Tanakpur and Lachhiwala (both healthy forests) than at Pantnagar (forest with mortality). Phylum *Proteobacteria* was abundant at Lachhiwala and Tanakpur whereas *Acidobacteria* predominated at Pantnagar. Amongst genera, *Pseudomonas, Flavobacterium, Bacillus, Paenibacillus, Sphingomonas, Nitospirae* and *Massilia* were abundant at Lacchiwala and Tanakpur. However, *Williamsia, Blastocatella, Methylobacterium,* and *Brevibacterium* at Pantnagar. Metagenomic bacterial diversity was linked to several soil biotic (population and enzyme activities) and abiotic properties (pH, organic carbon, EC and available N, P, K and micronutrients). Highest bacterial count was recorded at Tanakpur followed by Lachhiwala and Pantnagar. Soil enzyme activities such as acid and alkaline phosphatases and urease were highest at Lachhiwala. In contrast FDA and Dehydrogenase were highest at Tanakpur. A strong positive correlation was observed between all enzyme activities and soil nutrients (macro and micro). Since phosphatase activities and available phosphate in soil was higher so phosphate solubilizing bacteria (PSB) were recovered and characterized. Inorganic phosphorous solubilizing ability of bacteria ranged between 285.78 $\mu g\ ml^{-1}$ to 891.38 $\mu g\ ml^{-1}$ These PSB's exhibited multiple plant growth promoting traits also. In all 18 PSBs were distinguished into seven ARDRA groups. These were identified as *Pseudomonas sp.*, *Klebsiella sp., Streptomyces sp., Pantoea sp., Kitasatospora sp., Micrococcus sp.* and *Staphylococcus sp.* through 16SrDNA sequence analysis. Out of eighteen, sixteen bacterial isolates were positive for 82 bp *pqq* C gene whereas six for 72 bp *pqq* A gene.

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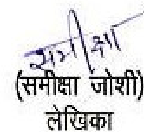
सत्र एवं प्रवेश का वर्ष	: द्वितीय, 2014–15	**उपाधि**	:	**पीएच.डी.**
मुख्य विषय	: सूक्ष्म जीव विज्ञान	**विभाग**	:	सूक्ष्म जीव विज्ञान
गौण विषय	: आण्विक जीव विज्ञान एवं जैव प्रौद्योगिकी			
शोध शीर्षकः	विभिन्न स्थानों के *डेलबर्जिया सिसो* रोक्सब. में राइजोस्फेरिक बैक्टीरियल विविधता			
सलाहकार	: डॉ. मानविका सहगल			

<u>सारांश</u>

डेलबर्जिया सिसो रोक्सब. अथवा शिशम एक मूल्यवान वृक्ष है, जो अंतर्राष्ट्रीय स्तर पर अपने मूल्यवान लकड़ी के लिये जाना जाता है। इसलिये, प्राकृतिक जंगलों में परिपक्व वृक्षों की बड़े पैमाने पर मृत्यु दर को नियंत्रित करने के कारण और तरीकों को समझने की तत्काल आवश्यकता है। वृक्षों की बढ़ोत्तरी में मृदा में पाये जाने वाले सूक्ष्मजीवों की महत्वपूर्ण भूमिका है। इसीलिये उत्तराखण्ड के तीन प्रमुख स्थानों लच्छीवाला, टनकपुर और पन्तनगर में शीशम मूलतंत्र के सूक्ष्मजीविय समुदायों का तुलनात्मक अध्ययन illumina-MiSeq अनुक्रमण के माध्यम से किया गया था। तीनों क्षेत्रों के शीशम मूलतंत्र की सूक्ष्मजीविय संरचना (16SrDNA के वी 3–वी 4 भाग) विविध और विशिष्ट थी। जीवाणु विविधता सूचकांक मान द्वारा संकेतित प्रजातियों की समृद्धि (चाओ मैट्रिक), प्रजातियों की समृद्धि और समानता (शैनन मैट्रिक) और विशिष्ट ओटीयू (प्रजाति मैट्रिक) की गिनती लच्छीवाला और टनकपुर की तुलना में पन्तनगर में सर्वाधिक थी, जिसमें लच्छीवाला के लिये उच्चतम आवृत्त क्षेत्र (88.86%) और सबसे कम टनकपुर (67.19%) के लिये थी। β विविधता विश्लेषण ने पन्तनगर (रोगग्रस्त जंगल) की तुलना में टनकपुर और लच्छीवाला (स्वस्थ जंगल) में राईजोस्फेरिक बैक्टिरियल विविधता के बीच घनिष्ठ संबंध दिखाई दिये। लच्छीवाला और टनकपुर में फाईलम प्रोटियोबैक्टिरीया सर्वाधिक संख्या में थे जबकि पन्तनगर में एसिडोबैक्टिरीया की संख्या सर्वाधिक थी। जैनेरा स्यूडोमोनास, फ्लेवोबैक्टिरीयम, बैसिलस, पैनिबैसिलस, स्फिंगोमोनास, नाईट्रोस्पोरा, और मैसिलिया लच्छीवाला और टनकपुर में सर्वाधिक मात्रा में थे। हांलाकि, विलियमसिया, ब्लास्टोकैटेला, मिथाईलोबैक्टिरीयम और ब्रेविबैक्टिरीयम पन्तनगर में ज्यादा थे। मैटाजिनोमिक जीवाणु विविधता, मिट्टी के कई जैविक (संख्या और एन्जाईम गतिविधियों) और अजैविक गुणों (pH, कार्बनिक कार्बन, E.C. और उपलब्ध N, P, K और सूक्ष्म पोषक तत्वों) पर आधारित है। सूक्ष्मजीवों की अधिकतम संख्या टनकपुर में और न्यूनतम पन्तनगर में दर्ज की गयी। लच्छीवाला से ली गई मृदा में एसिड फॉस्फेट, क्षारीय फॉस्फेट और यूरिएस एन्जाईम गतिविधियाँ सर्वाधिक थीं। इसके विपरीत टनकपुर में एफ0डी0ए0 और डीहाईड्रोजिनेज़ सर्वाधिक थे। सभी एंजाईम गतिविधियों और मिट्टी के पोषक तत्वों (मैक्रो और माइक्रो) के बीच एक मजबूत, सकारात्मक, सहसंबंध देखा गया था। चूंकि मिट्टी में फास्फेट की गतिविधियाँ और उपलब्ध फॉस्फेट अधिक था इसीलिये फॉस्फेट घुलनशील बैक्टिरीया (PSB) को पृथक तथा उनका रूपात्मक, कार्यात्मक और आणविक तकनीक से विवरण किया गया। बैक्टिरीया की अकार्बनिक फॉस्फोरस घुलनशील क्षमता 285.78 $\mu g\ ml^{-1}$ से 891.38 $\mu g\ ml^{-1}$ के बीच थी। इन फॉस्फेट घुलनशील बैक्टिरीया ने कई पादप वृद्धि गुणों का प्रदर्शन किया। सभी 18 पीएसबी को 7 ARDRA समूहों में विभाजित किया गया था। 16 S rDNA अनुक्रम विश्लेषण के माध्यम से इनकी पहचान *स्यूडोमोनास* एसपी., *क्लैबासीला* एसपी., *स्ट्रैप्टोमाइसीज* एसपी., *पैन्टोइ* एसपी., *कीटासाटोस्पोरा* एसपी., *माइक्रोकोकस* एसपी. और *स्टेफाइलोकोकस* एसपी. के रूप में की गई। 18 में से 16 सूक्ष्मजीवों में फॉस्फेट घुलनशील 82 bp *pqq* C जीन और छः में 72bp *pqq* A जीन को डीएनए अनुक्रमण तकनीक से प्रमाणित किया गया।

(मानविका सहगल)
सलाहकार


(समीक्षा जोशी)
लेखिका