Chapter 5
Phosphate-Solubilizing Fungi: Current Perspective and Future Need for Agricultural Sustainability

Deep Chandra Suyal, Manali Singh, Dipti Singh, Ravindra Soni, Krishna Giri, Satyajit Saurabh, Ajar Nath Yadav, and Reeta Goel

Contents

D. C. Suyal (✉)
Department of Microbiology, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

M. Singh
Invertis Institute of Engineering and Technology (IIET), Invertis University, Bareilly, UP, India

D. Singh
Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

R. Soni
Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chhatisgarh, India

K. Giri
Rain Forest Research Institute, Jorhat, Assam, India

S. Saurabh
DNA Fingerprinting Laboratory, Bihar State Seed and Organic Certification Agency, Patna, Bihar, India

Department of Bioengineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

A. N. Yadav
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

R. Goel
Department of Microbiology, College of Basic Sciences & Humanities, GBPUAT, Pantnagar, Uttarakhand, India

A. N. Yadav (ed.), *Recent Trends in Mycological Research*, Fungal Biology,
https://doi.org/10.1007/978-3-030-60659-6_5

5.1 Introduction

It is anticipated that world population will reach 9.7 billion by 2050, wherein Southeast Asia is the main contributor (Forbes 2017). The current dynamics of demographics suggest that the population in India will even surpass China by 2022. With our limited and shrinking agricultural land resources, the impetus is largely on the development of innovative and sustainable ways of transforming agriculture to feed the ever-increasing population. Currently, around 58% of the rural households depend upon agriculture as their principal source of livelihood agriculture is one of the largest contributors to GDP (gross domestic product) (IBEF 2017). According to Central Statistics Organization (CSO), the share of agriculture and allied sectors is expected to be 17% of the gross value added (GVA) in 2016–17 at 2011/12 prices. Green revolution ushered the successful implementation of Industrial Agriculture fueled by large-scale use of synthetic agro-chemicals and chemical fertilizers. The unavailability arising from modern agriculture is due to increase in cost of cultivation and rising food prices, both of which have to be extensively compensated by government which makes the economics of its perusal extremely costly and inefficient.

Phosphorus (P) is an essential macro-nutrient for plant growth and development. In spite of having an ample presence in the soil, its bioavailability is very low (Kour et al. 2020b). Mostly it is present in the form of the insoluble complexes and only 0.1% of the total P is reported to be present in the soluble form (Farhat et al. 2009; Tomer et al. 2016). Unfortunately, it is among the least mobile and most unavailable soil nutrient for the plants. Its solubility is reported to depend on several factors, namely, organic matter, pH, active sesquioxides, lime and nature and content of clay (Kour et al. 2019a). Soil pH is the important determinative factor and pH 6.7 is considered ideal for the same (Mehrvarz et al. 2008; Selvi et al. 2011). In tropics, it is observed to present as the inorganic compounds, i.e., iron–aluminum compounds (under acidic condition) and calcium compounds (under neutral to alkaline conditions) (Mehrvarz et al. 2008; Selvi et al. 2011). During summer and rainy seasons in the tropical countries including India, pH was found to go up to 10.5 units, salt level up to 2% temperature between 35 and 45 °C, which largely affects the mobility of the nutrients in the soil (Nautiyal 2000). Further, the major portion of the chemical fertilizers (75–90%) when applied to the agricultural fields get transformed into an insoluble oxide/silicate forms by reacting with Al^{3+}, Ca^{++}, Zn^{++}, Fe^{3+}, Co^{++}, etc.

(Selvi et al. 2011). This conversion decreases the efficiency of the fertilizers and ultimately increases the input cost for the agriculture. In this scenario, PSM provides a sustainable alternative to supplement the P to the crops. Application of PSM has shown up to 40% reduction in the need of chemical fertilizers when applied alone (Tomer et al. 2017) (Rajwar et al. 2018). This ability of the microorganisms has opened the new doors toward the exploration of microbial technologies in the agricultural sector.

India possesses a remarkable potential for the development of organic farming practices due to its agro-climatic conditions (Charyulu and Biswas 2010; Giri et al. 2015). Although India is one of the largest producers for agricultural commodities of the world, the productivity index in comparison to world benchmarks is extremely low. Shrinking agricultural land sizes are one of its major causes as the average plot size in India has fallen from 2.7 hectares in 1970 to under 1.2 hectares today (Economist 2015). Also, due to lack of proper education and awareness, there have been indiscriminate practices of chemical fertilizers and pesticides across the Indian Subcontinent thereby creating huge loss of natural soil productivity. It has been reported that excessive application of agro-chemicals leads to loss of soil fertility due to increase of salt content and thereby impacting on consumer's health (Swapna 2013). Based on numerous studies conducted, it is imperative that a transformation of large-scale conventional agriculture is required which in turn will need modification of biotic and abiotic factors in order to fulfill the agricultural demand of the future.

Replacement of chemical phosphatic fertilizers with PSM is the need of the hour to propagate organic input-based agriculture for improvement of overall human and environmental health. PSMs are the microbial inoculants or biological active products with formulations containing one or more beneficial strains of fungi or bacteria in an easy to apply and efficient carrier material which either add, conserve, or mobilize phosphate in soil (Mazid and Khan 2015; Dash et al. 2019). PSM-based biofertilizers are easy to use, non-toxic, and cost-effective (Kour et al. 2020c). They either manufacture the nutrients required by crops from soil or atmosphere or mobilize the nutrients pre-existing in soil media in forms most absorbable by crops. They have also been reported to act as biocontrol agents by conducting antagonistic activities against phytopathogenic bacteria. An example of one such activity is interference in the bacterial quorum sensing system. However, the primary function of PSM is reportedly for plant growth enhancement from which it excises more than one mechanism (Fig. 5.1) (Rani et al. 2013; Suyal et al. 2014a).

PSMs can solubilize the insoluble P complex into the bioavailable form through chelation, ion-exchange reactions, and acidification (Fig. 5.2). Several microbial groups including bacteria (*Pseudomonas, Thiobacillus, Azotobacter, Erwinia, Serratia, Agrobacterium, Arthrobacter, Bacillus, Enterobacter, Flavobacterium, Bradyrhizobium, Salmonella, Micrococcus, Alcaligens, Streptomyces, Chromobacterium,* etc.), cynabacteria (*Calothrix braunii, Westiellopsis prolific, Anabaena variabilis,* etc.), and fungi (*Aspergillus, Penicillium, Arthrobotrys, Trichoderma,* etc.) are known to solubilize the rock phosphates.

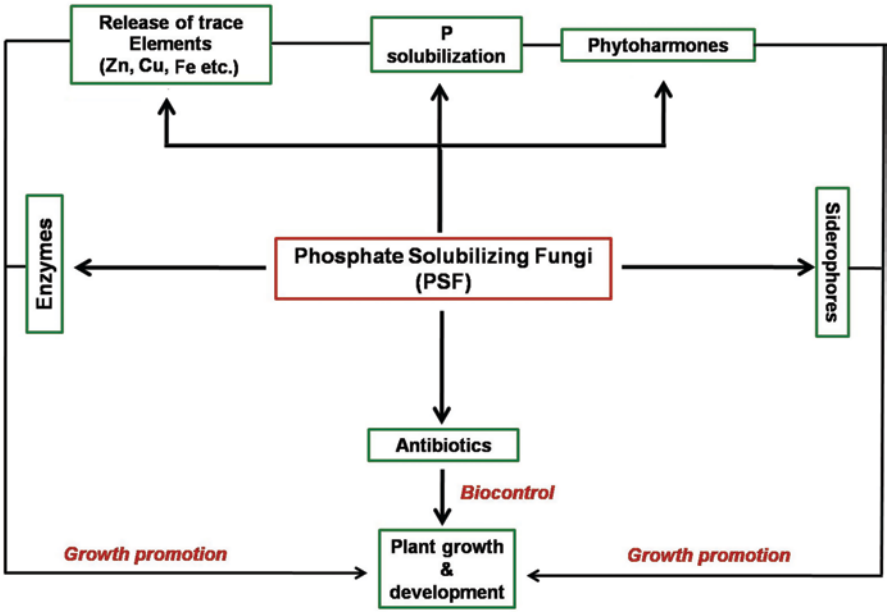

Fig. 5.1 Role of phosphate-solubilizing fungi in plant growth development

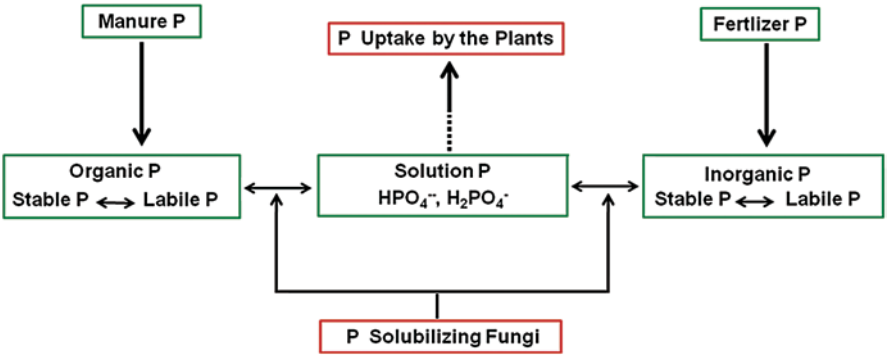

Fig. 5.2 Depiction of P-solubilization mechanism of the fungi

Among the fungi, *Aspergillus* and *Penicillium* are the most predominant genera which have shown their potential for P solubilization. Yu et al. (2005) have reported the P solubilization by *Penicillium oxalicum* and *Aspergillus niger* in liquid culture. Recently, Kalayu (2019) has reviewed several PSF, namely, *Aspergillus tubingensis, A. sydawi; A. ochraceus; A. versicolor, Penicillium bilaii, P. citrinum, P. digitatum; P. lilacinium; P. balaji; P. funicolosum, P. oxalicum, P. simplicissimum; P. rubrum, Arthrobotrys oligospora, Trichoderma viride*, *Rhizopus, Fusarium*, and *Sclerotium*.

5.2 Rhizospheric P-Solubilizing Fungi

Soil is the natural media that support vegetation by providing nutrients and other essential elements for growth. The thin area of soil surrounding the roots of the plants is known as rhizosphere. It is directly affected by root exudates and hence is rich in soil-related microorganisms. Root exudates are the compounds secreted by roots in its immediate proximity. The nature of microbial community in the rhizosphere is directly affected by the constituents of these root exudates. Exudates are majorly constituted by ions, sugars, aromatic and aliphatic acids, volatile aromatic compounds, vitamins, peptides, proteins, enzymes, plant hormones, alcohols, ketones, olefins, and urea. Root exudates contribute to 40% of the net fixed carbon by plant photosynthesis containing almost 200 different types of compounds. Root exudates perform ecological interactions with the soil microbial community by releasing signaling molecules, attractants, and stimulants. Moreover, they can be used by plants in their defense against various pathogens (Baetz and Martinoia 2014; Kobae 2019; Kour et al. 2019b).

The nature of the root exudates varies from one place to another because of the impact of various biotic and abiotic factors. The change in the nature of these exudates also changes the microflora of rhizosphere. Besides it, they also help the plant to compete with surrounding plants and promote plant–microbe symbiotic interactions (Yadav et al. 2017; Rajwar et al. 2018; Yadav et al. 2019; Rai et al. 2020). The microbes use these root exudates as a substrate and also contribute some of the metabolites that are absorbed by the plant to fulfill its nutritional requirements. Among the rhizospheric PSF, mycorrhizae are the most important groups of the microorganisms (Remy et al. 1994; Ezawa and Saito 2018). They are also known as fungal roots. Mycorrhizae are well efficient in the nutrient absorption from the soil, especially P (Harrison and van Buuren 1995; Harrison et al. 2002; Fonseca and Berbara 2008; Hart et al. 2017). They may be ectomycorrhiza (*Leccinum, Hebeloma, Lactarius, Suillus*, etc.) or endomycorrhizae (*Rhizophagus irregularis, Acaulospora, Gigaspora, Glomus, Entrophospora,* etc.) (Jansa et al. 2008; Kikuchi et al. 2016; Kobae 2019).

5.3 Mechanism of P-Solubilization

PSM employs the following three mechanisms (McGill and Cole 1981) to solubilize P: (a) by releasing compounds such as hydroxyl ions, protons, siderophores, organic acids, and CO_2 that assist the breakdown and solubilization of complex molecules; (b) biochemical mineralization by the discharge of extracellular enzymes; and (c) by releasing phosphorous during substrate degradation.

Further, on the basis of the nature of the substrate, the P-solubilization mechanisms can be explained as follows:

5.3.1 Organic

Three groups of enzymes are involved in the release of organic phosphorous from soil (Kaur et al. 2017).

5.3.1.1 Nonspecific Acid Phosphatases (NSAPs)

These are dephosphorylated phosphoester or phosphoanhydride compounds of organic matter. Phosphomonoesterases (phosphatases) are the most common among them. Acid phosphatases have been found to be present in several fungi, such as *Aspergillus, Penicillium, Fusarium,* and *Neurospora* (Shahab et al. 2009). These phosphatases were produced in media containing an inorganic nitrogen source [$NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3] and a very low concentration of inorganic phosphate (Pi). The fungal strain *Humicola lutea* 120-5 utilizes the phosphoprotein casein through biosynthesis of extracellular enzymes: acid proteinases (Aleksieva and Mutafov 1997; Aleksieva and Peeva 2000) and acid phosphatases (Micheva-Viteva et al. 2000). In some cases, mineralization of natural phosphorus and phosphate solubilization can exist together. Inoculation either only with phosphate solubilizer or with other potential rhizospheric organisms has been very much achieved (Ahemed and Kibret 2014).

5.3.1.2 Phytases

These are the enzymes having an ability to release at least one phosphate group from the phytic acid, a fixed organic form of P (Suyal and Tewari 2013a, 2013b; Kour et al. 2020a). The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC–IUB) distinguish two classes of phytate degrading enzymes, 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.28), initiating the dephosphorylation at the 3 and 6 positions of phytate, respectively (Guilan et al. 2009); completely hydrolyzing to inositol and inositol monophosphate.

Microbial phytase activity was most frequently detected in fungi. Mostly phytase producers are filamentous fungi, especially from the genus *Aspergillus*, *Penicillium,* and *Mucor*. Phytase from *A. niger* group is considered most active. Over 200 fungal isolates belonging to the genus *Aspergillus*, *Mucor, Penicillium*, and *Rhizopus, Trichoderma* have been tested for phytase production (Soni et al. 2010; Rawat et al. 2009). The phytase and its applications have recently been well reviewed by Sharma et al. (2020).

Several strains of yeasts, the eukaryotic fungi, contain biologically valuable proteins (40–60%), vitamin B-complex, important trace minerals, and several unique "plus" factors, such as ability to enhance P bio-availability (Sharma et al. 2020). It is reported that among yeasts, extracellular phytases are produced by *Schwanniomyces castellii* (Segueilha et al. 1992), *Arxula adeninivorans* (Sano et al. 1999), and

S. cerevisiae (Veide and Andlid 2006). Intracellular phytase occurs in several yeasts such as *Saccharomyces cereviseae* (Man-Jin et al. 2008; Iefuji et al. 2009) and *Cryptococcus laurentii* (Pavlova et al. 2008). Baker's yeast *S. cerevisiae* is generally recognized as safe (GRAS, defined by U.S. Food and Drug Administration) for food production, and has been widely used for the production of food-grade phytase (Veide and Andlid 2006; Yasoda et al. 2007).

5.3.2 Inorganic

The following are two main theories in this aspect:

(i) Acid production theory
(ii) Proton and enzyme theory

As per acid production theory, PSMs produce organic acids such as oxalic, fumaric, glyoxalic, malic, citric, gluconic, succinic, alpha-ketobutyric, 2-ketogluconic, and tartaric acid which lower the pH (Puente et al. 2004; Rodrigues et al. 2004). Its amount and type vary from fungus to fungus. Lowering of pH of the filtrates of PSMs is because of these organic acids (Rani et al. 2013). Fasim et al. (2002) observed the role of microbes in the solubilization of zinc oxide and phosphate through gluconic acid and 2-ketogluconic acid production (Table 5.1).

Proton and enzyme theory states that a group of enzymes such as esterase are responsible for the phosphorous solubilization from compounds containing organic phosphate. According to this theory, phosphorous solubilization, besides the

Table 5.1 Organic acids produced by P-solubilizing fungi

Fungi	Acids
Aspergillus candidus, A. flavus, A. niger, A. terreus, A. wentii, Fusarium oxysporum, Penicillium sp., *Trichoderma isridae, Ttrichoderma sp.*	Lactic, maleic, malic, acetic, tartaric, citric, fumaric, gluconic
A. flavus, A. candidus, A. fumigatus	Glutaric, oxalic, tartaric
Penicillium oxalicum	Malic, gluconic, oxalic
Aspergillus flavus, P. canescens	Oxalic, citric, gluconic, succinic
Penicillium rugulosum	Citric, gluconic
A. niger	Succinic, citric, oxalic, gluconic
Penicicllium variabile, Penicillium rugulosum, Penicillium radicum	Gluconic
A. awamori, A. foetidus, A. terricola, A. amstelodemi, A. Tamari	Oxalic, citric
A. japonicus, A. foetidus	Oxalic, citric, gluconic, succinic, tartaric
P. simplicissimum, P. bilaji	Citric, oxalic
A. awamori, P. digitatum	Succinic, citric, tartaric
Chaetomium nigricolor	2-Ketogluconic

generation of acid, involves release of protons in association with ammonium assimilation (Shahab et al. 2009). Other than these two systems, phytohormones such as indole acetic acid, cytokinin, and gibberellin also aid phosphate solubilization. Formation of chelating agents such as H_2S, CO_2, mineral acids, and siderophores also has indirect effect on phosphate solubilization (Shahab et al. 2009).

5.4 Genetics of P-Solubilizing Microbes

Generation of organic acids is likely to be involved in mineral phosphate solubilization in all the PSM including fungi. The genes mandatory for these acid productions were anticipated to affect this feature. A few genes involved in acid phosphatase have been represented (Rossolini et al. 1998). For example, the *acp*A gene communicates an acid phosphatase showing ideal activity at pH 6, with extensive substrate specificity (Reilly et al. 2006). Furthermore, broad-spectrum acid phosphatases containing class A gene *pho*C and class B gene *nap*A, separated from *Morganella morganii*, are extremely encouraging. Besides this, a little is known about the mechanism involved in the biochemical systems required for the union of the GDH-PQQ halo enzyme and the region for the variation in some microorganisms among constitutive and inducible phenotypes.

The conceivable inducers that show promising halo enzyme activity are glucose, gluconate, mannitol, and glycerol. 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). GDH requires pyrroloquinoline quinone (PQQ) as a cofactor, which is the product of a *pqq* operon comprised of 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 holoenzyme which leads to the production of gluconic acid from glucose. Han et al. (2008) have shown that the absence of 2-ketogluconic acid, due to inactivation of *pqq* genes in *Enterobacter intermedium* 60-2G, leads to insolubility of hydroxyl-apatite. PCR studies were conducted in *S. marcescens* CTM 50650 strain (Farhat et al. 2009) to check the presence of genes involved in the expression of MPS via activation of the direct oxidation pathway of glucose (GDH encode by *gdh* and *pqq* genes involved in the biosynthesis of the required PQQ cofactor). Rodrıguez et al. (2000), Rajwar et al. (2018), and Joshi et al. (2019) have reported *pqq* genes in the diazotrophs. A gene of phosphatase enzyme in *Burkholderia cepacia* is known to encode an outer membrane protein which increases the P transport within a cell (Rodrıguez et al. 2000). Two nonspecific periplasmic acid phosphatase genes (*nap*D and *nap*E) from *Sinorhizobium meliloti* were also cloned (Deng et al. 2001).

A MPS gene (*gab*Y) was isolated from *Pseudomonas cepacia* and its expression was studied in *E. coli* HB 101. Babu-Khan et al. (1995) have identified 396 *gab*Y ORFs of *P. cepacia* and evaluated their expression in *E. coli* K-12. They have found that this strain had synthesized apo-GDH but PQQ. Furthermore, JM109 (pSLY4)

and JM109 (pGAB1) were found to synthesize 10-fold more gluconic acid in the presence of 1 mM PQQ. In another study, a genetic construct using pKT230 and pMCG898 was prepared by Rodriguez et al. (2000), encoding PQQ synthase gene (responsible for MPS) from *Erwinia herbicola* and was transferred to *Pseudomonas* sp. and *Burkholderia cepacia* IS-16. The positive recombinant clones were able to produce higher insoluble phosphate in comparison to their respective wild type strains. A 7 kb fragment from *Rhanella aquatilis* was cloned by Kim et al. (1997) and transferred to *E. coli* strains so that hydroxyapatite-solubilization ability can be conferred and hence induce the production of gluconic acid. Presence of two open frames ORF1 and ORF2 and a partial ORF were revealed in nucleotide analysis. Among them, ORF2 encodes a protein of 44 kDa which has remarkable sequence resemblance to *pqq*E of *Klebsiella pneumonia, E. herbicola* and *Acinetobacter calcoaceticus* and were revealed in nucleotide analysis. Further, a 10 kDa protein was found to encode by ORF1 which has shown a strong sequence resemblance to the *pqq*D of *A. calcoaceticus* and *K. pneumoniae. E. coli* can produce GDH, without PQQ, and thus, does not produce GA.

The cloned 1.8 kb locus encodes a protein that shows striking resemblance to the gene III product of a *pqq* synthesis gene complex from *Acinetobacter calcoaceticus,* and to *pqq*E of *K. pneumoniae* (Liu et al. 1992). It has been observed that DNA fragment from *E. herbicola* worked as PQQ synthase gene. Further, few *E. coli* strains may possess cryptic PQQ which were supposed to complement by this ORF. These observations have revealed that although acid production is an essential way of P solubilization, it cannot be considered the only way to perform that. Numerous genes are reported which are responsible for solubilization of the insoluble phosphate. A *pcc* (phosphoenolpyruvate carboxylase) gene from *Synechococcus* was found to involve in P solubilization. To release Pi from the organic complexes, microorganisms have developed a specific system which possesses the alkaline and acid phosphatases. The genetic regulation of these enzymes has been studied. Under P limiting conditions, several genes are observed to induced and initiate the *pho* regulation, namely, *pho*A (for alkaline phosphatase), *pho*B (a positive regulator or an activator), *pho*T, *pst*S, and *pst*B, etc. They all constitute *pho* box (Torriani and Ludtke 1985; Makino et al. 1989; Ezawa and Saito 2018). PhoR protein regulates the Pho regulation both negatively and positively with excess and limited phosphate, respectively. Pho M is another protein showing inhibitory effect on the product of PhoR, into an inactive form, PhoM. In presence of Pi, PhoU exhibits a negative control.

The Pst-Pho U region constitutes an operon with a transcription attenuator between Pho S and Pho T (Wanner 1987). As organic acid production is among the key mechanisms of P solubilization, it is assumed that any change in structure/function of the respective genes will affect this property. In this scenario, genes of P uptake have been studied thoroughly in several PSM. It has been observed that *Sinorhizobium meliloti* possess at least two P transport systems – high- and low-affinity transport systems. The high-affinity system is observed to encode by the *pho* CDET operon, whereas low-affinity system is known to encode by orfA-pit operon. These genes are regulated by PhoB activator. In case of P-sufficient

conditions, *Pho*B becomes inactive and thus *pho*CDET genes are not expressed. Under P deficiency, *Pho*B becomes activated and thus *pit* permease system (low-affinity system) is suppressed while *pho* CDET system gets activated and predominantly acts as P transport (Bianco and Defez 2010). *pst*SCAB homologs have been found in some microorganisms that are known to serve as high-affinity P transporters (Behera et al. 2014). Recently, Ezawa and Saito (2018) have reviewed the genetics of P solubilization by arbuscular mycorrhizal fungi. The group has reported an SPX domain in the proteins which are involved in Pi homeostasis in eukaryotes.

5.5 Applications of Genetic Engineering for Potential Bioinoculants Development

PSF performance mainly depends on its potential to colonize under a certain habitat. Plate counting and most-probable-number techniques have been used for the study of fungal communities in the rhizosphere. It is considered that less than 1% of the microorganisms in the environment can generally be cultured by standard culture techniques. Spatial heterogeneity and culturing inability are the major limitations for identification of the fungus (Kirk et al. 2004, Mummey et al. 2006). Spatial heterogeneity occurs due to the temporal and spatial variability during the sampling. Moreover, improper sampling and handling may also affect the results. On the other side, culturing inability arises due to the lack of the suitable growth media. Therefore, microbial habitats, their interactions, and growth requirements need to be studied properly to overcome this problem.

Molecular biology techniques are extensively used for characterizing microbial community structures in different environments. Cloning and sequencing techniques are commonly used techniques to determine microbial community structure. Besides them, hybridization and probing techniques can also determine the same with the advantage that they are less time-consuming, however require a sufficient knowledge of the community to select the appropriate target sequence. Some other techniques such as ribosomal intergenic spacer analysis (RISA) and amplified ribosomal DNA restriction analysis (ARDRA) can be used to study PGF colonization or community structure. ARDRA and RISA have been used in the analysis of mixed bacterial populations from different environments. ARDRA can be used for taking an overview of genotypic changes occurred in the community over time. However, RISA provides a method of microbial community analysis for comparing differing environments or treatment effects without any kind of biasness imposed by culture-dependent approaches. In brief, RISA involves PCR amplification of an intergenic spacer region (ISR). These molecular techniques have greater quantitative efficiency and can be further extended to characterize PGF under in situ conditions.

Knowledge of the fungal genes governing the production of organic acids would make it possible to transfer the phosphate-solubilizing ability to various other microorganisms that are competent of colonizing a particular rhizosphere. As clear from the earlier discussions, rhizosphere competence is a most important factor that determines the fate of success or failure of microbial inoculant. The rhizosphere has

various amounts of carbon sources that can be utilized by the heterogeneous microbial communities in soil to produce various types of organic acids. Oxidative metabolism of glucose by glucose dehydrogenase (GDH) produces gluconic acid; glucose dehydrogenase (GDH) requires pyrroloquinoline quinine (PQQ) cofactor. Therefore, genes involved in the transport/biosynthesis of PQQ can be cloned from various microbes and transferred to the other (Bruto et al. 2014). If the genes involved in PQQ biosynthesis are transferred to *Trichoderma* sp. that possess apo-GDH and that is rhizosphere competent too, the resulting *Trichoderma* strains will show both phosphate-solubilizing activity as well as biocontrol activity. Similarly, Ambrose et al. (2015) have successfully characterized salicylate hydroxylase gene from the fungal endophyte *Epichloë festucae*.

5.6 Available Approaches and Methodologies to Study P-Solubilizing Microbes

For identification and characterization of the rhizospheric fungi, two different approaches can be explored, namely, culture-dependent and -independent (Soni et al. 2016; Suyal et al. 2019a, 2019b). Culture-dependent approaches involve the culturing of the fungi in the lab followed by their morphological characterization, carbon source utilization pattern, plasmid fingerprinting, FAME (fatty acid methyl esters) analysis, PLFA (phospholipid fatty acid analysis), DNA microarray, MLST (Multilocus sequence typing), mass spectrometry, etc. Unfortunately, all the fungi are not culturable and therefore, need another approach known as culture-independent approach. It involves metagenomics and other genetic fingerprinting techniques which provide a profile of the whole community and do not rely on the culturing of the fungi. These methods are rapid, accurate, and easy to perform. Furthermore, these methods involve the isolation of DNA directly from the soil samples followed by its restriction digestion, cloning, and metagenomic library construction (Goel et al. 2017). These shotgun clones can further be subjected to activity screening. The culture-independent approach also involves the in situ identification of microorganisms by FISH (fluorescent in situ hybridization) and PCR based identification by using different phylogenetic markers.

5.6.1 Morphological Characterization

Several morphological characteristics are useful in the fungal identification and characterization, namely, hyphal structure, mycelial growth, pigmentation, spores, etc. Morphological characterization is really rapid, easy, and does not need any sophisticated instrument. But it has several drawbacks too because the morphological expressions are dependent upon environmental factors (Li et al. 2009).

5.6.2 Biochemical Characterization

5.6.2.1 Carbon Source Utilization Patterns

The evaluation of carbon source utilization efficiency of the microorganisms is one of the oldest methods used for their identification and characterization. This technique is considered fast, reproducible, and cost-effective. The Biolog identification system is a commonly used microbial identification method based on their ability to oxidize a panel of 95 different carbon sources (Morgan et al. 2009). Thus, metabolic profile of the microorganisms is prepared and compared. The major disadvantage of this method is its biasness for cultivable microbial communities. Nevertheless, results may also vary according to the growth conditions of the microorganisms and inoculum density. Frąc et al. (2016) have developed a fast, accurate, and effective Microplate Method (Biolog MT2) for the detection of *Fusarium*.

5.6.2.2 FAME and PLFA Analysis

For many years, microbial lipids have been routinely used for their own identification. The two most common methods which are being used for this purpose are FAME and PLFA. FAME is rapid but indiscriminate while, PLFA is precise but time-consuming. These methods involve the analysis of the microbial fatty acids and identify them on the basis of signature molecules. Signature fatty acids are known to make a relatively constant proportion within a cell and can be differentiated among major taxonomic groups of the microorganisms (Frostegard and Baath 1996; Siles et al. 2018). Therefore, any variation in the fatty acid profile of the microorganisms represents the change in the microbial community structure. This technique is precise, high-throughput, and cost-effective with higher resolution capacity (Nelsona et al. 2010). The limitation of this technique is that cellular fatty acid composition depends on growth conditions, media, and temperature used to grow the organism, thus, may lead to misinterpretation.

5.6.3 Molecular Characterization

5.6.3.1 PCR-Based Methods

Random Amplification of Polymorphic DNA (RAPD)

It is a PCR-based fingerprinting technique. RAPD markers are the DNA fragments produced from the random amplification of the genomic DNA using single primer of arbitrary nucleotide sequence. After purifying the genomic DNA, PCR amplification can be done by using randomly designed primers (Clerc et al. 1998). By selecting the primers and amplification conditions judiciously, all such pairs of sequences

represented in the genome result in a set of fragments that is characteristic of the species or strain from which the DNA was prepared. These fragments are resolved by gel electrophoresis. The band pattern generated in the analysis represents genome characterization of a respective microbial strain. Recently, Hassan et al. (2019) have used this technique for rapid identification of the *Trichoderma* sp.

Amplified Fragment Length Polymorphism (AFLP)

It is a variation of RAPD technique, and able to detect polymorphic restriction sites without prior sequence knowledge using PCR amplification. Restriction enzyme (RE)-digested genomic DNA can be used as a template for PCR amplification. The primers contain the recognition sites of the RE as well as additional "arbitrary" nucleotides that extend beyond the restriction sites (Blears et al. 1998; Bertani et al. 2019). The fixed portion gives the primer stability and the random portion allows it to detect many loci. The amplified fragments are separated and visualized on denaturing polyacrylamide gels. This multiple-locus fingerprinting technique is highly sensitive and robust and has been evaluated for genotypic characterization of the fungi (Kathuria et al. 2015). Furthermore, it has higher reproducibility, resolution, and sensitivity compared to other techniques.

Repetitive Sequence-Based PCR (Rep-PCR)

Microbial genomes possess several low-copy-number repeated sequences, namely, rRNA operons, tRNA genes, insertion elements, etc. These sequences contribute to the evolution of the genome and function through DNA rearrangements. It also helps in creating the genetic fingerprints. Therefore, Rep-PCR fingerprinting is considered a well-established technique for microbial diversity analysis and identification (Shin et al. 2012; Masanto et al. 2019). This method is based on PCR-mediated amplification of DNA fragments located between specific interspersed repeated sequences in microbial genomes. It has high resolution, but results may vary due to the PCR biasness.

Multiple Locus Variable Number Tandem Repeat Analysis (MLVA)

It is a molecular technique which explores the natural variation in the number of tandem repeats found in the multiple loci of the microorganism. This method is extensively used for molecular typing of the microorganisms (Johansson et al. 2006). In this technique, variable number tandem repeats (VNTR) loci are subjected to PCR amplification followed by amplicon sequencing. The amplicon size is used to assess the number of repeated units in each locus (Singh et al. 2019). Thus, total numbers of repeats of the VNTR loci are combined and used to prepare the MLVA profile, which can be compared for the fungal identification. This technique has

high resolution and accuracy but often imperfect repeats containing mutations are encountered which affect the reproducibility of the results.

Multilocus Sequence Typing (MLST)

This technique identifies the microorganisms by analyzing the internal fragments of house-keeping genes present in multiple loci (Maiden et al. 1998; Gaiarsa et al. 2019). These fragments are then sequenced and compared. Fragments that differ are designated as separate alleles and thus the relatedness of the microorganisms is displayed in terms of their phylogenetic relationships.

Single-Strand Conformation Polymorphism (SSCP)

It is a conformational difference in single-stranded nucleotide sequences of identical length (Schwieger and Tebbe 1998). These nucleotide sequences with different confirmation can be separated by the gel electrophoresis technique. Moreover, the gel patterns thus observed can be used for fungal identification and characterization. The change in single nucleotide in the amplified region is sufficient to produce the distinct PCR-SSCP patterns. This technique is rapid and convenient for mutational analysis and allelic variance (Martynov et al. 2019). Problem in reproducibility is the major limitation for this SSCP technique.

Denaturing Gradient Gel Electrophoresis (DGGE) and Temporal Gradient Gel Electrophoresis (TGGE)

These two techniques are the well-known techniques for the microbial ecology analysis and involve both PCR as well as polyacrylamide gel electrophoresis (Rajwar et al. 2018; Rawat et al. 2019). The metagenomic DNA is amplified using GC clamp containing primers and allowed to separate on a polyacrylamide gel. The denaturation of the amplicons is achieved by urea and formamide in DGGE, while temperature in case of TGGE. The amplicons get denatured and separated on the basis of their nucleotide sequences. Thus, a profile can be generated which can be compared further for assessing the microbial diversity within a respective sample (Kumar et al. 2014). These techniques are fast and labor-intensive. However, primer selection, electrophoresis conditions, and PCR reactions require optimization to achieve reproducibility.

5.6.3.2 Restriction Enzyme-Based Methods

Pulsed-Field Gel Electrophoresis (PFGE)

This technique can be used for the separation of DNA fragments under the influence of an electric field by changing their directions periodically on the gel matrix. It is a powerful genetic fingerprinting technique to construct a genome amp of the microorganisms (Basim and Basim 2001; Kwon et al. 2019). Microbial DNA can be restricted digested using RE and allowed to separate through gel electrophoresis. However, the direction of the electric field is changed continuously to get a discrete band pattern. These patterns are then compared and matched with the available databases for the identification of the microorganisms.

Restriction Fragment Length Polymorphism (RFLP) Analysis

RFLP is a genetic fingerprinting technique that explores variations in homologous DNA molecules. It involves the restriction digestion of DNA followed by gel electrophoresis (Osborn et al. 2000; Florek et al. 2019). Digested fragments are then transferred from the gel matrix to the nitrocellulose membrane. The predesigned probes are then subjected to the hybridization with the membrane-bound DNA fragments. RFLP is considered very sensitive for microbial identification. However, incomplete restriction digestion of the DNA molecules may change the results.

Ribotyping

It involves the identification of microorganisms based on the restriction digestion of rRNA coding genes. In case of bacteria 16S rRNA genes are used for this purpose (Suyal et al. 2015a, b, 2019b), while intergenic transcribed spacer (ITS) regions are frequently used for ribotyping of the fungi (Suyal et al. 2013a). Furthermore, 18S rRNA genes can also be used for fungal identification (Goes et al. 2012). This technique is among the most powerful genetic fingerprinting techniques and is being used extensively worldwide. It is highly accurate and reproducible along with a high level of resolution.

Plasmid Fingerprinting with Restriction Enzymes

Plasmid is an extrachromosomal, covalently closed, double-stranded circular DNA molecule. Besides the bacteria, these are well known in the members of several fungal genera, namely, *Absidia, Agaricus, Alternaria, Claviceps, Epichloe, Erisyphe, Fusarium, Saccharomyces*, etc. Plasmids can be isolated, restricted

digested, and allowed to separate on gel electrophoresis to get a unique pattern (Owen 1989; Qin et al. 2019). These patterns are then compared for interpreting the fungal relatedness. It is a rapid, popular, easy, and cost-effective technique. However, plasmid instability is a major drawback for this genetic fingerprinting technique.

5.6.3.3 Hybridization-Based Methods

Fluorescent In Situ Hybridization (FISH)

This technique is extensively used for microbial identification under culture-dependent as well culture-independent approaches. It can detect the complementary DNA sequence within a chromosome with the help of a fluorescence tag (Amann et al. 2001; Witchley et al. 2019). For this purpose, fluorescent probes are designed which are actually the complementary sequence of the desired DNA fragment. The probe: DNA binding is detected by the fluorescent microscopy technique. This technique is highly reproducible with good resolution ability. Cellular permeability, sensitivity, target site specificity, and accessibility are the major concerns with this technique.

DNA Microarray

A DNA microarray can be defined as the collection of small DNA spots on a solid surface. It can be identified as a DNA chip, biochip, or gene chip (Wang et al. 2002). Initially, this technique was used for expressional analysis of the genes; however, nowadays, it is frequently used for microbial identification and characterization. In microarray, randomly fragmented microbial genomes are allowed to hybridize to with the microbial genome spotted on a solid surface (DNA chip) (Ye et al. 2001; Nilsson et al. 2019). Resulting hybridization profiles are then analyzed and compared. This genetic fingerprinting has shown high reproducibility, accuracy, and resolution. However, it is considered laborious to perform.

5.6.3.4 Protein-Based Characterization

Serotyping

Serotypes are the microbial strains with distinct immune cells and antigenicity. Thus, the identification and characterization of the microorganisms based on their serotypes is known as serotyping. Cell surface antigens are the major determinative factor for the serotyping. This approach involves western blotting, immunoprecipitation, ELISA, and other immunological techniques to generate the serotyping profiles which are then compared to get an idea about the genetic relatedness among the microorganisms (Li et al. 2006; Akins and Jian 2019).

Mass Spectrometry (MS)

It offers high-throughput, robust, and sensitive way of microbial identification and characterization. Fungal proteins can be extracted, purified, and allowed to mass spectrometric analysis for their detailed characterization (Demirev and Fenselau 2008; Welker 2011). In the field of microbial proteomics, MS can be used for both gel-based as well as gel-less approaches. In recent years, two-dimensional-gel electrophoresis (2D-GE) coupled with MS having ionization with matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) have shown its potential under gel-based proteomic approach (Soni et al. 2015; Suyal et al. 2014b, 2017). However, under gel-less approach, liquid chromatography analysis coupled with MS (LC-MS) is in great demand (Suyal et al. 2018, 2020). Both the approaches produce a profile of the microbial proteins which is then used to compare and characterize the respective strains.

5.6.3.5 Enrichments Methods

Bromodeoxyuridine (BrdU) Method

This method can be used to identify a metabolically active population within a niche (Sebastián and Gasol 2019). In this technique, BrdU (a labeled nucleotide) is added to the system and microbes are allowed to grow (Yin et al. 2000). Metabolically active individuals will incorporate BrdU into their nucleic acid and thus identified by using a label. This strategy is widely used in the bioremediation, especially for the isolation of the fungi which can use xenobiotics, heavy metals, and other compounds.

Stable Isotope Probing (SIP)

It is also an enrichment method in which ^{13}C-labeled substrate is provided to the microorganisms. Metabolically active microorganisms incorporate ^{13}C in their DNA and thus, make it denser than normal DNA. Density gradient centrifugation can be used to separate both the DNA which can be analyzed further with the help of the specific primers (Achouak and Haichar 2019). Therefore, SIP offers broad opportunity to study microbial communities and can be expanded further to stable isotopes of nitrogen and/or phosphorus (Buckley et al. 2007).

5.7 Conclusion and Future Prospects

To meet the ever-increasing demand of food due to population pressure, green revolution came into existence. It, however, brought remarkable gain in food production but with unnoticed concerns for sustainability due to disproportionate use of

chemical fertilizers. Moreover, future reliability on chemical fertilizers will persist to cause loss in soil fertility, pollution, and a lot of saddle on the fiscal system. Therefore, biofertilizers are being promoted alone or with combination with fertilizers. This integrated approach is vital to improve crop productivity and to maintain soil fertility.

PGFs not only exhibit plant growth promotion but they are also effective in bioremediation by detoxifying detrimental pollutants such as pesticides and heavy metal pollutants. Nevertheless, they are potential biopesticides, as they can control a wide variety of phytopathogens. In the case of controlled soil conditions, remarkable enhancement in yields of different crop plants has been reported through PGF applications. But soil is an unpredictable natural ecosystem. Efficacy of PGF in crop yield may vary under laboratory, greenhouse, and field trials, and therefore, the desired results are sometimes not achieved. Besides it, climatic variations influence the effectiveness of PGF. However, their performance can be optimized through acclimatization according to the prevailing natural soil environment. In the current scenario, where there is global reluctance toward genetically modified food crops, PGF-based farming practices might be an excellent alternative. This is a technology which is easy to access even to the farmers of developing nations including India. Thus, this trend of least possible input of chemicals in sustainable agricultural systems may help to achieve the food reliance for an ever-growing population. Further research in this perspective will widen the horizon of our knowledge and enable us to understand microbial responses to the diverse environments.

References

Achouak W, Haichar FZ (2019) Stable isotope probing of microbiota structure and function in the plant rhizosphere. Methods Mol Biol 2046:233–243

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Uni Sci 26(1):1–20

Akins PT, Jian B (2019) The frozen brain state of *Cryptococcus gattii*: a globe-trotting, tropical, neurotropic fungus. Neurocrit Care 30:272–279

Aleksieva P, Mutafov S (1997) Continuous culture of *Humicola lutea* 120-5 for acid proteinase production. W J Microbiol Biotechnol 13(3):353–357

Aleksieva P, Peeva L (2000) Investigation of acid proteinase biosynthesis by the fungus *Humicola lutea* in an airlift bioreactor. Enzy Microb Technol l2(5):402–405

Amann R, Fuchs BM, Behrens S (2001) The identification of microorganisms by fluorescence in situ hybridisation. Curr Opin Biotechnol 12:231–236

Ambrose KV, Tian Z, Wang Y, Smith J, Zylstra G, Huang B, Belanger FC (2015) Functional characterization of salicylate hydroxylase from the fungal endophyte *Epichloë festucae*. Sci Rep 5:10939

Babu-Khan S, Yeo TC, Martin WL, Duron MR, Rogers RD, Goldstein AH (1995) Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. Appl Environ Microbiol 61(3):972–978

Baetz U, Martinoia E (2014) Root exudates: the hidden part of plant defense. Trends Plant Sci 19(2):90–98

Basim E, Basim H (2001) Pulsed-Field Gel Electrophoresis (PFGE) technique and its use in molecular biology. Turk J Biol 25:405–418

Behera BC, Singdevsachan SK, Mishra RR, Dutta SK, Thatoi HN (2014) Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove- a review. Biocat Agric Biotechnol 3(2):97–110

Bertani G, Savo Sardaro ML, Neviani E, Lazzi C (2019) AFLP protocol comparison for microbial diversity fingerprinting. J Appl Genet 60:217–223

Bianco C, Defez R (2010) Improvement of phosphate solubilization and Medicago plant yield by an indole-3-acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76(14):4626–4632

Blears MJ, DeGrandis SA, Lee H, Trevros JT (1998) Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. J Indust Microbiol Biotechnol 21:99–114

Bruto M, Prigent-Combaret C, Muller D, Moenne-Loccoz Y (2014) Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. Sci Rep 4:6261

Buckley DH, Huangyutitham V, Hsu SF, Nelson TA (2007) Stable isotope probing with ^{15}N achieved by disentangling the effects of genome G+C content and isotope enrichment on DNA density. Appl Environ Microbiol 73:3189–3195

Charyulu KD, Biswas S (2010) Economics and Efficiency of Organic Farming vis-à-vis Conventional Farming in India. IIMA Working Papers WP2010-04-03, Indian Institute of Management Ahmedabad, Research and Publication Department

Clerc A, Manceau C, Nesme X (1998) Comparison of randomly amplified polymorphic DNA with amplified fragment length polymorphism to assess genetic diversity and genetic relatedness within genospecies iii of *Pseudomonas syringae*. Appl Environ Microbiol 64(4):1180–1187

Dash B, Soni R, Kumar V, Suyal DC, Dash D, Goel R (2019) Mycorrhizosphere: microbial interactions for sustainable agricultural production. In: Varma A, Choudhary D (eds) Mycorrhizosphere and Pedogenesis. Springer, Singapore, pp 321–338

Demirev PA, Fenselau C (2008) Mass spectrometry for rapid characterization of microorganisms. Annu Rev Anal Chem 1:71–93

Deng S, Elkins JG, Da LH, Botero LM, McDermott TR (2001) Cloning and characterization of a second acid phosphatase from *Sinorhizobium meliloti* strain 104A14. Arch Microbiol 176(4):255–263

Economist (2015) India is reforming other bits of its economy, but not farming.. Retrieved from https://www.economist.com/news/asia/21656241-india-reforming-other-bits-its-economy-not-farming-time-warp

Ezawa T, Saito K (2018) How do arbuscular mycorrhizal fungi handle phosphate? New insight into fine-tuning of phosphate metabolism. New Phytol 220:1116–1121

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

Fasim M, Ahmed N, Parsons R, Gadd GM (2002) Solubilization of zinc salts by bacterium isolated by the air environment of tannery. FEMS Microbiol Lett 213:1–6

Florek M, Krol J, Wozniak-Biel A (2019) Atypical URA5 gene restriction fragment length polymorphism banding profile in *Cryptococcus neoformans* strains. Folia Microbiol 64:857–860

Fonseca HM, Berbara RL (2008) Does *Lunularia cruciata* form symbiotic relationships with either *Glomus proliferum* or G. Intraradices? Mycol Res 112:1063–1068

Forbes (2017) How sensors, robotics and artificial intelligence will transform agriculture. Retrieved from https://www.forbes.com/sites/jenniferhicks/2017/03/19/how-sensors-robotics-and-artificial-intelligence-will-transform-agriculture/#73c4a393384b

Frąc M, Gryta A, Oszust K, Kotowicz N (2016) Fast and accurate microplate method (biolog MT2) for detection of *Fusarium* fungicides resistance/sensitivity. Front Microbiol 7(489):1–16

Frostegard A, Baath E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fert Soils 22:59–65

Gaiarsa S, Batisti Biffignandi G, Esposito EP, Castelli M, Jolley KA, Brisse S, Sassera D, Zarrilli R (2019) Comparative analysis of the two *Acinetobacter baumannii* Multilocus Sequence Typing (MLST) schemes. Front Microbiol 10(930):1–14

Giri K, Paliwal R, Suyal DC, Mishra G, Pandey S, Rai JPN, Verma PK (2015) Potential application of plant-microbe interaction for restoration of degraded ecosystems. In: Singh S, Srivastava K (eds) Handbook of research on uncovering new methods for ecosystem management through bioremediation. IGI Global, Hershey, pp 255–285

Goel R, Suyal DC, Narayan DB, Soni R (2017) Soil metagenomics: a tool for sustainable agriculture. In: Kalia V, Shouche Y, Purohit H, Rahi P (eds) Mining of microbial wealth and metagenomics. Springer Nature, Singapore, pp 217–225

Goes DKCGP, Fisher MLDC, Cattelan AJ, Nogueira MA, Carvalho CGPD, Oliveira ALMD (2012) Biochemical and molecular characterization of high population density bacteria isolated from sunflower. J Microbiol Biotechnol 22(4):437–447

Guilan L, Shaohui Y, Minggang L, Yake Q, Jiehua W (2009) Functional analysis of an Aspergillus ficuum phytase gene in *Saccharomyces cerevisiae* and its root-specific, secretory expression in transgenic soybean plants. Biotechnol Lett 31:1297–1303

Han SH, Kim CH, Lee JH, Park JY, Cho SM, Park SK, Kim YC (2008) Inactivation of *pqq* genes of *Enterobacter intermedium* 60-2G reduces antifungal activity and induction of systemic resistance. FEMS Microbiol Lett 282(1):140–146

Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413–2429

Hart MM, Antunes PM, Chaudhary VB, Abbott LK (2017) Fungal inoculants in the field: is the reward greater than the risk? Funct Ecol 32:126–135

Hassan MM, Farid MA, Gaber A (2019) Rapid identification of *Trichoderma koningiopsis* and *Trichoderma longibrachiatum* using sequence-characterized amplified region markers. Egypt J Biol Pest Control 29(13):1–8

IBEF (2017) Agriculture in India: information about Indian agriculture & its importance.. Retrieved from https://www.ibef.org/industry/agriculture-india.aspx

Iefuji H, Takashi W, Hiroko I, Kazuo M, Tsutomu F (2009) Cloning and characterization of a novel phytase from wastewater treatment yeast *Hansenula fabianii* J640 and expression in *Pichia pastoris*. J Biosci Bioeng 108(3):225–230

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol 177:779–789

Johansson A, Koskiniemi S, Gottfridsson P, Wistrom J, Monsen T (2006) Multiple-locus variable-number tandem repeat analysis for typing of *Staphylococcus epidermidis*. J Clin Microbiol 44(1):260–265

Joshi D, Chandra R, Suyal DC, Kumar S, Goel R (2019) Impact of bioinoculants Pseudomonas jesenii MP1 and Rhodococcus qingshengii S10107 on Cicer arietinum yield and soil nitrogen status. Pedosphere 29(3):388–399

Kalayu G (2019) Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int J Agron 4917256:1–7

Kathuria S, Sharma C, Singh PK, Agarwal P, Agarwal K, Hagen F, Meis JF, Chowdhary A (2015) Molecular epidemiology and in-vitro antifungal susceptibility of *Aspergillus terreus* species complex isolates in Delhi, India: evidence of genetic diversity by amplified fragment length polymorphism and microsatellite typing. PLoS One 10(3):e0118997

Kaur R, Saxena A, Sangwan P, Yadav AN, Kumar V, Dhaliwal HS (2017) Production and characterization of a neutral phytase of *Penicillium oxalicum* EUFR-3 isolated from Himalayan region. Nusantara Biosci 9(1):68–76

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K et al (2016) Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. New Phytol 211:1202–1208. https://doi.org/10.1111/nph.14016

Kim KY, Jordan D, Krishnan HB (1997) *Rahnella aquatilis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol Lett 153(2):273–277

Kim KY, Jordan D, Krishnan HB (1998) Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*. FEMS Microbiol Lett 159(1):121–127

Kim CH, Han SH, Kim KY, Cho BH, Kim YH, Koo BS, Kim YC (2003) Cloning and expression of pyrroloquinoline quinine (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. Curr Microbiol 47(6):457–461

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188

Kobae Y (2019) Dynamic phosphate uptake in Arbuscular Mycorrhizal roots under field conditions. Front Microbiol 6(159):1–12

Kour D, Rana KL, Yadav N, Yadav AN, Kumar A, Meena VS et al (2019a) Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion, and biotechnological applications for sustainable agriculture. In: Kumar A, Meena VS (eds) Plant growth promoting Rhizobacteria for agricultural sustainability: from theory to practices. Springer, Singapore, pp 19–65

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019b) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64

Kour D, Kaur T, Yadav N, Rastegari AA, Singh B, Kumar V et al (2020a) Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 157–176

Kour D, Rana KL, Kaur T, Sheikh I, Yadav AN, Kumar V et al (2020b) Microbe-mediated alleviation of drought stress and acquisition of phosphorus in great millet (*Sorghum bicolour* L.) by drought-adaptive and phosphorus-solubilizing microbes. Biocatal Agric Biotechnol 23:101501

Kour D, Rana KL, Sheikh I, Kumar V, Yadav AN, Dhaliwal HS et al (2020c) Alleviation of drought stress and plant growth promotion by *Pseudomonas libanensis* EU-LWNA-33, a drought-adaptive phosphorus-solubilizing bacterium. Proc Natl Acad Sci India B. https://doi.org/10.1007/s40011-019-01151-4

Kumar S, Suyal DC, Dhauni N, Bhoriyal M, 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. Afr J Microbiol 8(50):3931–3943

Kwon YJ, Shin JH, Byun SA, Choi MJ, Won EJ, Lee D, Lee SY, Chun S, Lee JH, Choi HJ, Kee SJ, Kim SH, Shin MG (2019) *Candida auris* clinical isolates from South Korea: identification, antifungal susceptibility, and genotyping. J Clin Microbiol 57(4):e01624–e01618

Li Y, Liu D, Cao B, Han W, Liu Y, Liu F, Guo X, Bastin AD, Feng L, Wang L (2006) Development of a serotype-specific DNA microarray for identification of some *Shigella* and pathogenic *Escherichia coli* strains. J Clin Microbiol 44(12):4376

Li W, Raoult D, Fournier P (2009) Bacterial strain typing in the genomic era. FEMS Microbiol Rev 33:892–916

Liu ST, Lee LY, Tai CY, Hung CH, Chang YS, Wolfram JH, Goldstein AH (1992) Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101: nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinine. J Bacteriol 174(18):5814–5819

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci 95(6):3140–3145

Makino K, Shinagawa H, Amemura M, Kawamoto T, Yamada M, Nakata A (1989) Signal transduction in the phosphate regulon of *Escherichia coli* involves phosphotransfer between PhoR and PhoB proteins. J Mol Biol 210(3):551–559

Man-Jin In, Sung-Won Seo, Nam-Soon Oh (2008) Fermentative production and application of acid phytase by *Saccharomyces cerevisiae* CY strain. Afr J Biotechnol 7(17):3115–3120

Martynov V, Chizhik V, Sokolova E, Kuznetsova M, Khavkin E (2019) Polymorphism of avirulence genes in potato late blight pathogen *Phytophthora infestans* as characterized by SSCP analysis. Agric Gene 13:100093

Masanto HA, Wibowo A, Subandiyah S, Shimizu M, Suga H, Kageyama K (2019) Genetic diversity of *Phytophthora palmivora* isolates from Indonesia and Japan using rep-PCR and microsatellite markers. J Gen Plant Pathol 85:367–381

Mazid M, Khan TA (2015) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3(3):1–14

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26(4):267–286

Mehrvarz S, Chaichi MR, Alikhani HA (2008) Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barely (*Hordeum vulgare* L.). American-Eurasian J Agric Environ Sci 3(6):822–828

Micheva-Viteva S, Tchorbanov B, Aleksieva P, Lazarova V (2000) Acid phosphatases excreted by *Humicola lutea* 120-5 in casein-containing medium. W J Microbiol Biotechnol 16(8–9):859–863

Morgan MC, Boyette M, Goforth C, Sperry KV, Greene SR (2009) Comparison of the Biolog omni log identification system and 16S ribosomal RNA gene sequencing for accuracy in identification of atypical bacteria of clinical origin. J Microbiol Methods 79(3):336–343

Mummey D, Holben W, Six J, Stahl P (2006) Spatial stratification of soil bacterial populations in aggregates of diverse soils. Microb Ecol 51:404–411

Nautiyal CS (2000) An efficient microbiological grown medium for screening phosphate solubilizing microorganisms. Fed Eur Mater Soc Microbiol Lett 170:265–270

Nelsona KY, Razbana B, Mc Martina DW, Cullimoreb DR, Takaya O, Patrick DK (2010) A rapid methodology using fatty acid methyl esters to profile bacterial community structures in microbial fuel cells. Bioelectrochemistry 78(1):80–86

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L (2019) Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17:95–109

Osborn AM, Moore ERB, Timmis KN (2000) An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ Microbiol 2:39–50

Owen RJ (1989) Chromosomal DNA fingerprinting-a new method of species and strain identification applicable to microbial pathogens. J Med Microbiol 30:89–99

Patel DK, Archana G, Kumar GN (2008) Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. DHRSS in the presence of different sugars. Curr Microbiol 56(2):168–174

Pavlova K, Gargova S, Hristozova T, Tankova Z (2008) Phytase from Antarctic yeast strain *Cryptococcuus laurentii* AL27. Folia Microbiol 53(1):29–34

Puente ME, Bashan Y, Li CY, Lebsky VK (2004) Microbial populations and activities in the rhizoplane of rock weathering desert plants root colonization and weathering of igneous rocks. Plant Biol 6:629–642

Qin L, Li A, Tan K, Guo S, Chen Y, Wang F, Wong KH (2019) Universal plasmids to facilitate gene deletion and gene tagging in filamentous fungi. Fungal Genet Biol 125:28–35

Rai PK, Singh M, Anand K, Saurabhj S, Kaur T, Kour D et al (2020) Role and potential applications of plant growth promotion rhizobacteria for sustainable agriculture. In: Rastegari AA,

Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 49–60

Rajwar J, Chandra R, Suyal DC, Tomer S, Kumar S, Goel R (2018) Comparative phosphate solubilizing efficiency of psychrotolerant *Pseudomonas jesenii* MP1 and *Acinetobacter* sp. ST02 against chickpea for sustainable hill agriculture. Biologia 73(8):793–802

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

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS (2009) Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. Genet Resour Crop Evol 56:53–64

Rawat N, Sharma M, Suyal DC, Singh DK, Joshi D, Singh P, Goel R (2019) Psyhcrotolerant bioinoculants and their co-inoculation to improve *Cicer arietinum* growth and soil nutrient status for sustainable mountain agriculture. J Soil Sci Plant Nutr 19(3):639–647

Reilly TJ, Felts RL, Henzl MT, Calcutt MJ, Tanner JJ (2006) Characterization of recombinant *Francisella tularensis* acid phosphatase A. Protein Exp Purif 45(1):132–141

Remy W, Taylor TN, Hass H, Kerp H (1994) Four-hundred-million year-old vesicular-arbuscular mycorrhizae. Proc Natl Acad Sci U S A 91:11841–11843

Rodriguez H, Rossolini GM, Gonzalez T, Glick BR (2000) Isolation of a gene from *Burkholderia cepacia* IS-16 encoding a protein that facilitates phosphatase activity. Curr Microbiol 40(6):362–366

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth promoting bacteria *Azospirillium spp*. Naturwissenschaften 91:552–555

Rossolini GM, Schippa S, Riccio ML, Berlutti F, Macaskie LE, Thaller MC (1998) Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology. Cell Mol Life Sci 54(8):833–850

Sano K, Fukuhara H, Nakamura Y (1999) Phytase of the yeast *Arxula adeninivorans*. Biotechnol Lett 21:33–38

Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-Single-Strand Conformation Polymorphism for 16S rRNA-based microbial community analysis. Appl Environ Microbiol 64:4870–4876

Sebastian M, Gasol JM (2019) Visualization is crucial for understanding microbial processes in the ocean. Phil Trans R Soc B 374(1786):1–7

Segueilha L, Lambrechts C, Boze H, Moulin G, Galzy P (1992) Purification and properties of the phytase from *Schwanniomyces castellii*. J Ferment Bioeng 74:7–11

Selvi K, John-Paul B, Ravindran JA, Vijaya V (2011) Quantitative estimation of insoluble inorganic phosphate solubilization. Int J Sci Nat 2(2):292–295

Shahab S, Ahmed N, Khan NS (2009) Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. Afr J Agric Res 4(11):1312–1316

Sharma A, Ahluwalia O, Tripathi AD, Singh G, Arya SK (2020) Phytases and their pharmaceutical applications: mini-review. Phytases and their pharmaceutical applications: mini-review. Biocat Agric Biotechnol. https://doi.org/10.1016/j.bcab.2019.101439

Shin HD, Kim DU, Seong CN, Song HG, Jong-Ok K (2012) Genetic and phenotypic diversity of carbofuran-degrading bacteria isolated from agricultural soils. J Microbiol Biotechnol 22(4):448–456

Siles JA, Ohlinger B, Cajthaml T, Kistler E, Margesin R (2018) Characterization of soil bacterial, archaeal and fungal communities inhabiting archaeological human-impacted layers at Monte Iato settlement (Sicily, Italy). Sci Rep 8(1903):1–14

Singh M, Malik MA, Singh DK, Doimari S, Bhavna SR (2019) Multilocus variable number tandem repeat analysis (MLVA)-typing of *Brucella abortus* isolates of India reveals limited genetic diversity. Trop Anim Health Prod. https://doi.org/10.1007/s11250-019-02110-x

Soni SK, Magdum A, Khire JM (2010) Purification and characterization of two distinct acidic phytases with broad pH stability from *Aspergillus niger* NCIM 563. World J Microbiol Biotechnol 26(11):2009–2018

Soni R, Suyal DC, Agrawal K, Yadav A, Souche Y, Goel R (2015) Differential proteomic analysis of Himalayan psychrotolerant diazotroph *Pseudomonas palleroniana* N26 strain under low temperature diazotrophic conditions. CryoLetters 36(2):74–82

Soni R, Suyal DC, Sai S, Goel R (2016) Exploration of nifH gene through soil metagenomes of the western Indian Himalayas. 3Biotech 6(1):1–4

Suyal DC, Tewari L (2013a) *In vitro* degradation of natural animal feed substrates by intracellular phytase producing Shiwalik Himalayan budding yeasts. Afri J Microbiol Res 7(47):5374–5383

Suyal DC, Tewari L (2013b) Phytase and its applications. Int J Curr Res 5(10):3042–3043

Suyal DC, Shukla A, Goel R (2014a) Growth promotory potential of the psychrophilic Diazotroph *Pseudmonas migulae* S10724 against native *Vigna radiata* (L.) Wilczek. 3Biotech 4:665–668

Suyal DC, Yadav A, Shouche Y, Goel R (2014b) Differential proteomics in response to low temperature diazotrophy of Himalayan psychrophilic nitrogen fixing *Pseudomonas migulae* S10724 strain. Curr Microbiol 68(4):543–550

Suyal DC, Yadav A, Shouche Y, Goel R (2015a) Diversified diazotrophs associated with the rhizosphere of Western Indian Himalayan native red kidney beans (*Phaseolus vulgaris* L.). 3Biotech 5(4):433–441

Suyal DC, Yadav A, Shouche Y, Goel R (2015b) Bacterial diversity and community structure of Western Indian Himalayan red kidney bean (*Phaseolus vulgaris* L.) rhizosphere as revealed by 16S rRNA gene sequences. Biologia 70(3):305–313

Suyal DC, Kumar S, Yadav A, Shouche Y, Goel R (2017) Cold stress and nitrogen deficiency affected protein expression of psychrotrophic *Dyadobacter psychrophilus* B2 and *Pseudomonas jessenii* MP1. Front Microbiol 8(430):1–6

Suyal DC, Kumar S, Joshi D, Soni R, Goel R (2018) Quantitative proteomics of psychotrophic diazotroph in response to nitrogen deficiency and cold stress. J Proteome 187:235–242

Suyal DC, Joshi D, Debbarma P, Soni R, Dash B, Goel R (2019a) Soil metagenomics: unculturable microbial diversity and its function. In: Varma A, Choudhary D (eds) Mycorrhizosphere and pedogenesis. Springer, Singapore, pp 355–362

Suyal DC, Kumar S, Joshi D, Yadav A, Shouche Y, Goel R (2019b) Comparative overview of red kidney bean (*Phaseolus valgaris*) rhizospheric bacterial diversity in perspective of altitudinal variations. Biologia 74(10):1405–1413

Suyal DC, Joshi D, Kumar S, Soni R, Goel R (2020) Differential protein profiling of soil diazotroph *Rhodococcus qingshengii* S10107 towards low-temperature and nitrogen deficiency. Sci Rep. https://doi.org/10.1038/s41598-019-56592-8

Swapna AL (2013) Development of biofertilizers and its future perspective. J Pharm 4:327–332

Tomer S, Suyal DC, Goel R (2016) Biofertilizers: a timely approach for sustainable agriculture. In: Choudhary DK, Varma A, Tuteja N (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer Nature Singapore Pvt Ltd, Singapore, pp 375–395

Tomer S, Suyal DC, Rajwar J, Yadav A, Shouche Y, Goel R (2017) Isolation and characterization of phosphate solubilizing bacteria from Western Indian Himalayan soils. 3Biotech 7(2):1–5

Torriani A, Ludtke DN (1985) The pho regulon of Escherichia coli. In: Schaechter M, Neidhart FC, Ingraham J, Kjeldgaard NO (eds) The molecular biology of bacterial growth. Jones and Bartlett Publishers, Boston, pp 224–242

Veide J, Andlid T (2006) Improved extracellular phytase activity in *Saccharomyces cerevisiae* by modifications in the PHO system. Int J Food Microbiol 108:60–67

Wang RF, Beggs ML, Robertson LH, Cerniglia CE (2002) Design and evaluation of oligonucleotide-microarray method for the detection of human intestinal bacteria in fecal samples. FEMS Microbiol Lett 213:175–182

Wanner BL (1987) Control of phoR-dependent bacterial alkaline phosphatase clonal variation by the phoM region. J Bacteriol 169(2):900–903

Welker M (2011) Proteomics for routine identification of microorganisms. Proteomics 11:3143–3153

Witchley JN, Penumetcha PM, Noble SM (2019) Visualization of *Candida albicans* in the murine gastrointestinal tract using fluorescent in situ hybridization. J Vis Exp 153:e60283

Yadav AN, Verma P, Kour D, Rana KL, Kumar V, Singh B, Chuahan VS, Sugitha TCK, Saxena AK, Dhaliwal HS (2017) Plant microbiomes and its beneficial multifunctional plant growth promoting attributes. Int J Environ Sci Nat Resour 3(1):1–18

Yadav AN, Gulati S, Sharma D, Singh RN, Rajawat MVS, Kumar R, Dey R, Pal KK, Kaushik R, Saxena AK (2019) Seasonal variations in culturable archaea and their plant growth promoting attributes to predict their role in establishment of vegetation in Rann of Kutch. Biologia 74(8):1031–1043

Yasoda N, Hirimuthugoda ZC, Longfei WI (2007) Probiotic yeasts with phytase activity identified from the gastrointestinal tract of sea cucumbers. SPC Beche de Mer Information Bulletin 26:1–3

Ye RW, Wang T, Bedzyk L, Croker KM (2001) Applications of DNA microarrays in microbial systems. J Microbiol Methods 47:257–272

Yin B, Crowley D, Sparovek G, De Melo WJ, Borneman J (2000) Bacterial functional redundancy along a soil reclamation gradient. Appl Environ Microbiol 66:4361–4365

Yu SL, Liu YN, Jing GL, Zhao BJ, Guo SY (2005) Analysis of phosphate accumulating organism cultivated under different carbon sources with polymerase reaction denaturing gradient gel electrophoresis assay. J Environ Sci 17:611–614