Chapter 3
Current Perspectives on Phosphate-Solubilizing Endophytic Fungi: Ecological Significances and Biotechnological Applications

Edla Sujatha, Kuraganti Gunaswetha and Pallaval Veera Bramhachari

Abstract Phosphorus is one of the essential nutrients for optimum plant growth after nitrogen. Their structural and chemical complexity greatly reduces their availability to the plants and is one of the major limiting macroelements to plant growth. Phosphorus is present in both organic and inorganic forms. Though abundant amount of phosphorus is present in the soil, its availability is reduced by various environmental factors that influence bio-geo-cycling of phosphorus. Current research is mainly focused on the exploitation of endophytic fungi for solubilization of phosphorus in an efficient way. Endophytic fungi including the genera *Aspergillus, Penicillium, Piriformospora, Trichoderma, Curvularia,* and other class of endophytic symbionts such as AM fungi are identified as potent Phosphate solubilizers. Endophytic fungi promote plant growth by a variety of mechanisms such as solubilization of "P"-like macronutrients by different reactions, able to produce bio-control agents, i.e., antibiotics and siderophores and plant protecting agents against pathogens, synthesis of growth hormones such as gibberellins, cytokines, and auxins. Phosphate-solubilizing endophytic fungi are promising and efficient organisms capable of increasing "P" availability and the best alternative approach to chemical fertilizers.

Keywords Endophytic fungi · Biotechnological applications · Ecological significance · Inorganic and organic phosphates solubilization

3.1 Introduction

Soil microorganisms greatly influence the nature of the soil and its health through beneficial and harmful activities. Microorganisms present in the rhizosphere mediate certain functions, for instance, decomposition, nutrient immobilization, mineralization, nitrogen fixation, and release of nutrients. In addition to these, microorganisms

E. Sujatha (✉) · K. Gunaswetha
Department of Microbiology, Kakatiya University, Warangal, Telangana, India
e-mail: sujathaedla_1973@kakatiya.ac.in

P. V. Bramhachari
Department of Biotechnology, Krishna University, Machilipatnam 521001, Andhra Pradesh, India

A. N. Yadav et al. (eds.), *Plant Microbiomes for Sustainable Agriculture*, Sustainable Development and Biodiversity 25, https://doi.org/10.1007/978-3-030-38453-1_3

also possess phosphate-solubilizing ability by converting insoluble phosphates to soluble phosphorus (Pradhan and Sukla 2005) in soil and make them readily accessible to plants.

The father of plant pathology de Bary proposed the term endophyte to refer any organism that resides within plant tissue exclusive of any disease manifestations in host plant (Nisa et al. 2015). All types of plants harbor a wide variety of microorganisms, for instance, fungi, bacteria, and cyanobacteria that posses endophytic properties and play an imperative role in plant metabolism and physiology (Hardoim et al. 2015). During symbiotic association established between endophytes and host plants, both the organisms get mutually benefited, the plant provides nutrients to colonizing endophytes, while the endophytes accelerate biosynthetic pathways for metabolite synthesis that have many applications in agronomy. For example, management of plant growth and novel disease-resistant mechanisms against pathogens.

Endophytes can be isolated from exterior or interior part of sterilized plant tissues. Significant biochemical molecules such as Terpenoids, isoflavonoids, flavonoids, and phenolics are released from plant roots. They may attract the fungi from root region to colonize within the plant as an endophyte. However, the endophytes are depicted to be colonized in different plant tissues associated with the different ecosystems. Fungi can be classified into diverse groups derived from their role and survival, such as epiphytic, endophytic, pathogenic, and mycorrhizal fungi (Porras-Alfaro and Bayman 2011). Some endophytic fungi can find their way to either vertical or horizontal root region and penetrate to the deeper regions of plants.

Endophytic fungi colonize the tissues of host plant by particular route of transmission and this can be either vertical or horizontal method. Endophytic fungi transmit from the mother plant to offspring via seeds (true endophytes). A study carried out by Hodgson et al. (2014) in forbe species, common poppy, knapweed, cornflower, sheep's sorrel, groundsel, and ribwort plantain, and two endophyte species, *Cladosporium sphaerospermum* and *Alternaria alternata* primarily investigated the vertical transmission of species. Horizontal transmission occurs by airborne spores or through soil. Endophytes colonize forbes via leaves of the host through horizontal transmission.

According to earlier fossil records, evolutionary tendency reveals an association between a diverse group of plants and endophytic fungi. Plant endophyte communications resulted in plant growth promotion, uptake of micronutrients, and synthesis of different types of secondary metabolites and bioactive compounds with potential applications in industry, medicine, and agriculture. Endophytic fungi provide protection to plants against plant pathogens, reduce biotic and abiotic stresses, and for the reason that these organisms are considered as eco-friendly bioresources. Endophytic fungi may enhance plant growth by solubilization of potassium, phosphorus and zinc, produces phytohormones, viz., cytokines, gibberellic acids, indole acetic acids, hydrolytic enzymes and Fe-chelating compounds, ammonia and hydrogen cyanide (Rai et al. 2014). Different classes of fungi, for instance, Mucoromycota, Basidiomycota, Oomycota, and Ascomycota were depicted as plant growth promoters and protect the plants under anomalous and abiotic stress conditions. Natural products produced by endophytes were previously reported with potential anti-bacterial, anti-fungal, and anti-protozoal properties. Nonetheless, the secondary metabolites produced by endophytes are proved to have remarkable anti-cancer, anti-microbial, insecticidal properties, pharmaceutical sciences, and in other biotechnological applications (Kusari et al. 2011; Aly et al. 2010; Uzma et al. 2018; Mishra et al. 2017).

3.2 Diversity of Phosphate-Solubilizing Microorganisms

A great extent of microbial species exhibit phosphate-solubilizing ability, these include archaebacteria, bacteria, actinomycetes, and fungi. These microbes reside in the plant tissues exclusive of causing any harmful effects to the host. Generally, they prevail in tissues of the host plant with the symbiotic association. These microbes were previously isolated from different types of plants, including *Triticum* (Yadav et al. 2018a; Verma et al. 2015, 2016a, b), *Oryza sativa* (Piromyou et al. 2015), *Zea mays, Capsicum annuum L.*, *Saccharum officinarum* (Montanez et al. 2012; Thanh and Diep 2014), mustard, citrus (Kasotia and Choudhary 2014), *Solanum tuberosum* (Rado et al. 2015; Manter et al. 2010;), *Glycine max* (Mingma et al. 2014), *Pisum sativum* (Narula et al. 2013; Tariq et al. 2014), *Phaseolus vulgaris* (Suyal et al. 2015), *Helianthus* (Forchetti et al. 2010; Ambrosini et al. 2012), and *Cicer arietinum* (Saini et al. 2015). Fungal endophytes pertaining to diverse genera including *Acremonium, Aspergillus, Paecilomyces, Cryptococcus, Fusarium, Curvularia, Rhodotorula, Cladosporium, Alternaria, Phaeomoniella, Chaetomium, Colletotrichum, Berkleasmium, Rhizoctonia, Geomyces, Leptospora, Phyllosticta, Microdochium, Neotyphodium, Ophiognomonia, Glomus, Penicillium, Rhizopus, Trichoderma, Xylaria,* and *Wallemia* have been isolated from various host plants (Suman et al. 2016; Verma et al. 2017; Yadav et al. 2018a, b). Recently, a nematode fungus *Arthrobotrys oligospora* was identified to solubilize rock phosphate Togo, Tilemi rock phosphate, Kodjari phosphate rock. The fungi solubilized all three types of rock phosphates. Given these, Duponnogs and group (2006) demonstrated the phosphate solubilization ability in vivo conditions.

3.3 Biotechnological Applications of Natural Products from Endophytic Fungi

Biotechnology has opened up numerous avenues for exploitation of endophytic microorganisms in medicine, agriculture, and industry from diverse ecological niches and their applications in agriculture are aptly essential for plant growth, plant protection, and yield (Yadav et al. 2018a; Rana et al. 2019c). Because of their ability to promote plant growth and adapt under extreme abiotic stresses, the endophytic microorganisms have, in fact, captured the attention of the scientific community

(Soni et al. 2018; Yadav et al. 2019b, c, d). With the exceptional capacity to produce secondary metabolites, the endophytic fungi may perhaps unearth novel applications in pharmaceuticals, industrial processes, and horticulture (Joseph and Priya 2011). Endophytic bacterial and fungal organisms possess extensive applications as bio-control agents, bio-inoculants, and bio-fortification of micronutrients (Yadav 2019; Yadav et al. 2019a; Yadav and Yadav 2019).

The excessive usage of chemical phosphorus (P) fertilizers to increase agricultural yield sequentially to meet the requirements of escalating global food demand potentially causes soil and water pollution, eutrophication, depletion of soil fertility, and deposition of toxic heavy metals such as arsenic (As), lead (Pb), and selenium (Se) in the soil. Abundant soil microbes together with bacteria, fungi, actinomycetes, and algae are efficient in solubilizing insoluble soil phosphate to convert into the soluble P and making it available to plants. Strikingly, these microbes promote the growth and yield of a wide variety of crops. Thus, it is essential to inoculate phosphate-solubilizing microorganisms (PSM) via the seeds, to the crop and soil, which is a potential strategy to improve the crop yield. Despite their immense significance in the improvement of soil fertility, phosphorus-solubilizing microbes are yet to replace conventional chemical fertilizers in commercial agriculture. Extensive studies are obligatory to comprehend recent approaches in a diversity of phosphate-solubilizing endophytes and their colonizing ability and application to enhance agronomic yield.

3.4 Endophytic Fungi as P-Solubilizers and Growth Promoters

Phosphate-solubilizing fungi enhance plant growth by different mechanisms and those are (Fig. 3.1): provide nutrients to plant by solubilization process, produce biological control substances, i.e., antibiotics and siderophores, provide protection against the plant pathogens, and stimulate growth hormones production (auxins, gibberellins, and cytokines). In agriculture, phosphate-solubilizing fungi play a significant role as bio-inoculants for improvement of plant growth (Khan et al. 2010; Kour et al. 2019b, c; Rana et al. 2019a, b). The competent phosphate-solubilizing fungi were tested under in vitro conditions and selected for large-scale production and eventually distributed to farmers. In addition to that, suitable carrier selection is also paramount for the development of fungal inoculants such as peat, farmyard manure, soil, cow dung, and cafe powder which are being used as suitable carriers. However, a perfect carrier is designed to possess some unique qualities like good absorption ability, sufficient level of moisture pH, aeration stability, pH buffering capacity, and porousness. In addition to these characteristics, the carrier must be eco-friendly, non-hazardous to microbes, plants, animal, and humans. In addition to this, it should be easy to handle, mix, sterilizable, and store. Keeping in view of cost–benefit ratio, the carriers ought to be cheaper and easily available. The carrier enhances the persistence of phosphorus-solubilizing activity; fungal spores after mixing with a carrier can be

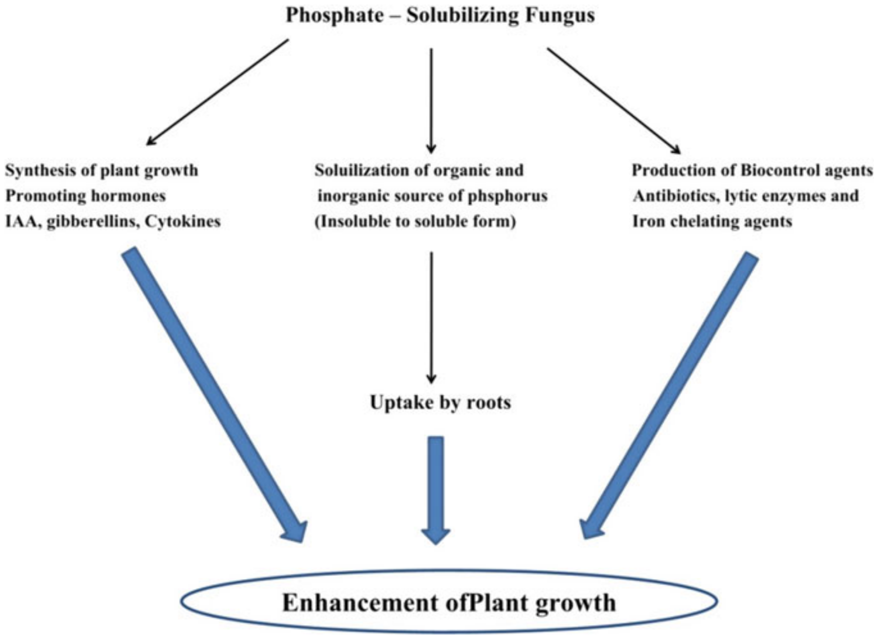

Fig. 3.1 Mechanisms of plant growth acceleration by endophytic P-solubilizing fungi

stored for about 3 months at 30 ± 2 °C. Plethora of examples of commercially available inoculants, i.e., *Penicillium radicum* and *Penicillium bilaiae* were demonstrated in large-scale production and shown to possess "P" absorption ability. Various stages involved in bulk scale production and application of P-solubilizing fungi are clearly depicted (Fig. 3.2).

Various studies indicating the effect of a single culture and/or mixed culture of phosphate-solubilizing fungus on different plant growth parameters observed the effect of mixed inoculation of P-solubilizing fungal strains (two strains of *A. awamori* and four of *P. citrinum*) on growth and seed production of chickpea in pot experiment. Notably, all the isolates were shown synergistic effect and resulted in noteworthy stimulation of root and shoot lengths of legume, height of the plant, seed weight, and number compared to the un-inoculated control.

Phosphorus is a vital nutrient and a part of structural compounds and mediates catalytic reactions in plant metabolism. Phosphorus plays a major role in capturing solar energy and is converted into useful plant compound. Phosphorus is a key component of DNA and RNA. Two phosphate-solubilizing fungi, i.e., *Penicillium oxallicum* P4 and *Aspergillus niger* P85 were isolated by Yin et al. (2015) from calcium-rich soils of China. A remarkable increase in plant fresh weight was observed in strain p24 when rock phosphate was supplemented externally. A study carried out on *Aspergillus aculeatus* P93 has also shown a significant increase in the availability of soluble phosphorus of maize grown in non-amended soil (Yin et al. 2017).

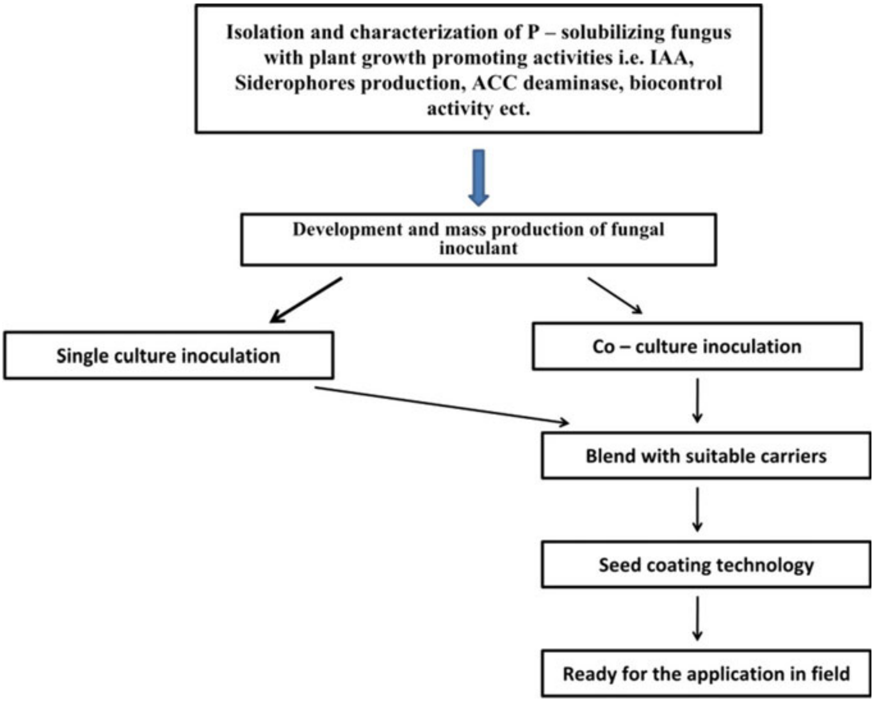

Fig. 3.2 Production and application of phosphate-solubilizing endophytic fungal inoculants

In a recent study, phosphate-solubilizing microorganisms depicted a synergistic effect on plant growth parameters and leaf chlorophyll content (Senthil kumar et al. 2018). In addition to solubilizing phosphates, some may produce potential bio-control agents against plant pathogens. PSM can produce anti-fungal compounds such as flavonoids and phenolics, siderophores, antibiotics and hydrolytic compounds. All of which inhibits growth of plant pathogens.

3.5 Phosphate Solubilization Mechanism by Endophytic Fungi

Based on the availability of type of phosphates (organic or inorganic), endophytic fungi employ suitable mechanism for solubilization of phosphates and endophytic fungi are capable to synthesize organic acids, proteins, OH^- ions, Ca^{+2} exopolysaccharides, CO, siderophores, and enzymes, those may play a significant role in phosphate solubilization (Fig. 3.3).

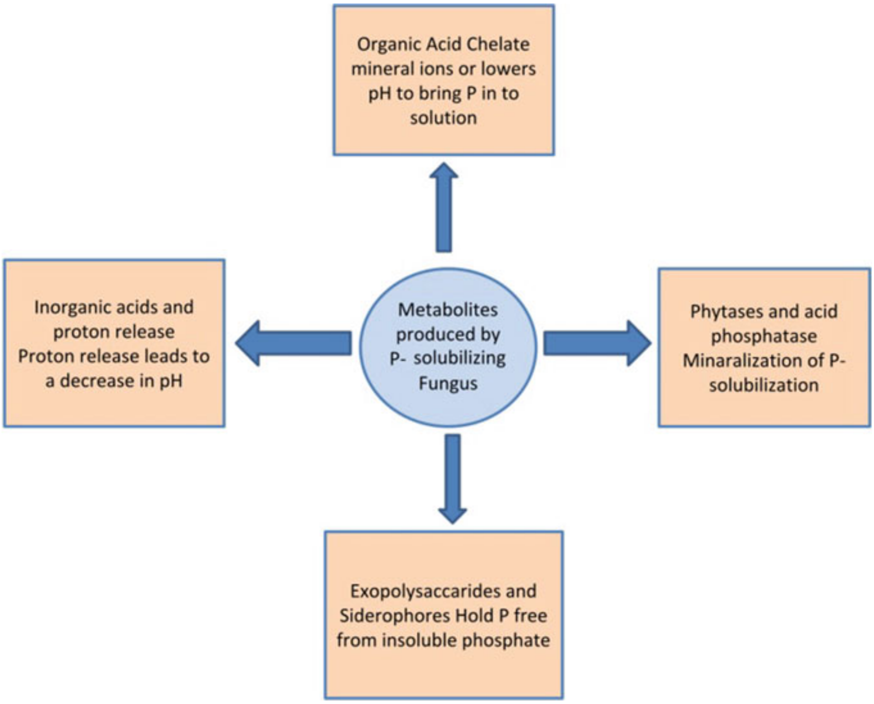

Fig. 3.3 Production of metabolites by P-solubilizing fungi

3.5.1 Inorganic P-Solubilization

Various theories explained the key mechanism underlying the inorganic phosphate solubilization. The principal mechanism is dissolving compounds such as hydroxyl ions, siderophores, organic acids, carbon monoxides, proteins, extracellular enzymes, and exopolysaccharides (Sharma et al. 2013). Secretion of organic acids by endophytic fungi into soil seems to be a most important mechanism for inorganic phosphate solubilization primarily organic acids such as glycolic, maleic, formic, lactic acid, gluconic acid, oxalic, tartaric propionic, and succinic acids. The quantity of organic acid varies with the endophytic fungal strain and also the type of organic acid produced is greatly determined by source of insoluble phosphorus. According to Mendes et al. (2013), *Aspergillus niger* FS1 primarily secrete oxalic acid in higher quantity in treatments with $FePO_4$ and $AlPO_4$, whereas gluconic acid was produced in meager quantity in $AlPO_4$. In contrast to this, *Penicillium canescens* FS23 produced citric and gluconic acids after treatment with Ca_3 $(PO_4)_2$, $AlPO_4$, and rock phosphate. Organic matter present in soil is an excellent source of organic phosphorus. The total quantity of organic phosphorous present in soil is as high as 30–50% of total phosphorus. Organic phosphorus in the soil is principally in the form of inositol phosphate (or) soil phytates. Organic phosphorus can be mineralized with the involvement of enzymes such as phosphatases, phytases.

Many studies explained about p-solubilization based on an organic acid concept. Several genes are involved in production of organic acids. Among all organic acids, gluconic acid is more essential in P-solubilization produced by endophytic fungi. The genetic basis of P-solubilization was studied by Kusari et al. (2012). He observed upregulation of pyrroloquinoline quinine and glucose dehydrogenase genes in solubilization of phosphorous. PQQ-dependent glucose dehydrogenase present on cytoplasmic membrane stimulates oxidation of glucose to gluconic acid. Because of production of gluconic acid, the pH of the soil further decreases which make the following ions HO_4^{-2} and HPO_4^{-3} (soluble forms of phosphorus) more available. An array of genes is involved in the production of organic acids.

3.5.2 Organic P-Solubilization

Mineralization of organic phosphate carried out by involvement of various enzymes, i.e., phytases, phosphonatases, and phosphatases. Phosphatases dephosphorylation or hydrolyze and phosphoanhydride and organic phospho-ester bonds of organic matter. Among all phosphatases, predominant types of enzymes are phosphomonoesterases (Nannipieri et al. 2011). Based on pH optima they are classified as acid and alkaline phosphatase (Behera et al. 2014). Several genes encoding for alkaline and acid phosphatases with broad substrate specificity were cloned and characterized. However, a considerable amount of phosphatases was secreted by plant roots, and it has been reported that microbial phosphates possess a stronger affinity for substrate when compared to derived by plant phosphatases.

A large quantity of phosphorus is found in fruits and seeds for the reason that it is important for the development of seeds. Phytin is a significant form of "P" in seeds. Phytin is naturally degraded by phytases. This is the main source of inositol phosphate and constitutes for more than fifty percent of organic phosphorus in the soil. Phytases act upon phytate and make available free form of phosphorus. Noteworthy that the phosphonatases and carbon-phosphorus lyases hydrolyze carbon-phosphorus bond of organophosphates and release free phosphate (Rodriguez et al. 2006). Because of scarcity, organo-phosphatases do not add much to the soluble form of phosphate in the soil solution. A plethora of studies revealed that organic acids released by P-solubilizing fungi are much superior to bacteria; therefore, endophytic fungi exhibit greater P-solubilization activity. Motsara et al. (1995) revealed that solubilization of rock phosphate was much higher under in vitro conditions by *Rhizoctonia solani*, Penicillium*, Fusarium oxysporum,* and *Aspergillus niger*.

3.6 Development of Phosphate-Solubilizing Endophytic Fungal Inoculants

Phosphates-solubilizing endophytic fungal inoculants are used as major bio-fertilizers. These bio-inoculants are more eco-friendly when compared to chemical fertilizers. Fungi are depicted as predominant P-solubilizing inoculants when compared to other bacterial inoculants; hence, these strains hold much importance in agriculture. Nonetheless, several fungal bio-fertilizers were already developed by IARI as bio-inoculants which comprises *Aspergillus awamori, A. niger,* and *P. digitatum, P.bilaii* strains which were commercialized by Novozymes Biologicals Limited (Canada). Interestingly, the strain *P.radicum* was recently developed by Bio-Care Technology (Australia) by Gupta and Rodriguez Couto (2018). Similarly, in India, P-bio-fertilizers were produced by Ambika Biotech and Agro Services (Madhya Pradesh) (Pal et al. 2015).

For production of P-solubilizing fungal inoculums, huge amount of endophytic fungal strains are required. There are broadly three phases in development of bio-fertilizers. In the first phase, there is a selection and screening of potential phosphate solubilizers followed by a selection of fungal inoculants. The screening process can be carried but by the cultivation of fungi in modified Pikovskaya's medium. In second phase, proper endophytic fungal bio-fertilizers can be developed and the third phase includes checking the quality and persistence of P-solubilizing microorganisms and distribution to farmers (Khan et al. 2010; Kumar et al. 2017). Notably, few potent microorganisms are selected, screened, and cultivated in large scale for production of bio-fertilizers under optimized conditions in a suitable fermentation broth. For cultivation of fungi, lower pH (acidic condition) is more suitable at the same time inhibits the bacterial contamination (Nelofer et al. 2016). Once an adequate amount of growth is obtained, the biomass can be extracted and mixed with suitable pre-sterilized carrier material, purified, packed under aseptic conditions, and stored under appropriate conditions before commercialization. At every stage of bio-fertilizer production, it is mandatory to assess the level of contamination as well as for the amount of desired microorganisms.

Definite problems may also be associated with the commercial-scale synthesis of bio-fertilizers, among those sometimes microorganisms unable to survive under in vivo conditions. This may be due to the fact that the bio-inoculants are either difficult to survive under unfavorable environmental conditions or outcompeted by presented microflora (Walia et al. 2017). One of the important strategies to surmount this problem is amalgamation of bio-fertilizers with suitable carriers.

Carriers being used in production of bio-fertilizers should possess definite characteristics like it should be easily mixed with microbes to enhance the sustainability and survival of microorganisms by maintaining optimum pH, an adequate level of moisture and aeration, etc. Thus, the carrier material should possess an excellent moisture absorption ability and pH buffering capacity, nonetheless, it should also be non-toxic to microorganisms, and eco-friendly to plants, animals, and humans. In addition to there, it is easy to sterilize, and easy to mix, handle, and store. In view of the cost–benefit ratio, the carrier should be effortlessly available and cheap. Nowadays, different types of carriers are being used for the production of bio-fertilizers. Smith (1995) has classified the carriers into different categories, first category comprises different types of soils, peat and coal, waste cake powder farmyard manure, plant debris and second group comprises barnyard compost, soya bean oil, shelled nut oil, barnyard compost. The last group consists of inert materials like perlite, rock phosphate, and calcium sulfate vermiculite. These carriers have also been used in combinations. Wang et al. (2015) reported the utilization of different carriers for developing a bio-fertilizer of *A. niger* and reported a mixture of wheat husk and perlite to enhance the availability of "P" content.

Among soil microorganisms, AM fungi have been found to be a noteworthy component of soil–plant systems (Schreiner and Bethlenfalvay 2003). An AM fungus plays a major role in nutrient and water uptake by plants and provides other benefits to host, such as tolerance under adverse environmental conditions and disease resistance (Pal et al. 2014). Due to their obligatory symbiosis, it is highly difficult to produce AM-based bio-fertilizers in *in vitro* conditions.

Mass scale production AMF is highly difficult because of its specific nutritional requirements (Pal et al. 2015). The different strategies were reported by Berruti et al. (2016) for the utilization of AMF as bio-fertilizers. According to first strategy AMF-harboring rhizosphere, soil can be used as bio-inoculants; however, this method may not be reliable and may perhaps result in colonization of weeds and pathogens. In other strategies, AM spores that were isolated from root region can be used for this AM fungal organism inoculated on a host trap plant in an inert medium. The trap plant is highly susceptible for Arbuscular mycorrhizal fungal growth, and therefore it is used for production of AM fungi for bulk scale. This is frequently used inoculums for inculcation to crop plants in large scale. This consists of a set of some kind of AM spores present in soil inoculants.

In this method, trap plant should be highly amicable for inoculation of desired AM fungi and also should be ideal for large-scale production of propagates. In addition to these, the trap plant should show intense root development within a short period of time and resist to harsh environmental conditions, suitable for synthesis of fungal propagules (Sadhana 2014). An important observation came from a study of Selvakumar et al. (2016) that maize could be the suitable host trap plant when compared to the Sudan grass for the propagation of *Claroideoglomus etunicatum*. Other trap plants including *Chloris gayana, Sorghum vulgare, Zea mays*, *Sorghum bicolor var. sudanense,* and *Ipomea batatas* are most common trap plants used for mass scale culturing of Arbuscular mycorrhizal fungi (Sadhana 2014).

By wet sieving and decantation, the AM fungi is regularly isolated from soil (Singh et al. 2010) followed by microscopic observation of AM fungi. Mass multiplication is carried out by collecting a large number of spores by pot culture method. Host trap plant and AM fungi were cultured in natural solid medium containing clay, peat, sand, perlite soil, and different types of composted plant debris. Tamil Nadu Agricultural University designed a method, and according to this, a trench lined with polythene sheet is being used as plant growth pot or tub. Fifty kg of vermiculite and 5 kg of sterilized soil are filled in trench up to 20 cm height. To this 1 kg of AM spores inoculated 2–5 cm below the surface of vermiculite. Sterilized seeds of trap plant are sown in a trench along with an appropriate dosage of nitrogen source urea and superphosphate. After the period of 60 days roots of trap, plants are cut, spores, a mixture of vermiculite, hyphal fragments, and infected root pieces obtained were used as AM fungal inoculants. Without using soil also some researchers developed hydroponics and aeroponics for the cultivation of AM fungi in the presence of trap plants. The major advantage of these methods is that there is a feasibility to produce pure and clean AM spores (Ijdo et al. 2011).

3.7 Application of Phosphate-Solubilizing Endophytic Fungal Bio-Inoculants

Treatments of seed surface with suitable bio-inoculants are the most common choice of inoculation prior to seeding and reported to be the popularly used method (Walia et al. 2013b). However, there are few techniques that are widely used for the inoculation of endophytic microorganisms, viz., soil application, seed treatment, and foliar spraying. In seed treatment process, carrier-coated fungal inoculums are immersed with seeds in a liquid culture medium. In this method, a fungus adhered firmly to the seed surface. Conversely, there are some constraints in this method. Amount of viable fungi adhered on to the seed surface may not be adequate. The plant species are under cultivation at commercial scale by vegetative propagation, and the endophytic P-solubilizing bio-inoculants are usually applied to plant parts before planting in the field (Panhwar et al. 2013; Kour et al. 2019a; Kumar et al. 2019; Yadav et al. 2019e). The shoots developed from such plants are deemed to be more amenable for bacterization by endophytic microorganisms. Application of endophytes to the soil is another method of bio-inoculants application (supplementation of soil with endophytic bio-inoculants). These methods have many advantages which include the following:

- A high number of P-solubilizing fungi may disseminate per unit area.
- Less number of interactions may occur between bio-inoculants and chemically treated seeds.
- This method is more rapid in comparison with seed inoculation technique.
- These bio-inoculants are more tolerant to dry and desiccated conditions.

In view of above aspects, phosphate-solubilizing endophytic fungal inoculants can be applied by two approaches.

1. Single culture of phosphate-solubilizing fungi can be used as inoculants as single culture approach (SCA).
2. Two cultures can be used as bio-inoculants are called mixed culture approach (MCA).

3.8 Effect of Phosphate-Solubilizing Fungal Bio-Inoculants

Soil is a natural habit for wide variety of microbial communities. The interactions occurring between microbial communities basically impact a physico-chemical property of the soil, and soil fungi performs several imperative roles in the maintenance of soil biochemistry directly or indirectly. Direct endophytic fungi accelerate plant growth promotion through the production of phytohormones, mineralization of salts and ions (Guleria et al. 2014) and in the indirect mechanism; it plays an important role in bio-control agents against phytopathogenic microbe (Walia et al. 2013a). Mehta et al. (2011) studied the growth enhancement of groundnut in association with endophytic fungi. They studied two fungi, i.e., *Aspergillus niger and Penicillium notatum* supplement of soils tri-calcium phosphate (TCP) under pot culture conditions and reported that there is a remarkable improvement in dry weight and height of the plant. When a mixed culture of fungal strains was employed as inoculants, a substantial improvement was observed in plant height as 81% and plant dry weight as 105% compared to controls (Prasanna et al. 2011; Mehta et al. 2011).

It is pertinent that a number of plants and weight of seeds enhanced remarkably with single or multiple inoculations of fungal strains. Other studies carried out by Priyadharsini and Muthukumar (2017) on pigeon pea revealed that when inoculated with the fungi *Curvularia geniculata* has shown a significant impact on growth parameters. *C. geniculata* inoculated seedlings of pigeon pea were taller (26.53%) and showed increased shoot and root dry weight (16.67–33.33%) as compared to un-inoculated control seedlings. In addition to P-solubilization, endophytic fungi also play an imperative role in phytohormone production that can remarkably enhance plant growth. Thus, the exploitation of phosphate-solubilizing fungi is considered eco-friendly, profitable, and sustainable approach for enhancement of crop yield.

3.9 Application of RDNA Technology in Developing Phosphate-Solubilizing Endophytic Fungi

Interaction of endophytic fungi with host plant is relatively an intricate process. Set of genes are involved in such interaction including nitrogen, phosphorus, and other nutrient exchanges between endophytic fungi and host plant tissues were studied; nonetheless, widespread research is desirable to better comprehend the genetic aspects of such interactions. Comprehensive and enhanced knowledge is essential for the involvement of genes and their regulation to undertake genetic manifestation of fungi, which consecutively can be employed for better phosphorus uptake and improved plant growth. The molecular approaches signify a vital role in understanding the genetic aspects of host fungal interactions. Among several molecular approaches, the cloning and gene sequencing methods are most promising and consent to determine which techniques are time-consuming. Nucleic acid hybridizations and probing techniques are required to possess sufficient knowledge of microbial community. However, other molecular methods such as amplified ribosomal DNA restriction analysis or ribosomal intergenic spacer analysis can be employed for the endophytic fungal colonizations.

3.10 Future Applications of Endophytic Fungal Phosphate Solubilizers

In recent times, usage of phosphate fertilizers is highly expensive that cannot be afforded by farmers, particularly in developing countries. Scientists thus have a great responsibility toward society to find some innovative ways from biological sources to make available "P" to crop plants, by an economically efficient alternative for chemical fertilizers. Most of the soils are deficient in available phosphorus to plants and chemical fertilizers are expensive. Due to this interest has been developed in the application of rhizosphere microbes and endophytic fungi with phosphate-solubilizing capacity as bio-inoculants to solubilize phosphate from poorly available sources in soil. Although the potentiality increased for developing such inoculants, their vast applications remain incomplete by intricacy in an understanding of microbial inoculants. These endophytic fungi not only enhance the phosphate availability to the plants but also provide protection to the plants against plant pathogens and stimulate plant growth. The major challenge associated with endophytic fungi is a commercial-scale application, in fact, managing microbial communities to favor plant colonization by beneficial endophytic microorganisms. The contribution of endophytic fungal research may have environmental and economic impacts. Molecular-level research in this aspect is necessary for a better understanding of host endophytic interaction.

Acknowledgements The authors sincerely acknowledge the support extended from Kakatiya University, Warangal and Krishna University, Machilipatnam.

Conflict of Interest We declare no Conflict of Interest.

References

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41:1–16

Ambrosini A, Beneduzi A, Stefanski T, Pinheiro F, Vargas L, Passaglia LP (2012) Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). Plant Soil 356:245–264. https://doi.org/10.1007/s11104-011-1079-1

Behera BC, Singdevsachan SK, Mishra RR, Dutta SK, Thatoi HN (2014) Diversity, mechanism and biotechnology of phosphate solubilizing microorganisms in mangrove—a review. Biocatal Agric Biotechnol 3:97–110

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural bio-fertilizers: let's benefit from past successes. Front Microbiol 6:1559

Duponnois R, Kisa M, Plenchette C (2006) Phosphate solubilizing potential of the nematofungus *Arthrobotrys oligospora*. J Plant Nutr Soil Sci 169:280–282
Forchetti G, Masciarelli O, Izaguirre MJ, Alemano S, Alvarez D, Abdala G (2010) Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. Curr Microbiol 61:485–493
Guleria S, Sharma K, Walia A, Chauhan A, Shirkot CK (2014) Population and functional diversity of phosphate solubilizing bacteria from apricot (*Prunus Armeniaca*) of mid and high regions of Himachal Pradesh. Bioscan 9(2):1435–1443
Gupta VG, Rodriguez-Couto S (eds) (2018) New and future developments in microbial biotechnology and bioengineering: penicillium system properties and applications. Elsevier
Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320
Hodgson S, Cates C, Hodgson J, Morley NJ, Sutton BC, Gange AC (2014) Vertical transmission of fungal endophytes is widespread in forbs. Ecol Evol 4:1199–1208
IJdo M, Cranenbrouck S, Declerck S (2011) Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21:1–16
Joseph B, Priya RM (2011) Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am J Biochem Mol Biol 1(3):291–309
Kasotia A, Choudhary DK (2014) Role of endophytic microbes in mitigation of abiotic stress in plants. In: Ahmad P, Rasool S (eds) Emerging technologies and management of crop stress tolerance. Elsevier, New York, pp 97–108
Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi-current perspective. Arch Agron Soil Sci 56:73–98
Kour D, Rana KL, Yadav AN, Yadav N, Kumar V, Kumar A, Sayyed RZ, Hesham AE-L, Dhaliwal HS, Saxena AK (2019a) Drought-tolerant phosphorus-solubilizing microbes: biodiversity and biotechnological applications for alleviation of drought stress in plants. In: Sayyed RZ, Arora NK, Reddy MS (eds) Plant growth promoting rhizobacteria for sustainable stress management, Volume 1: Rhizobacteria in abiotic stress management. Springer, Singapore, pp 255–308. https://doi.org/10.1007/978-981-13-6536-2_13
Kour D, Rana KL, Yadav N, Yadav AN, Kumar A, Meena VS, Singh B, Chauhan VS, Dhaliwal HS, Saxena AK (2019b) 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. https://doi.org/10.1007/978-981-13-7553-8_2
Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA, Saxena AK (2019c) 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, Volume 2: Perspective for value-added products and environments. Springer, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1
Kumar V, Yadav AN, Verema P, Sangwan P, Abhishake S, Singh B (2017) β-Propeller phytases: diversity, catalytic attributes, current developments and potential biotechnological applications. Int J Biol Macromolec 98:595–609
Kumar M, Kour D, Yadav AN, Saxena R, Rai PK, Jyoti A, Tomar RS (2019) Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. Biologia 74:287–308. https://doi.org/10.2478/s11756-019-00190-6
Kusari S, Zuhlke S, Spiteller M (2011) Effect of artificial reconstitution of the interaction between the plant *Camptotheca acuminata* and the fungal endophyte *Fusarium solani* on camptothecin biosynthesis. J Nat Prod 74(4):764–775
Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798
Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60:157–166

Mehta P, Walia A, Chauhan A, Shirkot CK (2011) Accelerated solubilization of inorganic phosphate and production of antifungal activity in soil by plant growth promoting rhizobacteria isolated from apple rhizosphere. J Mycol Plant Pathol 41(3):342–349

Mendes GO, Moreira de Freitas AL, Pereira OL, da Silva IR, Vassilev NB, Costa MD (2013) Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Ann Microbiol 64:239. https://doi.org/10.1007/s13213-013-0656-3

Mingma R, Pathom-aree W, Trakulnaleamsai S, Thamchaipenet A, Duangmal K (2014) Isolation of rhizospheric and roots endophytic actinomycetes from Leguminosae plant and their activities to inhibit soybean pathogen, *Xanthomonas campestris* pv. *glycine*. World J Microbiol Biotechnol 30:271–280

Mishra VK, Passari AK, Chandra P, Leo VV, Kumar B, Gupta VK, Singh BP (2017) Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from Mirabilis jalapa L. using UPLC-ESI-MS/MS and TD GC-MS. PLoS One 12(10):1–24. https://doi.org/10.1371/journal.pone.0186234

Montanez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. Appl Soil Ecol 58:21–28

Motsara MR, Bhattacharyya PB, Srivastava B (1995) Bio-fertilizers their description and characteristics. In: Biofertilizer technology, marketing and usage, a sourcebook-cum-glossary, Fertilizer development and consultation organization 204–204. A Bhanot Corner, 1–2 Pamposh Enclave, New Delhi, 110048, India, pp 9–18

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bunemann E, Oberson A, Frossard E (eds) Phosphorus in action: biological processes in soil phosphorus cycling, soil biology, vol 26. Springer, Heidelberg, pp 251–244

Narula S, Anand R, Dudeja S, Pathak D (2013) Molecular diversity of root and nodule endophytic bacteria from field pea (*Pisum sativum* L.). Legum Res 36:344–350

Nelofer R, Syed Q, Nadeem M, Bashir F, Mazhar S, Hassan A (2016) Isolation of phosphorus-solubilizing fungus from soil to supplement biofertilizer. Arab J Sci Eng 41:2131–2138

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Mic Pathog 82:50–59

Pal S, Singh HB, Rakshit A (2014) The arbuscular mycorrhizal symbiosis: an underground world wide web. In: Singh DP, Singh HB (eds) Microbial communities for sustainable soil health and ecosystem productivity. Studium Press LLC, Houston, pp 219–253

Pal S, Singh HB, Farooqui A, Rakshit A (2015) Fungal biofertilizers in Indian agriculture: perception, demand and promotion. J Eco-friendly Agric 10:101–113

Panhwar QA, Jusop S, Naher UA, Othman R, Razi MI (2013) Application of potential phosphate-solubilizing bacteria and organic acids on phosphate solubilization from phosphate rock in aerobic rice. Sci World J 2013:272409

Piromyou P, Greetatorn T, Teamtisong K, Okubo T, Shinoda R, Nuntakij A, Tittabutr P, Boonkerd N, Minamisawa K, Teaumroong N (2015) Preferential association of endophytic *Bradyrhizobia* with different rice cultivars and its implications for rice endophyte evolution. Appl Environ Microbiol 81:3049–3061

Porras-Alfaro A, Bayman P (2011) Hidden fungi, emergent properties: endophytes and microbiomes. Annu Rev Phytopathol 49:291–315

Pradhan S, Sukla LB (2005) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol 5:850–854

Prasanna A, Deepa V, Murthy PB, Deecaraman M, Sridhar R, Dhandapani P (2011) Insoluble phosphate solubilization by bacterial strains isolated from rice rhizosphere soils from southern India. Int J Soil Sci 6(2):134–141

Priyadharsini P, Muthukumar T (2017) The root endophytic fungus *Curvularia geniculata* from *Parthenium hysterophorus* roots improves plant growth through phosphate solubilization and phytohormone production. Fungal Ecol 27:69–77

Rado R, Andrianarisoa B, Ravelomanantsoa S, Rakotoarimanga N, Rahetlah V, Fienena F, Andriambeloson O (2015) Biocontrol of potato wilt by selective rhizospheric and endophytic bacteria associated with potato plant. Afr J Food Agric Nutr Dev 15:9762–9776

Rai M, Rathod D, Agarkar G, Dar M, Brestic M, Pastore GM, Junior MRM (2014) Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. Symbiosis 62:63–79

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, Rastegari AA, Singh K, Saxena AK (2019a) Endophytic fungi: biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi, vol 1. Diversity and enzymes perspectives. Springer, Switzerland, pp 1–62

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V, Singh BP, Dhaliwal HS, Saxena AK (2019b) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer, Cham, pp 105–144. https://doi.org/10.1007/978-3-030-03589-1_6

Rana KL, Kour D, Yadav AN (2019c) Endophytic microbiomes: biodiversity, ecological significance and biotechnological applications. Res J Biotechnol 14:142–162

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant and soil 287:15–21

Sadhana B (2014) Arbuscular mycorrhizal fungi (AMF) as a bio-fertilizer-a review. Int J Curr Microbiol App Sci 3:384–400

Saini R, Dudeja SS, Giri R, Kumar V (2015) Isolation, characterization, and evaluation of bacterial root and nodule endophytes from chickpea cultivated in Northern India. J Basic Microbiol 55:74–81

Schreiner RP, Bethlenfalvay GJ (2003) Crop residue and Collembola interact to determine the growth of mycorrhizal pea plants. Biol Fertil Soils 39(1):1–8

Selvakumar G, Kim K, Walitang D, Chanratana M, Kang Y, Chung B, Sa T (2016) Trap culture technique for propagation of arbuscular mycorrhizal fungi using different host plants. Korean J Soil Sci Fertil 49:608–613

Senthil Kumar CM, Jacob TK, Devasahayam S, Thomas S, Geethu C (2018) Multifarious plant growth promotion by an entomopathogenic fungus *Lecanicillium psalliotae*. Microbiol Res 207:153–160

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587

Singh SR, Singh U, Chaubey AK, Bhat MI (2010) Mycorrhizal fungi for sustainable agriculture—a review. Agric Rev 31:93–104

Smith SR (1995) Agricultural recycling of sewage sludge and the environment. CAB international

Soni R, Yadav SK, Rajput AS (2018) ACC-deaminase producing rhizobacteria: prospects and application as stress busters for stressed agriculture. In: Panpatte DG, Jhala YK, Shelat HN, Vyas RV (eds) Microorganisms for green revolution. Springer, New Delhi, pp 161–175

Suman A, Yadav AN, Verma P (2016) Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. In: Singh DP, Abhilash P, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, research perspectives. Springer, New Delhi, pp 117–143. https://doi.org/10.1007/978-81-322-2647-5_7

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

Tariq M, Hameed S, Yasmeen T, Zahid M, Zafar M (2014) Molecular characterization and identification of plant growth promoting endophytic bacteria isolated from the root nodules of pea (*Pisum sativum* L.). World J Microbiol Biotechnol 30:719–725

Thanh DTN, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in maize (*Zea mays* L.) cultivated on Acrisols of the Southeast of Vietnam. Am J Life Sci 2:224–233

Uzma F, Hashem A, Murthy N, Mohan HD, Kamath PV, Singh BP, Venkataramana M, Gupta VK, Siddaiah CN, Chowdappa S, Alqaeawi AA, Abd Allah EF (2018) Endophytic fungi alternative sources of cytotoxic compounds: a review. Front Pharmacol 9(309):1–37. https://doi.org/10.3389/fphar.2018.00309

Verma P, Yadav AN, Shukla L, Saxena AK, Suman A (2015) Hydrolytic enzymes production by thermotolerant *Bacillus altitudinis* IARI-MB-9 and *Gulbenkiania mobilis* IARI-MB-18 isolated from Manikaran hot springs. Int J Adv Res 3:1241–1250

Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK, Suman A (2016a) Molecular diversity and multifarious plant growth promoting attributes of bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. J Basic Microbiol 56:44–58

Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK, Suman A (2016b) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J Biol Sci. https://doi.org/10.1016/j.sjbs.2016.01.042

Verma P, Yadav AN, Kumar V, Singh DP, Saxena AK (2017) Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crops improvement. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives. Springer Nature, Singapore, pp 543–580. https://doi.org/10.1007/978-981-10-6593-4_22

Walia A, Mehta P, Chauhan A, Shirkot CK (2013a) Antagonistic activity of plant growth promoting rhizobacteria isolated from tomato rhizosphere against soil borne fungal plant pathogens. Int J Agri Environ Biotechnol 6(4):587–595

Walia A, Mehta P, Chauhan A, Shirkot CK (2013b) Effect of *Bacillus* sp. strain CKT1 as inoculums on growth of tomato seedlings under net house conditions. Proc Natl Acad Sci India Sect B Biol Sci 84(1):144–155

Walia A, Guleria S, Chauhan A, Mehta P (2017) Endophytic bacteria: role in phosphate solubilization. In: Maheshwari DK, Annapurna K (eds) Endophytes: crop productivity and protection, sustainable development and biodiversity. Springer, Berlin, pp 1–33

Wang H, Liu S, Zhai L, Zhang J, Ren T, Fan B, Liu H (2015) Preparation and utilization of phosphate bio-fertilizers using agricultural waste. J Int Agric Adv 14:158–167

Yadav AN (2019) Endophytic fungi for plant growth promotion and adaptation under abiotic stress conditions. Acta Sci Agric 3:91–93

Yadav N, Yadav AN (2019) Actinobacteria for sustainable agriculture. J Appl Biotechnol Bioeng 6:38–41

Yadav AN, Kumar V, Prasad R, Saxena AK, Dhaliwal HS (2018a) Microbiome in crops: diversity, distribution and potential role in crops improvements. In: Prasad R, Gill SS, Tuteja N (eds) Crop improvement through microbial biotechnology. Elsevier, New York, pp 305–332

Yadav AN, Verma P, Kumar V, Sangwan P, Mishra S, Panjiar N, Gupta VK, Saxena AK (2018b) Biodiversity of the genus penicillium in different habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering, penicillium system properties and applications. Elsevier, Amsterdam, pp 3–18. https://doi.org/10.1016/b978-0-444-63501-3.00001-6

Yadav AN, Gulati S, Sharma D, Singh RN, Rajawat MVS, Kumar R et al (2019a) Seasonal variations in culturable archaea and their plant growth promoting attributes to predict their role in establishment of vegetation in Rann of Kutch. Biologia 74:1031–1043. https://doi.org/10.2478/s11756-019-00259-2

Yadav AN, Yadav N, Sachan SG, Saxena AK (2019b) Biodiversity of psychrotrophic microbes and their biotechnological applications. J Appl Biol Biotechnol 7:99–108

Yadav AN, Mishra S, Singh S, Gupta A (2019c) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019d) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019e) Recent advancement in white biotechnology through fungi. Volume 3: Perspective for sustainable environments. Springer, Cham

Yin Z, Shi F, Jiang H, Roberts DP, Chen S, Fan B (2015) Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. Can J Microbiol 61(12):913–923

Yin Z, Fan B, Roberts DP, Chen S, Shi F, Buyer JS, Jiang H (2017) Enhancement of maize growth and alteration of the rhizosphere microbial community by phosphate-solubilizing fungus *Aspergillus aculeatus* P93. J Agric Biotechnol 2(2):1–10