

Chapter 16

Role of Endophytic Microorganisms in Phosphate Solubilization and Phytoremediation of Degraded Soils



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Abstract Phosphorus (P) is considered as the second most important element in plant nutrient profile after nitrogen. It primarily exists incorporated in organic compounds or as mineral salts in soil. Despite these, phosphorus compounds are disbursed abundantly in agricultural soil, and the majority of them are of insoluble form. With the assistance of plant-associated bacteria, the inorganic phosphate solubilization is one of the significant mechanisms for plant growth promotion. The mechanism involves the solubilization of phosphate complexes into more available forms such as orthophosphate ions by organic acid secreted by microbes. The employment of plant growth promoting P bacterial inoculants as biofertilizers can provide favourable alternative to replace chemical fertilizer to some extent. Some examples of phosphate solubilizer are *Bacillus*, *Pseudomonas* and *Aspergillus*, while the phosphate absorber includes arbuscular mycorrhizal fungi (e.g. *Glomus*). Phytoremediation of heavy metals in association with phosphate-solubilizing bacteria are known to overcome metal stress on plants due to the contaminated substrate. In case of mine-degraded soils, endophyte assisted P-solubilization enhances the bioavailability of insoluble P to plants which in turn enhances the plant growth. Therefore, this chapter covers endophytes assisted

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sustainable in-situ remediation of contaminated site which stimulates plant growth, defence against metal toxicity and soil fertility.

Keywords Biofertilizers · Soil · Heavy metals · Amendment · Phytoremediation · Microbes

16.1 Introduction

Mining activity causes complete loss of soil profile, vegetation and the biodiversity of a land. It also causes air and water pollution, disturbs drainage and permanently affects a landform (Ghosh and Maiti 2020; Mohapatra et al. 2020). Mine spoil is characterized by impoverished nutrient content, low organic content and cation exchange capacity and disturbed ambient soil physicochemical and biological properties (Basu et al. 2015; Ahirwal et al. 2021; Ghosh and Maiti 2021a). A degraded mine spoil is devoid of soil organic matter, microbial activity and the enzymatic activities associated with the soil fauna (Maiti 2013; Ghosh and Maiti 2021b). A mine spoil is devoid of essential soil nutrients and often the storehouse of potentially toxic elements (Ahirwal and Maiti 2017; Ghosh and Maiti 2021c). Phosphorous (P) is a crucial component for overall plant development and productivity (Rawat et al. 2021). Its properties constrain its free accessibility and make it a restraining nutrient for vegetation development (Mehta et al. 2017). Thus, an efficient amendment technique is required for mitigation of phosphate deficiency and heavy metal contaminations in mine spoils/tailings and technosol. Some common restoration practices for post-mining coal mine degraded land are forestry, agricultural practices, grass-legume seeding, fly ash amended plantation and biochar aided plantation (Šebelíková et al. 2019; Shukla and Lal 2005; Kumari et al. 2022; Świątek et al. 2019; Fellet et al. 2011; Ghosh et al. 2020).

In a natural soil ecosystem, plants interact with a number of symbiotic microorganisms (Domka et al. 2019). The plant–soil interaction includes synergy of plants with rhizobacteria and endophytic fungi (Maiti 2013; Domka et al. 2019; Varma et al. 2019a, b). Actinomycetes, bacterial and fungal endophytes perforate the plant through root zones along with flower, leaf, stem and cotyledon (Li et al. 2012). The microbiomes are such integral part of plants that they can be used as proxy to study the phenotypic variation of the plant genotype. The knowledge of plant–microbiome interactions can help improving the economic and environmental sustainability of mine spoil restoration through agriculture and forestry. A reduction in inputs, in terms of fertilizer, water, or chemical pesticides, would lead to significant cost savings (Prasad 2017, 2018; Prasad et al. 2021).

Endophytic microbes have the ability to grow throughout the host plant tissues and releases phytochemicals that provide resistance to disease and help in nutrient mineralization for host plant (Maiti 2013). Some endophytic fungi can also solubilize P and supply it to their non-mycorrhizal counterparts, encouraging its growth under nutrient environment (Mehta et al. 2017; Rawat et al. 2021). Thus, they help in improving the overall plant growth under stressed environmental conditions (Maiti 2013). *Curvularia geniculata* isolated from *Parthenium hysterophorus* roots is a dark septate root

endophytic fungus which can improve plant growth by promoting P-solubilization and certain phytohormone secretion (Priyadharsini and Muthukumar 2017). Another important role played by endophytes includes resistance to heavy metals and assistance in phytoremediation of a metal contaminated site. Endophyte-assisted phytoremediation technology has been reported to be an efficient technique for in situ remediation of potentially toxic elements contaminated soils (Mastretta et al. 2009; Domka et al. 2019; Guerrero-Zúñiga et al. 2020). During the phytoremediation of polluted sites, heavy-metal contamination enduring endophytes can also improve plant growth, reduce metal phytotoxicity and influence translocation and accumulation of metal. Thus, this chapter focuses on the beneficial role of endophytes for phosphate solubilization and heavy metal remediation. In conclusion, this chapter provides an insight on how endophytes-assisted phytoremediation enhances soil properties.

16.2 Role of Endophytes for Mine Spoil Reclamation

16.2.1 *Phyostimulation and Nutrient Cycling*

Essential nutrients such as C, N, H, O and P are absolutely necessary for plant growth and development. These nutrients are in chemical form through atmosphere, soil, water and organic matter. Endophyte facilitates the uptake of nutrients by the roots of the plants (Nair and Padmavathy 2014). They have been reported to elicit different modes of actions for plant adaptation in P-deficient soil and facilitation of N uptake (Arachevaleta et al. 1989). Certain endophytic bacteria have been reported to produce phytohormones such as cytokinins, auxins and gibberellic acids which are essential plant growth regulators (Xin et al. 2009). Endophytes play vital role in biodegradation of the debris of its host flora (Mehta et al. 2017).

16.2.2 *Enzyme Production, Antimicrobial Activity and Source of Bioactive*

Soil micro-organisms are the source of a number of commercially important enzymes. This quest for alternative source of enzyme production has led to the discovery of certain endophytes which can produce vital enzymes. Endophytic fungi such as *Aspergillus japonicas*, *Cladosporium sphaerospermum*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *P. glandicola* and *Xylaria* sp. have been reported to produce enzymes such as pectinases, cellulases, xylanases and proteases (Nair and Padmavathy 2014). *Acremonium zeae*, isolated from maize, has also been reported to produce the enzyme hemicellulase (Bischoff et al. 2009). A number of isolated endophytes from plants have been reported to possess antimicrobial activity (He et al. 2020). Most endophytes show antimicrobial activity; however, the ones obtained from medicinal plants affects a broad spectrum of pathogenic microbes (Nair and Padmavathy 2014).

16.2.3 Bioremediation

Bioaccumulation, bio-stimulation, bio-deterioration, bio-leaching, bio-reduction and bio-sorption are some common bioremediation techniques used for heavy metal contamination. Endophytes possess the ability to breakdown complex compounds. Mastretta et al. (2009) reported that the inoculation of *Nicotiana tabacum* with endophytes resulted in improved plant growth under Cd toxicity and the phytoavailable Cd concentration was high in comparison with the one having no endophytic growth. According to Basu et al. (2015), a number of microorganisms catalyse the reduction of Cr (VI) to Cr (V) or Cr (III) in various environmental conditions. Cr (VI) reduction is shown to be metabolic in some species of bacteria but can also be dissimilatory/respiratory when exposed to anaerobic conditions. Although, most microbes are sensitive to Cr (VI), some microbes are highly resistant and can tolerate Cr (VI) toxicity in the soil. Metal reductase genes found on plasmids and chromosomes impart the resistance to these microbes for growth in Cr (VI) environment (Patra et al. 2017). Some common endophytes that have the potential for Cr remediation include *Acinetobacter*, *Arthrobacter*, *Bacillus* spp., *Cellulomonas* spp., *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas* and *Ochrobactrum* (Hossan et al. 2020). A review conducted by Pushkar et al. (2021) reported that major bacterial communities found at chromium contaminated sites are *Gammaproteobacteria*. Other bacteria reported to inhabit chromite contaminated sites includes *Serratia marcescens*, *Pseudomonas aeruginosa*, *Alcaligenes faecal* and *Klebsiella oxytoca*.

16.3 Role of Endophytes for Phosphate Solubilization

Phosphorus is an essential macronutrient for the proper metabolism, growth and plant development. Phosphorus is abundantly available in both inorganic and organic forms in soil; however, due to the complex formation with metal ions in soil, it is unavailable for plant uptake. Phosphate-solubilizing endophytes have the ability to solubilize the complex phosphates in the soil by various mechanisms. Some commonly used mechanisms used by these microbes include production of enzymes, organic acids and siderophores that have the ability to chelate the heavy metal ions and form complexes, making bioavailable phosphates for vegetation uptake (Rawat et al. 2021). These endophytes also produce certain phytohormones such as auxins, cytokinins and gibberellins which promote plant growth. 1-aminocyclopropane-1-carboxylic acid deaminase produced by endophytes has been reported to improve plant growth under stressful environment which improves its resistance to heavy metal toxicity (Fig. 16.1). A few examples of endophytes, their host plant and the role they play are given in Table 16.1.

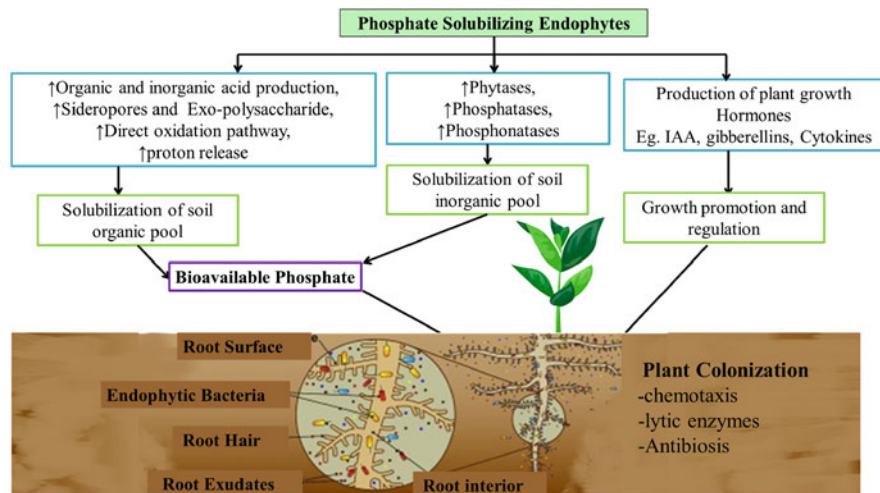


Fig. 16.1 Mechanism by which endophytes promotes plant growth and phosphate solubilization

16.4 Role of Endophytes for Phytoremediation

A number of endophytes have been reported to be heavy metals resistant. Endophyte-assisted phytoremediation is an effective technique for in situ remediation of contaminated soils (Prasad 2022). Microbes develop symbiotic relationships with their plant hosts and promote phytoremediation. Some common hyperaccumulating plants such as *Brassica juncea* (L.) Czern., *Pteris vittata*, *Sedum alfredii* and non-hyper-accumulators, such as *Arabidopsis thaliana*, *Brassica napus* and *Glycine max* have been reported to house a number of important endophytes (He et al. 2020). During pollutant phytoremediation association of heavy-metal-resistant endophytes can result in enhancement of plant development followed by decrease in metal phytotoxicity and affect translocation of metals in plants. They even produce certain enzymes which help in the degradation of contaminants that reduces the phytotoxicity of the potentially toxic elements. Application of endophytes for phytoremediation and their significance for the host plant growth has been given in Table 16.2.

16.5 Case Studies

16.5.1 Fungal Root Endophytes in Metal-Polluted Tailings

Flores-Torres et al. (2021) conducted a research identifying and assessing the plant and fungal root endophytes in bioremediation of polymetallic polluted tailings. The study revealed the significant role of native plants such as *Tagetes lunulata*, *Cordia*

Table 16.1 Phosphate-solubilizing endophytes, their respective host, phosphate-solubilizing ability and their significance

Endophyte	Host	Isolation of endophytes and colonization	Phosphorous solubilized ($\mu\text{g mL}^{-1}$)	Significance	References
<i>Serratia plymuthica</i> BMAI	<i>Vicia faba</i> L.	<ul style="list-style-type: none"> Strain isolated from the extracted rock phosphate (RP) stockpiles (operated by the company of phosphate of Gafsa: CPG Tunisia). Direct strain inoculation in plant at a cell concentration of approximately 3×10^6 CFU g^{-1} of soil 	450	<ul style="list-style-type: none"> Phosphorus uptake improved Improved plant biomass and height 	Borgi et al. (2020)
<i>Pseudomonas</i> sp.	<i>Triticum aestivum</i>	–	101	<ul style="list-style-type: none"> Production of Siderophore Improved biomass and dry matter 	Liu et al. (2019)
<i>Bacillus megaterium</i>	<i>Brassica napus</i>	–	119	Phosphorus content and biomass production increased	Zheng et al. (2019)
<i>Staphylococcus sciuri</i> , <i>Bacillus pumilus</i> ,	<i>Oryza sativa</i> L.	<ul style="list-style-type: none"> From fresh soil (attached to roots of <i>O. sativa</i>) Colonization of PSB in glucose based N-free medium for Azotobacter type free living N_2 fixers 	192	Enhanced phosphorus uptake in shoot, root, grains	Rajapaksha and Senanayake (2011)
<i>Enterobacter asburiae</i> <i>Acinetobacter rhizosphaerae</i>	<i>Zea mays</i> L.	<ul style="list-style-type: none"> Rhizosphere of <i>Hippophae rhamnoides</i> Charcoal based bacterial inoculants (CFU of approx. $10^7/\text{g}$) 	750	<ul style="list-style-type: none"> Plant height and root shoot length increased Available ca and P content high in soil with endophytes. 	Gulati et al. (2010)
<i>Penicillium</i> spp.	<i>Triticum aestivum</i>	<ul style="list-style-type: none"> From disinfected wheat roots (3–7 mm root segments) 	–	Plant fertility improved	Wakelin et al. (2004)

<i>Bacillus</i> sp.	<i>Cicer arietinum</i>	<ul style="list-style-type: none"> • Cultured in semiselective medium for <i>Penicillium</i> spp. (Dichloran, rose bengal, chloramphenicol agar) • Pre isolated bacterial strain 	753	<ul style="list-style-type: none"> • Improved phytohormones • Dry weight of nodules, root shoot biomass increment 	Ditta et al. (2018)
<i>Pseudomonas putida</i>	<i>Pisum sativum</i>	N-fixers isolation from nodules of pea plants Isolated and colonized in yeast extract mannitol medium +2.5% Congo red dye	319	<ul style="list-style-type: none"> • Shoot biomass increased • High seed protein content and increased phosphorus in shoots 	Ahmad et al. (2013)
<i>Penicillium oxalicum</i>	<i>Triticum aestivum</i> , <i>Zea mays</i> L.	<ul style="list-style-type: none"> • Rhizospheric soil from <i>Jatropha curcas</i> (rock phosphate landfills of Rajasthan state mines and minerals Ltd) • Fungi isolated in PVK agar +50 mg P₂O₅/100 ml medium 	586	<ul style="list-style-type: none"> • Shoot height enhanced 1.5 times compared to control • 42% increase in yield of wheat compared to control • 82% increase in root shoot biomass compared to control 	Singh and Reddy (2011)
<i>Streptomyces laurentii</i>	<i>Sorghum bicolor</i> L.	Aerobic bacteria isolated from rhizospheric soil (Amaranthus, buckwheat, millets and maize soil attached to roots) by serial dilution method in growth medium	206	Siderophore production, enzymatic activity potassium solubilization	Kour et al. (2020)
<i>Pantoea vagans</i> , <i>Pseudomonas psychrotolerans</i> , <i>Bacillus subtilis</i> , <i>Bacillus safensis</i> and <i>Pantoea agglomerans</i>	<i>Festuca arundinacea</i> ; <i>Solanum lycopersicum</i> and <i>Capsicum annuum</i>	<ul style="list-style-type: none"> • Endophytes isolated from plants grown in the foothills of Appalachian Mountains of USA (37.125372, 79.298415) unfertilized soil. • Sterilized random plants were sectioned in root leaf and 	274–372	<ul style="list-style-type: none"> • Promoted <i>Festuca arundinacea</i> growth in vitro • Promoted pepper and tomato growth • The extracellular enzymes such as acid phosphatase and phytase secreted by the 	Mei et al. (2021)

(continued)

Table 16.1 (continued)

Endophyte	Host	Isolation of endophytes and colonization	Phosphorous solubilized ($\mu\text{g mL}^{-1}$)	Significance	References
<i>Enterobacter</i> sp. J49 or <i>Serratia</i> sp. S119	<i>Zea mays</i> , <i>Glycine max</i> and <i>Arachis hypogaea</i> L.	Isolated from cultivated peanut plants Two endophytic strains colonization was analysed by isolating bacterial cells from internal tissues of stem, leaves and roots of peanut, soybean and maize plants from the microcosm assay.	–	<p>bacteria helped in decomposition and nutrient cycling</p> <ul style="list-style-type: none"> • Promoted the growth of maize and soybean plants and contributed significantly P to their tissues • Root exudates of the three produced changes in pectinase and cellulase activities • Potential sources for the formulation of biofertilizers 	Lucero et al. (2021)

Table 16.2 Application of endophytes for phytoremediation and their significance for the host plant growth

Endophytes	Heavy metals (mg kg ⁻¹)	Host plant	Isolation of endophytes	Significance	References
Enterobacter sp. CBSB1 (OGM – gene)	Cd (45.2); Pb (876.7)	<i>Brassica juncea</i>	Isolation of endophytes • Strain was isolated from the surface-sterilized <i>B. juncea</i> roots	Improved shoot length. A decrease in Cd and Pb concentration was observed in shoot biomass.	Qiu et al. 2014
Bacillus sp.	Cd (5.9), Zn (1236) Pb (153)	<i>Sedum plumbizincicola</i>	Isolated from the rhizospheric soil of <i>S. plumbizincicola</i> by serial dilution method	Plant biomass and chlorophyll content increased. Increased Cd, Pb and Zn soil bioavailability	Ma et al. 2015
<i>Pseudomonas azotoformans</i>	Cd (400) Ni (350) Zn (500)	<i>Trifolium arvense</i>	Strain isolated from the leaves of Ni hyperaccumulator plant <i>Alyssum serpyllifolium</i> (subsp. malactianum)	Improved plant biomass and increased [Cd], [Ni] and [Zn] in shoots	Ma et al. 2017
<i>Pseudomonas korensis</i> AGB-1	As (827) Cd (103) Cu (396) Pb (2431) Zn (3991)	<i>Miscanthus sinensis</i>	Isolated from fine roots of <i>M. Sinensis</i> growing in the mine site soil following Sun et al. (2010)	Improved shoot and root biomass, increased chlorophyll, protein content. Decrease in plant stress and increase in [As] and [Cd] in roots	Babu et al. 2015

congestiflora and *Lupinus campestris* as well as the exotic plant species *Asphodelus fistulosus*, and *Cortaderia selloana* in phytoextraction and/or phytostabilization of Zn, Pb and Cd. Molecular studies of fourteen endophytic fungi isolated from root inner zones of *Pennisetum villosum* and *T. lunulata* showed the prevalence of *Alternaria* and other *Pleosporales*. The dominance of endophytes in several plant root systems indicates the interaction and functioning of mycorrhiza in mine tailings. Exotic invasive plants *A. Fistulosus* and *P. villosum* showed more than 50% root colonization intensity by endophytes, which could ascertain its invasive capacity. The study reported that these endophytes could facilitate the advancement of *Ambrosia artemisiifolia* growing at polluted sites; therefore, mycorrhizal interactions can help in promoting local adaptation and/or reducing environmental stress. Thus, the study indicated that the employment of native endophytic fungi could emphasize the establishment of plants for reclamation of mine waste in semi-arid climate in biologically sustainable manner. Also, high efforts are needed to enhance the vegetation practice of mine wastes under study, which can efficiently reduce, in turn, their potential ecotoxicological impact on organisms, human populations and agricultural areas.

16.5.2 Root Colonizing Endophytes for Succession in a Mine Degraded Land

Kolaříková et al. (2017) studied the fungal community assembly during spontaneous primary succession in Sokolov brown-coal mining in Czech Republic. The fungal communities associated with the roots of *Betula pendula* and *Salix caprea* were studied in a mine spoil chronosequence (12–50 years old sites) site. The study showed that the fungal root endophytes, fungal plant pathogens and ectomycorrhizal fungi changed significantly along the age of reclamation. Ectomycorrhizal fungi and fungal plant pathogens communities have a direct impact on the development of the vegetation cover and the properties of the reclaimed mine spoil. Thus, the study concluded that plant community structure changed along the various stages of succession which was directly impacted by the endophyte and pathogen communities of the soil. The study provided a better understanding of community assembly of root-associated fungi and provided insight of fungal ecology in various stages of succession.

16.6 Conclusion

Phytoremediation with endophyte assistance can be a promising technique for the restoration of a degraded and contaminated soil. They are known to improve nutrient uptake, enhance growth, decrease phytotoxicity of heavy metals and effect their

assimilation in plants body. These endophytes also solubilize the unavailable phosphorus in soil and restore the deficiency in soil. These endophytes also play a vital role in phytoremediation of heavy metal contaminated sites. Thus, endophytes as a mean to remediate contaminated sites should be explored for eco-friendly and effective remediation of heavy metal contaminated sites. Selection of potent endophytes with multifunctional role is essential for the commercialisation and reduction of cost of restoration of mine-degraded land. Thus, future researches should be done to develop and discover new strains from various ecological niches and for employing in degraded soil restoration.

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