Chapter 6
Endophytic Microbes and Their Role in Land Remediation

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6.1 Introduction

Food security and "zero hunger challenge" are the most thriving topics of present era. It is listed second in the United Nations sustainable development goals. It aims to eradicate hunger, and malnutrition, that was estimated to be 12.5% of current global population i.e., 7.6 billion people in the year 2010–2012 (FAO 2012). According to UN report, 26.4% of global population affected by food insecurity in the year 2018; with an estimated demand of 9.7 billion by 2050 (DESA 2015). The pandemic apart from other factors like population rise, climate change, environmental stressors, land-use patterns, irrigation, post-harvest management techniques have been affecting the food production and supply chain throughout the world. Rapid industrialization and urbanization are also affecting agricultural productivity. The green revolution started in 1950–1960s targeted to increase agricultural productivity through adoption of new technologies, using high yielding crop varieties, increased use of inorganic fertilizers, agrochemicals and irrigated water supply. In 2014–15, with 250 million tons of food grain production, India is on the verge of becoming a food basket for the world. However, the challenge remains with over exploitation of agricultural lands resulting into loss of top-soil and reduced yield. At the same time, agricultural residues pose a grave danger to our fragile ecosystem. Prolonged application of inorganic fertilizer and agrochemicals exerts deleterious impact on soil health. Persistent pesticides, herbicides, fungicides etc. tend to cause loss of soil fertility over the years. These groups of contaminants are termed as emerging organic contaminants (EOCs). Apart from agricultural residues, emerging organic contaminants (EOCs) include industrial chemicals, surfactants, personal care products and pharmaceutical products etc. EOCs severely affect soil health (Hu et al. 2017; Usman et al.

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2017). Therefore, researchers have been extensively working on alternative bio-based options for sustainable pollution management without compromising the soil health and its fertility (Das et al. 2021).

Pollutants like toxic metals present in industrial wastes and effluents contaminate soil and surface water. These metals undergo phase distribution and speciation under variable environmental conditions. They enter the food chain, bioaccumulate, and magnify, thus create serious problems at trophic levels. For-example, in the year 1956, 1784 people died from consumption of organic mercury contaminated fish from Minamata bay of Japan, the episode infamously known as “Minamata disease”, where the origin of methyl mercury was traced back to a chemical factory effluent (Nabi 2014). Similarly, other effluents with toxic metals like Cr, As, Pb, Se etc. also show tendencies toward bioaccumulation under different environmental condition (Gorai et al. 2020). However, there are few incidences where they entered into the food chain, causing harmful effect on living organisms. Therefore, safe removal techniques are the need of the hour.

Several researchers have been looking for sustainable remediation techniques. It involves both chemical and biological methods. Bioremediation is an emerging technique where biological methods are applied for a synergistic interaction between environmental contaminants and their cleaning process. Bioremediation techniques are of two types: microbe assisted remediation and phytoremediation. Microbe assisted remediation technique includes biostimulation, bioaugmentation, and intrinsic bioremediation. On the other hand, phytoremediation includes phytoextraction, phytostabilization, phytodegradation, phytostimulation, phytovolatilization, rhizofiltration, biological hydraulic containment, phytodesalinization. In the present chapter, we will discuss about few microbe-assisted remediation techniques, its present status and future scope.

Previous researchers have extensively worked with rhizospheric bacteria; but endophytic interaction and its implication in terms of bioremediation is a relatively new topic. Endophytes colonize easily, promote plant growth and enable to remediate the surrounding soil surface (Tong et al. 2017; Gorai et al. 2020). Therefore, they are more efficient and preferable over rhizospheric bacteria. At times, endophyte-plant interaction may lead to change in host plant metabolism and physiology (He et al. 2019). The altered metabolism can facilitate phytoextraction process and/or pollutant degradation in the substrate (Tripathi et al. 2017; Tong et al. 2017; Afzal et al. 2017). Among, all existing bioremediation techniques, endophyte assisted remediation techniques have tremendous scope and future use (Feng et al. 2017; Srivastava et al. 2020).

6.2 What Are Endophytic Microbes?

In the year 1886, Bary discovered the term “Endophyte”. The term was originally derived from Greek words “endon” means “within”, and “phyton” means “plants”. Therefore, endophytes are those microbes that reside inside different parts of plant body. Later on, 1904, this was further re-discovered in Darnel, Germany (Tan and Zou 2001). Endophytes have been defined in various ways by several researchers depending upon their source of origin. Bacon and White 2000 defined endophytic microorganisms as “microbes that reside within living internal tissues of plants without causing any instant and overt negative effects”. An alternative definition of endophytic fungi is “fungi that live for all or at least a significant part of their life cycle asymptomatically within plant tissues” (Wilson 1995). According to Carrol, endophytes are asymptomatic microbes that reside inside plants; while Petrini (1991) described that endophytic microbe are those microorganisms that living at least one part of their life cycle within the internal parts of plant tissues without imparting any harmful effects to the host plant. Wilson and Carrol (1997) depicted additional information regarding endophytes implied that a part or total life cycle of endophytic bacteria or fungi reside in the living tissues of host plants without causing any apparent or symptomatic infections. Different group of organisms are involved in endophytic association. These are bacteria, fungi, algae and oomycetes. Mostly bacteria and fungi are found to present as endophytic organisms in plants.

6.3 Effect of Endophytes in Soil Fertility Management

Change in land use pattern and simultaneous agricultural intensification exerts an unbearable pressure to the environment. Loss of agricultural land, results in extensive use of the remaining ones. However, with time, soil tends to lose its fertility. Continuous and rampant use of inorganic fertilizer including other agrochemicals contributes to the cause of fertility loss. Therefore, researchers around the Globe are looking for sustainable options to increase the crop yield without depleting any soil properties. It is quite obvious that consecutive cultivation without a fallow period or crop rotation leads to loss of top soil and eventually decreases fertility. Therefore, a gradual shift toward biological fertilizers like composts, organic manures, vermicompost, microbial consortiums, biofertilizers etc. is being explored. All biological techniques are found to be cost-effective, feasible and less harmful. Among biological techniques, endophytic microbes, in single inoculum or consortium, play vital role in soil quality improvement, when applied in a strategic manner. Therefore, the present study focuses on what role endophytic microorganisms play in soil fertility management, plant growth promotion and land remediation. Figure 6.1 represents how endophytic association regulates plant functioning and rhizo-spheric soil conditioning results in land remediation.

6.4 Nitrogen Fixation

Plants are unable to utilize atmospheric nitrogen directly. But nitrogen is an essential element for plant growth; therefore, atmospheric nitrogen needs to be fixed and

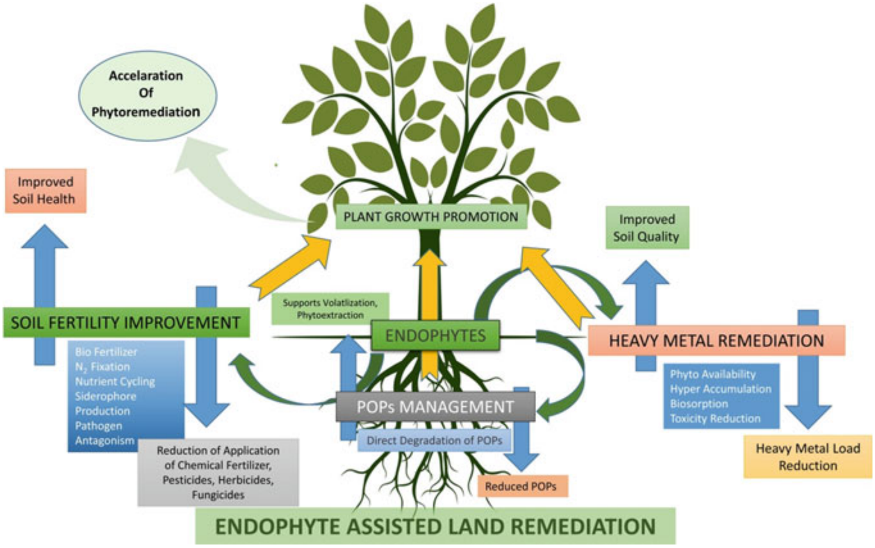

Fig. 6.1 Endophyte-assisted land remediation techniques

convert into bioavailable forms for plant uptake. Usually, to meet nitrogen requirement of crops, farmers apply inorganic nitrogen i.e., urea as N source following standard management practice. Rigorous application of nitrogen-based chemical fertilizer increases the risk of environmental pollution and decreases soil fertility. It also releases a great amount of greenhouse gases (NO_x) at the production site.

Soil microbes play an important role in N_2 fixation, assimilation and denitrification process. Exploration and strategic application of these microorganisms can assist in reducing soil nitrogen deficiency. Hurek and Reinhold-Hurek (2003) observed that under N_2-stressed condition, endophytic microorganisms are better promoter of plant growth than rhizospheric microbes.

Diazotrophs fix atmospheric nitrogen into ammonia. This ammonia gets oxidized to form nitrate that gets dissolved in the nutrient pool and become bioavailable. It undergoes further assimilation in plant body, forms amino acids that finally participates in protein synthesis, eventual plant growth. During endophytic nitrogen fixation, these microbes form nodule or oxygen free structure. These nodules are mostly infected with both Gram-positive and Gram-negative bacteria depending upon the host plant. All these microbe populations inside the root nodule, contribute to nitrogen fixation and are symbiotic in nature. According to Dobereiner et al. (1993) and Muthukumarasamy et al. (2007), *Gluconoacetobacter diazotrophicus* contribute approximately 150 kg N/H/year. During banana cultivation, an increase of bioavailable nitrogen at 79% and 11% was reported, when inoculated with *Agrobacteria* and *Azospirillum* respectively (Zuraida et al. 2000). Soybeans are extensively cultivated legumes around the world. The roots of soybean are found to form nodules with different strains of *Bradyrhizobium*, *Mesorhizobium*. Here also, different strains have different growth rate and different nitrogen fixing ability. Sainz et al. (2005) calculated a total of 142 kg N/H/year fixed nitrogen in Soybeans. These endophyte-plant symbiotic associations play impeccable role in N fixation and availability in soil. Table 6.2 listed N_2-fixing endophytic microorganisms with their respective host plants.

6.5 Biofertilizer

Biofertilizers are substances containing beneficial microbial inoculum. These microbes are efficient in enhancing N availability, P-solubilization and K-exchange in soil surface. They are environment friendly, and cost-effective (Kumar et al. 2017; Singh et al. 2011). The combined action of living microorganisms with soil or mineral substrate results in slow release of nutrients, and thus, enhance the rate of nutrient absorption by plants (Roychowdhury et al. 2017). It not only promotes plant growth but also increases soil fertility (Pal et al. 2015). Endophytic bacteria are capable of intensifying growth of non-leguminous crop improvement (Long et al. 2008; Sturz et al. 2000; Iniguez et al. 2004). Ngamau et al. (2014) described potential use of endophytic organisms as effective biofertilizer in the cultivation of banana. *Azospirillum brasiliens, Bacillus* sp., *Barkholderia* sp., *Citrobacter* sp., *Enterobacter* sp. are some known endophytic bacteria isolated from banana plants. Shen et al. (2019) introduced the efficiency of *Rhizobium larrymoorei, Bacillus aryabhattai, Pseudomonas granadensis* and *Bacillus fortis* as potent biofertilizer in rice cultivation.

6.6 Pathogen Antagonism

A large number of endophytic organisms exhibit broad spectrum antimicrobial activities. Therefore, another beneficial trait of endophytes is pathogen antagonism i.e., reducing the pathogen load in soil and thus, improves soil fertility. They suppress plant pathogen growth via combined action of metabolite release and abiotic changes. They release metabolites like antibiotics, HCN, phenazines, pyoleutorin, pyrrolnitrin, 2, 4-diacetylphloroglucinol etc. (Lugtenberg and Kamilova 2009). Endophytes improve host plant resistance against the pathogens by delaying or defending the entry of pathogen into the plant systems (Walters et al. 2007). Endophytes present in the host plant tend to stimulate a group of elicitors to trigger plant's induced or innate defence mechanism. In due course, they also release a wide range of enzymes like phenylalanine ammonialyase, peroxidase, beta-glucanase, chitinase, ascorbate peroxidase, polyphenol oxidase and superoxide dismutase etc. Workers reported that *Pseudomonas fluroscence* are capable of inducing resistance in olive and tomato plant by activating defence enzymes (Siddiqui and Shaukat 2003; Gómez-Lama Cabanás et al. 2014). Endophytes use of plant secondary metabolites like alkaloids, steroids, terpenoids, flavonoids, phenols, phenolic acids and peptides against pathogens. Several studies confirmed the production of secondary metabolites could successfully reduce pathogen load in potato and turmeric cultivation (Sturz and Kimpinski 2004; Sessitsch et al. 2004; Vinayarani and Prakash 2018). Gorai et al. (2021) reported the control of early blight of potato caused by *Alternaria alternata* using endophytic bacteria *Bacillus velezensis* SEB1.

6.7 Siderophore Production

Siderophore is a low molecular weight iron chelating compound secreted by different microorganisms. It has very high and specific affinity to iron. It primarily forms complex with iron (Fe^{2+}) molecules and increases the availability and mobility of iron to the plants. Siderophores are produced by a number of plant growth promoting rhizobacteria as well as endophytic microbes. In *Cicer areatinum* and *Pisum sativum* endophytic bacterial strains are potent to produce more than 65 siderophore production units (Maheswari et al. 2019). Loaces et al. (2011) studied the diversity of siderophore producing endophytic strains in rice where *Pantoea* sp. was predominant over *Burkholderia sp., Pseudomonas sp., Enterobactor sp.* and *Sphignomonas sp.*

6.8 Nutrient Cycling

Nutrient cycling is of utmost important with regard to soil fertility management. It involves a continuous transfer of energy and mass among biotic and abiotic systems. Though energy transfer is unidirectional, mass transfer occurs in a continuous cycle. The process begins with degradation of dead biomass into smaller and simpler fractions. Such processes are managed by catalysts like different enzymes to facilitate faster break down of complex macromolecules and gradual microbial propagation. With eventual release of water-soluble fractions, nutrient fractions get dissolved in the soil nutrient pool and become readily available for the plants and other heterotrophs. Many saprophytic fungi and bacteria play important role in the degradation process (Carroll 1988). Promputtha et al. (2010) showed that endophytes can regulate nutrient cycling process. During litter degradation, Nair and Padmavathy (2014) observed that the endophytic organisms trigger the activities of saprophytic organisms to quicken the process. It has been observed that release of enzymes like cellulase, hemi-cellulase etc. accelerates the decomposition process and nutrient release. He et al. (2012) reported that the presence of endophytic microbes in the host body expedites the release of enzymes and their activity. Chen et al. (2020) reported that association of *Epichloe* endophytes promoting growth, metabolic activity and nutrient uptake in perennial ryegrass (*Lolium perenne*) in a low fertile soil environment. Presence of endophytes showed distinct positive impact on organic carbon content, major nutrient like N, P, K content, micronutrients like manganese (Mn) concentration in both root and shoot portions.

6.9 Plant–Endophytic Interaction and Their Role in Plant Growth Promotion

6.10 Plant–Endophytic Interactions

Figure 6.2 shows a detailed mechanism of plant–endophyte interaction and various mechanisms involved. Complex endophytic microbial communities colonize within

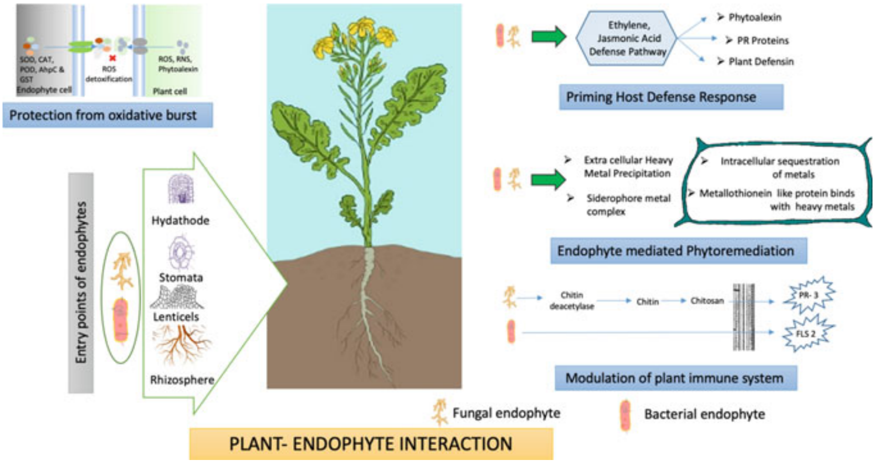

Fig. 6.2 Endophyte and plant interactions: mode of entry and mechanism of action. It is a pictorial representation showing multifaceted interaction of endophytes with host plants. (1) Endophytes prime the host plant's defensive responses against phytopathogens mediate intracellular responses and trigger ethylene/jasmonic acid transduction pathway. (2) Reactive oxygen species (ROS) and reactive nitrogen species (RNS), generated by the plant, are neutralized by the production of enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), alkyl hydroperoxide reductase (AhpC) and glutathione-S-transferase (GSTs) in endophytes. (3) Fungal endophytes modulate the plant's immune system by the production of chitin deacetylases, which deacetylate chitosan oligomers and, hence, prevent themselves from being recognized by chitin-specific receptors (PR-3) of the plants that recognize chitin oligomers. Perception of flagellin (FLS 2) from endophytes also differs from phytopathogens. (7) Endophytic microbes alleviate metal phytotoxicity via extracellular precipitation, intracellular accumulation, sequestration, or biotransformation of toxic metal ions to less toxic or non-toxic forms. Where, ET, ethylene; JA, jasmonic acid; ROS, reactive oxygen species; SOD, superoxide dismutases; CatA, catalases; POD, peroxidases; AhpC, alkyl hydroperoxide reductases; GSTs, glutathione-s-transferases; EF, effector protein; PR-3, chitin-specific receptors; FLS 2, flagellin; MT, metal transporters; IC, ion channels; CW, bacterial cell wall. (Adapted from source: Khare et al. 2018, Frontiers in Microbiology)

the plant tissues. They play major roles for the promotion of plant growth and development (Stone et al. 2000; Kobayashi and Palumbo 2000). Endophytes are ubiquitous in nature. Yet the mechanisms behind the endophytic microbe–plant interactions are hitherto unknown. They are in the primary stages of investigation and need more detailed works to understand these interactions (Strobel et al. 2004; Thomas and Soly 2009). On a simpler note, endophytic colonization means entry, growth and multiplication of endophytic organisms within host plants. Both the endophytic microbes and pathogen follow same mechanism during the entry within the host plant tissues (Gorai et al. 2020). But, one of the interesting points of endophytic microbial entry which markedly differs from pathogenic entry, host plant does not develop any resist power against the endophytes. Natural openings of plants like stomata, lenticels and hydathodes or any wounds caused by various pathogenic attack, soil particles or abiotic stresses generally use as routes for the entry of endophytes within host plant (Reinhold-Hurek and Hurek 1998). Behind these natural openings they are also eligible to take part in direct entry by releasing various plant cell wall degrading enzymes (Quadt-Hallmann et al. 1997; Reinhold-Hurek and Hurek 1998). In order to establish a successful endophytic colony, they need to cross few important steps like selection of the host, host-recognition and colonization on the targeted part and final entry into the host tissues respectively (Gorai et al. 2020). Plant secretes secondary metabolite in the form of root exudates. Some of these molecule act as signaling molecule which helps the chemotactic movements of endophytic microbes (Gorai et al. 2020). At first, they reach their destination site with the help of flagella and finally adhere with the surface using pilli (Zeidler et al. 2004). One of the excellent abilities of endophytic microbes is adaptive capabilities in highly diverse environment. Gorai et al. (2020) mentioned that with changes of different environmental factors like sudden changes in pH, carbon source, osmotic pressure, and oxygen availability of the surroundings, they can easily sustain and survive (Gorai et al. 2020). Endophytic microorganisms are very important to the plants, thus colonization of endophytes within the plant is very important for providing the benefits to the host plant. However, process of endophytic bacterial colonization within tissues of plant is quite complex and this includes several stages (Stępniewska and Kuźniar 2013).

Rhizosphere area around the plant root is inhabited by unique populations of microorganisms (Gorai et al. 2020). It was reported that plants release significant amounts of photosynthates or exudates like amino acids, organic acids, proteins etc. which act as signaling molecules to help the endophytic organisms in their chemotactic movements. Pattern and sites of colonization are specific for each endophytic strain (Zachow et al. 2015). When an endophytic strain attached to the host surface, it starts the penetration process for entering within the host tissues. Penetration process can occur through either active or passive ways. Penetration of endophytes occurs passively through the cracks of root tips or root regions caused by harmful organisms (Hardoim et al. 2008). On the other hand, active penetration occurs via attachment and proliferation of exogenous polysaccharides, lipopolysaccharides, structural components, quorum sensing that helps the endophytes to migrate and multiplication inside the tissues of plant (Böhm et al. 2007; Dörr et al. 1998; Duijff et al. 1997;

Suárez Moreno et al. 2010). After entering within the roots of host plant, endophytic bacteria can now migrate systematically to intercellular spaces of adjacent tissues by producing cell wall degrading enzymes like pectinase and cellulase (Compant et al. 2010) as well as to above ground tissues using flagella or through perforated plates of xylem tissues during transpiration (Compant et al. 2005; Sapers et al. 2005).

During the endophytic colonization process, microorganisms usually prefer the site of plant having thin surfaces such root hairs or apical part of root meristem. Reinhold-Hurek et al. (2006) described that endophyte *Azoarcus* sp. BH72 secretes lytic enzyme endoglucanase at entry site during colonization process. Suzuki et al. (2005) reported that endophyte *Streptomyces galbus* colonize in *Rhododendron* by using a non-specific wax degrading enzyme. Process of endophytic colonization depends upon several factors such as type of microbial strains, genotype of host plant, different biotic and abiotic factors, nutrients limitation etc. Till date, several researchers have indicated about the various routes of endophytic colonization inside the plants. For examples, endophyte *Ralstonia solanacearum* firstly attached to different parts of roots of host plant and enters by invasion of roots, then it migrates upwardly through xylem vessels (Alvarez et al. 2012). Another study stated that endophyte *Paraburkholderia phytofirmans* PsJN enters into the host cells through the layer exodermis of roots and crosses the cortical tissues, endodermal layers and finally moves upper part through xylem vessels (Compant et al. 2005, 2010). After the successful colonization within host tissues, endophytic microbes play multifaceted beneficial roles for the host plants. Endophytes can directly help the host plant by producing various plant growth promoting factors (Afzal et al. 2019) and by increasing nutrient uptake of the host plant (Vacheron et al. 2013). Indirectly, endophytic bacteria keep the host plant healthy by killing the pathogens and pests by nutrient restraint, by producing different kinds of antibiotics (Glick et al. 2007), siderophores (Lodewyckx et al. 2002), hydrolytic enzymes (Fan et al. 2002; Myo et al. 2019) and/or by inducing systemic resistance in plants (Kloepper and Ryu 2006).

6.11 Plant Growth Promotion

Diverse groups of beneficial microbial communities are found to inhabit in different locations of plant's body or its surface which are ranging from rhizosphere, phyllosphere to the endospheric regions (Feng et al. 2016). Most of these symbiotic organisms produce various substances which may promote plant growth and development. In endophyte–plant symbiotic relationships, both the partners are benefitted in which plants supply nutrients and provide shelter to the endophytes while indirectly endophytic organisms help the plants by increasing resistance against pathogen and herbivores (Bamisile et al. 2018). In addition, endophytes also increase the plant growth and development by increasing stress tolerance and nutrient uptake like nitrogen, iron and phosphorus by the plants especially in nutrient deficient conditions (Ji et al. 2014; Martinez-Klimova et al. 2017). It has been also reported earlier those different kinds of phytohormone such as auxin, gibberellin, cytokinine etc. are produced by some endophytes (Gohain et al. 2015; Pimentel et al. 2011). Beside the phytohormone production, some of them possess other plant growth properties like synthesis of 1-aminocyclopropane-1-carboxylate deaminase (ACCD), production of siderophores, solubilization of phosphates and production of antimicrobial metabolites etc. (Serepa-Dlamini 2020).

There are several endophytic bacteria play significant beneficial roles for the host plant growth promotion by various ways (Table 6.1) like production of phytohormones like Indole acetic acid (Gao and Tao 2012); ACC deaminase, (Karthikeyan et al. 2012; Ali et al. 2014; Glick, 2014), phosphate (P) solubilization, nitrogen fixation etc. For examples, endophytic bacteria *Bacillus subtilis* CNE 215 and *Bacillus lichenoformes* CRE1 isolated from chickpea were able to solubilize P and produce ammonia respectively (Saini et al. 2015). On the other hand, Egamberdieva et al. (2017) described that endophytic bacteria *Bacillus subtilis* NNU4 and *Archomobacter xylosoxidans* NNU2 isolated from chickpea show PGP properties like P solubilization, IAA production, siderophore production and HCN production. In addition, some of plant growth promoting endophytes showed excellent antagonistic activity against phytopathogens (Table 6.2).

6.12 Identification of Endophytes and Their Utilization Against Persistent Organic Pollutants

From last few decades of the twentieth century, impact of persistent organic pollutants became a matter of concern. Persistent organic pollutants (POPs) are toxic organic compounds present in the environment, used for anthropogenic purposes, transported by means of air or water. Transboundary movement of persistent organic pollutants makes them more dangerous than any other pollutant. During Stockholm convention, 1972, the “Dirty Dozen” term was coined to twelve POPs used extensively for industrial purpose. Their presence and magnification disrupt proper functioning of the ecosystem. Those synthetically produced toxic chemical substances are aldrin, endrin, dichlorodiphenyltrichloroethane (DDT), polychlorinated dibenzofurans (PCBs), hexachlorocyclohexane, mirex etc. They persist for a long time in the environment, hence termed as persistent organic pollutants (POPs) (Boudh et al. 2019). According to Oonnittan and Sillanpää (2020), POPs show salient features like acute toxicity, biomagnification and long-range transport. Most of the POPs are the outcome of different anthropogenic activities and the waste thus generated. POPs are resistant to any form of physical, chemical or photolytic degradation. Direct exposures of such POPs have drastic effect on living organism. In mammals, they behave as xenoestrogens, thus causes endocrinal malfunction, loss of body weight, ovarian cancer, congenital disease, low sperm count, damage of central nervous system etc. In other living organism, disruption in sexual reproduction, retarded growth, mutation

Table 6.1 List of various endophytic isolates and their plant growth promoting attributes

Host plants	Endophytes isolates	P solubilization	IAA production	Siderophore activity	N_2 fixation	Ammonia production	References
Oryza sativa	*Paenibacillus kribbensis*		+	+			Puri et al. (2018)
	Klebsiella pneumonia	+	+	+			
	Pseudomonas putida	+	+	+			
	Bacillus amyloliquefaciens	+	+	+	+		Verma et al. (2015)
Maize	*Pseudomonas putida*		+	+			Sandhya et al. (2017)
	Pseudomonas lini		+				
	Pseudomonas thivervalensis		+				
	Pseudomonas aeruginosa		+	+			
	Pseudomonas montelli		+	+			
Wheat	*Acinetobacter guillouiae*	+	+				Rana et al. (2020)
	Achromobacter xylosoxidans	+	+		+		Jha and Kumar (2009)
	Bacillus amyloliquefaciens	+	+	+	+		Verma et al. (2015)
	Acinetobacter lwoffii	+	+		+		Verma et al. (2015)
Chickpea	*Archomobacter xylosoxidans* NNU2	+	+	+			Egamberdieva et al. (2017)

(continued)

Table 6.1 (continued)

Host plants	Endophytes isolates	P solubilization	IAA production	Siderophore activity	N_2 fixation	Ammonia production	References
	Bacillus subtilis NNU4	+	+	+			
	Bacillus subtilis CNE215	+				+	Saini et al. (2015)
	Bacillus licheniformis CRE1	+				+	
	Bacillus licheniformis	+					
	Burkholderia cepacia		+				Shahid and Khan (2018)
Soybean	*Pseudomonas aeruginosa*	+	+	+			Kumawat et al. (2019)
	Bacillus subtilis			+			Singh et al. (2017)
	Bacillus megaterium	+	+	+	+		Subramanian et al. (2015)
	Methylobacterium oryzae	+	+	+	+		
Peanut	*Enterobacter asburiae*		+				Wang et al. (2013)
	Pantoea agglomerans		+				
	Sphingomonas azotifigens		+				
	Bacillus megaterium		+				

(continued)

Table 6.1 (continued)

Host plants	Endophytes isolates	P solubilization	IAA production	Siderophore activity	N_2 fixation	Ammonia production	References
	Bacillus arbutinivorans		+				
Faba bean	*Pseudomonas yamanorum* B12		+	+			Bahroun et al. (2018)
	Pseudomonas fluorescens B8P		+	+			
	Rahnella aquatilis B16C		+	+			

“ + ” indicates positive result and “–”indicates negative result

Table 6.2 List of N_2 fixing endophytic isolates and respective host plants

Endophytic organism	Host plant	Reference
Gluconacetobacter diazotrophicus	Sugarcane	James and Olivares (1998)
Azospirillum sp	Pineapple	Weber (1999)
Burkholderia sp	Pineapple	Weber (1999)
Herbaspirillum	Banana	Weber (1999)
Burkholderia sp	Rice	Baldani et al. (2000)
Microbacterium sp.	Sugarcane	Lin et al. (2012)
Paenibacillus sp	Poplar	Scherling et al. (2009)
Klebsiella oxytoca	Sugarcane	Govindarajan et al. (2007)
Klebsiella pneumoniae	Sugarcane	Govindarajan et al. (2007)
Rhizobium leguminosarum bv. trifolii	Rice	Yanni et al. (1997)
Frankia sp	*Alnus glutinosa*	Li et al. (1996)
Glomus fasciculatus	*Hippophaë rhamnoides*	Gardner et al. (1984)

etc. can directly be linked with the adverse effect of these persistent organic pollutants. Researchers have reported POPs multidirectional effect on basic agronomy like soil health, accumulation and contamination of food, genetic changes of soil microorganisms, disruption of normal soil biodiversity (Saha et al. 2017; Guo et al. 2012).

Because, POPs are resistant to other forms of physical, chemical or photolytic degradation methods, therefore, researchers have been concentrating on biological remediation of these pollutants. However, owing to their recalcitrant nature, POP bioavailability is almost negligible. Presence of excessive amount of POP in the environment, hinders plant growth and development, thus limiting phytoremediation process (Doty 2008). Therefore, even phytoremediation needs a co-metabolism assistant for this group of pollutants. In this part, endophytes and plant act synergistically. Here, metabolome i.e., mixed community of the host plant initiate oxidation of organic compounds present in the substrates and provide carbon and energy source for the microbes (Feng et al. 2017; Gerhardt et al. 2009; Pandey et al. 2009). Table 6.3 enlists groups of endophytic bacteria with their respective host plants that degrade persistent organic pollutants. The presence of endophytic microorganisms can be beneficial in two different ways. One is indirectly by supporting the plant growth and other is by direct degradation. Endophytes support plant growth and inhibit persistent organic pollutants through phytoremediation techniques like phytoextraction i.e., pollutants are absorbed and accumulate inside the plant tissue (Ali et al. 2013); phytovolatilization i.e., organic pollutants or contaminants are absorbed and released

Table 6.3 Endophytes and their host plant association for persistent organic pollutant degradation

Pollutants	Endophyte	Plant	References
Diesel	*Pseudomonas* sp. strain ITRI53 *Rhodococcus* sp. strain ITRH43	Ryegrass	Andria et al. (2009)
	Enterobacter ludwigii	Italian ryegrass, birds foot trefoil and alfalfa	Yousaf et al. (2011)
Hydrocarbon	*Bacillus* sp. *Pseudomonas* sp.	*Azadirachta indica*	Singh and Padmavathy (2015)
	Pseudomonas sp. strain ITRI53, *Pseudomonas* sp. strain MixRI75	Italian ryegrass (L. multiflorum var. Taurus)	Afzal et al. (2011), Afzal et al. (2012)
	Enterobacter ludwigii strains	*Lolium multiflorum, Lotus corniculatus*, and *Medicago sativa*	Yousaf et al. (2011)
	Pantoea sp. strain ITSI10, *Pseudomonas* sp. strain ITRI15	Italian rye grass (*L. multiflorum* var. *Taurus*) and birdsfoot trefoil (*L. corniculatus* var. *Leo)*	Yousaf et al. (2010a, b)
TCE	*Pseudomonas putida* W619-TCE	Poplar	Weyens et al. (2010a)
	Burkholderia cepacia VM1468 possessing (a) the pTOM-Bu61 plasmid	Yellow lupine	Weyens et al. (2010b)
	Enterobacter sp. strain 638	Poplar	Taghavi et al. (2011)
	Enterobacter sp. strain PDN3	Poplar	Kang et al. (2012)
Toluene	*Burkholderia cepacia*	*Zea mays* *Triticum aestivum*	Wang et al. (2010)
Chlorobenzoic acids	*Pseudomonas aeruginosa* R75; *Pseudomonas savastanoi* CB35	*Elymus dauricus*	Siciliano et al. (1998)
Pyrene	*Staphylococcus* sp. BJ106	*Alopecurus aequalis*	Sun et al. (2014)

(continued)

Table 6.3 (continued)

Pollutants	Endophyte	Plant	References
	Enterobacter sp. 12J1	Wheat (*Triticum* sp.) maize (*Z. mays*)	Sheng et al. (2008a, b)
2,4-Dichlorophenoxyacetic Acid	*Pseudomonas putida* VM1450	*Pisum sativum*	Germaine et al. (2006)
Catechol and phenol	*Achromobacter xylosoxidans*	*Ipomoea aquatica, Chrysopogon zizanioides, Phragmites australis*	Ho et al. (2009)
Naphthalene	*Pseudomonas putida*	*Pisum sativum*	Germaine et al. (2009)
2,4,6-Trinitrotoluene, hexahydro-1,3, 5-trinitro-1,3,5-triazine, octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine	*Methylobacterium populi* BJ001	*Populus alba*	Van Aken et al. (2004)
n-Hexadecane, PAH	*Pseudomonas* spp., *Brevundimonas* sp, *Pseudomonas rhodesiae*	*Medicago sativa, Puccinellia nuttaalliana, Festuca altaica, Lolium perenne, Thinopyrum ponticum*	Phillips et al. (2008)
Hexachlorocyclohexane	*Rhodococcus erythropolis* ET54b, *Sphingomonas* sp. D4	Cytisusstriatus	Becerra-Castro et al. (2013)
Fenpropathrin	*Klebsiella terrigena* E42; *Pseudomonas* sp. E46	*Spirodela polyrhiza*	Xu et al. (2015)

as volatile in atmosphere (Ferro et al. 2013), and/or transformation of complex toxic contaminants into simpler or non-toxic forms (Wiszniewska et al. 2016).

The intercellular spaces of plant tissue are enriched with sugars, nutrients, amino acids etc., therefore, it provides a safe environment for the endophytes to grow and populate (Bacon and Hinton, 2007). Studies have shown that endophytes readily use secondary metabolites like terpenes, flavonoids, salicylic acids and lignin derivatives synthesized inside host plant body and release a cluster of POP degrading enzymes (Feng et al. 2017; Jha et al. 2015). All these secondary metabolites serve either as an analogue of the POPs owing to their structural similarities or act as an intermediate, thus stimulate endophytic degradation (Jha et al. 2015). For example, metabolite like salicylate is involved in activating acquired systemic resistance in the host plant. It also tends to stimulate enzymes for naphthalene degradation (Singer et al. 2003). These metabolites provide carbon and energy source for microbial proliferation. And, these endophytic microbes receive pollutant degrading genes through horizontal gene transfer like pTOM-Bu61 plasmid (representing Toluene and TCE degradation) inside the host and can modulate the gene expression (Thijs et al. 2016; Taghavi et al. 2005). They release diverse array of catabolic enzymes like cytochrome P450 monooxygenase and co-enzymes like NAD/NADPH for metabolic degradation and detoxification of POPs (Liu et al. 2014; Zhu et al. 2016; Doty 2008). According to Siciliano et al. (2001), those endophytes, isolated from plants grown in hydrocarbon contaminated soil, are mostly capable of degrading hydrocarbons. It has been found that population of hydrocarbon degrading bacteria is much higher inside host plant tissue especially in root system, as compared to their rhizospheric soil. Researchers were able to isolate a number of potent crude oil degrading bacterial strains from the plants grown in crude oil contaminated soil (Yousaf et al. 2010a, b; Phillips et al. 2008). Germine et al. (2009) reported endophytic bacterial strains, isolated from poplar trees, were capable of degrading herbicide. Apart from this, several bacterial strains, isolated from the poplar trees, were capable of activating metabolic degradation of aromatic hydrocarbons like benzene, toluene, xylene etc. (Taghavi et al. 2011; Moore et al. 2006). Similarly, endophytic strains isolated from different wetland plants were capable of detoxifying a group of pesticides and organic hydrocarbons (Chen et al. 2012; Zhang et al. 2013).

The first in vitro study of POP degradation by endophyte was performed by Germaine et al. (2006). Here, the researcher inoculated *Pisum sativum* with endophytic bacterial strain *Pseudomonas putida* VM1450. It showed successful degradation of 2, 4-dichlorophenoxy acetic acid. Later on, Germaine et al. (2009) reported that another endophytic bacterial strain *Pseudomonas putida* VM1441 efficiently degraded naphthalene compounds from the soil surface. According to Andria et al. (2009) presence of organic pollutant in soil directly affects the colonization of endophyte in endo-sphere and POP degrading gene expression. Becerra-Castro et al. (2013) successfully exhibited cohort application of plant and endophytes to remediate hexachlorocyclohexane contaminated soil. They remarkably put an exemplary use of endophytes via consortium of *Rhodococcus erythropolis* ET54b and *Sphingomonas sp.* D4 inoculated inside the plant *Cytisuss triatus* grown in hexachlorocyclohexane contaminated soil. The consortium was successful in accelerating degradation of target pollutant.

Endophytes are getting attention for last few decades in the field of remediation. They show plant growth promoting activities, genetic diversity and stress tolerance. The synergistic action of host plant and endophytic bacteria for remediation of POPs is a very effective and sustainable approach. Selected endophytes can be genetically engineered for increased efficiency as a sole endophytes or cohort design for co-metabolism under different phytoremediation techniques. These models are now very promising and show an effective lineage, not only for the remediation of POPs but also for food safety.

6.13 Effect of Endophytes Against Heavy Metal Contaminated Soil

Soil pollution due to heavy metal contamination is a serious environmental hazard. Presence of heavy metals shows adverse effect on the trophic levels. It contaminates soil, surface water, agricultural crops, microbial ecosystem (Kidd et al. 2012). Presence of cadmium, lead, copper, chromium, and nickel above the permissible limit in the environment, exert harmful impact on living organism (Hemambika et al. 2011). Heavy metal toxicity is associated with long range contamination, non-degradation and bioaccumulation. Heavy metal toxicity retards plant growth by suppressing carbohydrate metabolism and photosynthesis (Becerril et al. 1988). It also affects the process of respiration (Keck 1978). The conventional methods to remediate heavy metal contamination are metal stabilization using soil amendments, soil washing with acid or chelators, reverse osmosis, evaporation, precipitation, electrochemical treatment, ion exchange and sorption (Kadirvelu et al. 2002; Luo et al. 2010). But these conventional methods are, chemical-dependent, exorbitant, also energy-expensive. They also contribute to generation of toxic sludge (Hemambika et al. 2011). Under such perspectives, bioremediation techniques are highly preferred over any other existing chemical technique. Endophyte mediated phytoremediation is an alternative approach for heavy metal removal from contaminated lands (Burges et al. 2016). Here, endophytes reduce the metal stress through reduced phytotoxicity and improved metabolic capabilities as growth promoter (GP) (Feng et al. 2017). Examples of potential endophytes are usually members of the genera *Pseudomonas* (Feng et al. 2017); *Rahnella* (He et al. 2019), *Bacillus* (Gorai et al. 2021) among all other microbes. These workers highlighted successful association of endophytes and plants for promising biological control methods. Table 6.4 elucidates endophytic association with their host plants actively involved in heavy metal remediation and potential mechanism involved.

Ma et al. (2016) described that endophyte plays an active role in metal detoxification via direct or indirect plant growth promotion and altered metal uptake mechanism. Govarthanan et al. (2016) reported a root endophytic bacteria *Paenibacillus* sp from *Tridax procumbens* were significantly able to remove Cu, Pb, As and Zn when incubated in vitro. Any change in temperature, pH and incubation period shows direct effect on the amount of heavy metal removal. The study recorded element removal percentage of up to 61.4% Cu, 37.3% As, 54.5% Zn and 37.5% Pb. Metal resistant endophytes promote plant growth via nitrogen fixation, production of siderophores, other phytohormones, solubilization of major nutrients viz. N, P, K, utilizing single N source in the form of 1-aminocyclopropane-1-carboxylic acid and biotransformation of N, P, K (Rajkumar et al. 2009).

Table 6.4 List of endophytes used for heavy metal remediation in soil

Host plant	Endophyte	Metal remediated	Mechanism	Reference
Brassica napus	*Pseudomonas fluorescens* G10, *Microbacterium* G16	Pb, Cd, Zn, Cu and Ni	Increased solubility, uptake of Pb	Sheng et al. (2008a, b)
Pteris vittata *Pteris multifida*	Proteobacteria and actinobacteria	As	As-V reduction, As-III oxidation	Zhu et al. (2014)
Alnus firma *Brassica napus*	*Bacillus* sp. MN3-4	Pb, Cd, Zn, Ni	Bio-removal, phytotoxicity reduction	Shin et al. (2012)
Alnus firma	*Bacillus thuringiensis* GDB-1	As, Cu, Cd, Ni, Zn, Pb	Bio-removal, increased bioaccumulation	Babu et al. (2011)
Solanum nigrum	*Serratia marcescens* LKR01, *Arthrobacter* sp. LKS02, *Flavobacterium* sp. LKS03, *Chryseobacterium* sp. LKS04	Pb, Zn, Cu, Cd	Decreased phytotoxicity, increased metal accumulation	Luo et al. (2011)
Solanum nigrum	*Pseudomonas* sp. Lk9	Cr, Cu, Cd, Zn	Improved heavy metal availability	Chen et al. (2014)
Lupinus luteus	*Burkholderia cepacia* L.S.2.4, *Herbaspirillum seropedicae* LMG2284	Pb, Cd, Co, Cu, Ni	Bio-removal, reduction of phytotoxicity	Lodewyckx et al. (2001)
Lycopersicon esculentum	*Methylobacterium oryzae* CBMB20, *Burkholderia* sp. CBMB40	Cd, Ni	Biosorption, removal of toxicity	Madhaiyan et al. (2007)
Miscanthus sinensis	*Pseudomonas koreensis* AGB-1	As, Cd, Pb, Zn	Increased metal uptake	Babu et al. (2015)
Sorghum bicolor	*Bacillus* sp. SLS18	Cd, Mn	Improved biomass production and total metal uptake	Luo et al. (2012)
Pelargonium graveolens	*Pseudomonas monteilii* PsF84, *Pseudomonas plecoglossicida* PsF610	Cr	Increased biomass, help Cr(IV) sequester	Dharni et al. (2014)

6.14 Phytoavailability

Transfer of heavy metals from soil to plant is dependent on bioavailability of the metals in the soil (Glick 2010). Other limiting factors such as redox potential, organic matter contents, soil particle size, nutrient dynamics, pH of soil etc. regulate metal availability in soil (Lebeau et al. 2008). Endophytes tend to reduce toxicity of pollutants inside host plant through several interwinding biochemical pathways. Studies showed that isolated heavy metal resistant endophytes promote plant growth and assist in phytoremediation of contaminated soil (Chen et al. 2014). Rajkumar et al. 2009 demonstrated that, by the secretion of low molecular weight organic acids and metal specific ligands, endophytic bacteria can increase metal and mineral solubilization. Production of organic acids by the root exudates alters soil pH. It plays a vital role in eventual nutrient solubilization and uptake. Endophytic bacteria can produce a wide range of chemicals like fatty acids, glycol lipids, mycolic acids, lipopeptides, polysaccharide protein complex, phospholipid etc. (Bannat et al. 2010) which can fasten the rate of phytoremediation as they increase the phytoavailability of the metals (Bacon and Hilton 2011). Rajkumar et al. 2009 stated that several biosurfactants are produced and released by groups of endophytic bacteria. These biosurfactants interact and form organo-metallic complex with insoluble metals. These metals are then gradually desorbed from the soil matrix. This process alters mobility and phytoavailability of metals. Hence, it accelerates the phytoremediation, especially phytoextraction of heavy metals. Application of such bacteria in soil can be beneficial from the aspect of heavy metal remediation. Babu et al. (2013) observed that endophytic *Bacillus thuringiensis* GDB-1 inoculation in *Alnus firma* removal up to 77% of Pb, 64% Zn, 34% As, 9% Cd, 8% Cu, and 8% Ni in metal amended mine tailing extract. The inoculum also facilitated P solubilization, ACC deaminase, Indole acetic acid production and activation, siderophore production. This resulted in 141% increase in root length, 144% increase in shoot height and 170% of dry biomass; thus, promoting overall crop health.

6.15 Hyper Accumulation and Biosorption

Other efficient bioremediation techniques are biosorption and hyper accumulation of pollutants. Hyperaccumulator plants effectively remove metals from contaminated surfaces. They are able to absorb selective metals even when their presence is below 1% in the substrate. Baker (2000) defined that if a plant is able to absorb 1% of Zn, 0.1% of nickel, cobalt, copper, lead and 0.01% of cadmium from the substrate, then that plant can be termed as a hyper accumulator. Hyperaccumulator plants reduce toxicity by reducing intracellular M-Cysteine and M-Methionine concentration (M representing metal), at times interfere with plant metabolism. For example, in selenium hyperaccumulator plant *Astagalus bisulcatus*, it has been observed that inoculation of genetically engineered *E. coli* increased its Se tolerance and decreased non-specific binding of Se to the proteins (Terry et al. 2000). Hyperaccumulators tend to uptake exceedingly high amount of one or more metals from the growing substrates and translocate, eventually accumulate in the shoot. Endophytes present in the hyperaccumulators tend to modulate the process of phytoextraction of heavy metals from the contaminated soil (Chen et al. 2014). In this study by Chen et al. (2014), bacterial endophyte *Pseudomonas* sp. Lk9 was found to increase the efficiency of *Solanum* *nigrum* L. for Cd accumulation up to 64% in the dry shoot. Similarly, another report suggested that *Serrartia* sp LRE07 is able to absorb more than 60% of cadmium and 35% of zinc in a mono metallic culture solution (Luo et al. 2011). In active biosorption, metal is slowly accumulated in intracellular space crossing the cell membrane. These metals are sequestered and accumulated inside the host body (Ma et al. 2011). On the contrary, in passive biosorption, entry of metal ions into a cell occurs without metabolite interactions (Vijayaraghavan and Yun 2008). Here, metals react with different functional groups on cell surface like hydroxyl, carbonyl, amine, phosphonate, sulfhydryl (Ma et al, 2011) and form complex structures, thus become unavailable.

According to Shin et al. (2012), inoculation of heavy metal resistant endophyte *Bacillus* sp. MN3-4 contributes to increase in the phytoremediation efficiency through intracellular Pb accumulation. Another report says *Bacillus thuringiensis* GDB-1 isolated from the root of *Pinus sylvestris* enhances the metal accumulation efficiency of *Alnus firma* (Babu et al. 2013). Sheng et al. (2008a, b) isolated and identified two endophytic bacterial strains capable of promoting Pb accumulation in *Brassica napus*. According to the report of Ma et al. (2015), the enhanced accumulation of cadmium (Cd), zinc (Zn) and lead (Pb) in plants was found to be controlled by the presence of heavy metal resistant endophytic bacteria *Bacillus* sp. It is evident from the past and recent studies that the endophytes play remarkable role in metal accumulation process supporting the phytoremediation methods and finally push to an improved and efficient heavy metal remediation technique.

6.16 Toxicity Reduction

Phytotoxicity is one of the critical factors for successful phytoremediation. Association of bacteria and plant plays a vital role in balancing the phytoremediation techniques and reducing metal toxicity. In this scenario, endophytic bacteria have some excellent host plant cohort backup that either leads to toxicity reduction or increased plant tolerance (Rajkumar et al. 2009). Recent studies revealed that mechanisms like extracellular precipitation (Babu et al. 2015), biotransformation of metal ions to non-toxic or less toxic forms (Zhu et al. 2014), intracellular accumulation (Shin et al. 2012) make those endophytes more relevant to metal remediation. Mindlin et al. (2002) said that, microorganisms develop heavy metal and antibiotic resistance, if they are synchronized with the ability to perform horizontal gene transfer (HGT). Recent studies revealed that endophytes tend to modulate activities of plant antioxidant enzymes like peroxidase (PO_x), catalase (CAT), super oxide dismutase (SOD), ascorbate peroxidase, as well as lipid peroxidation. These ROS activated enzymes play important role in plant defence mechanism. It has been reported that some endophytes promote DNA methylation in the form of metal resistance or detoxification process. Brown et al. (2003) and Cursino et al. (2000) stated that endophytic bacteria express different genes to convert mercury into non-toxic form. Studies showed genetically engineered endophyte-plant symbionts tend to improve phytoremediation efficiency of hyperaccumulator plants. Qiu et al. (2014) reported that introduction of gcsgs i.e., bifunctional glutathione-synthetase gene into *Enterobacter* sp. present as an endophyte symbiont in *Brassica juncea* increases plant's efficacy to remediate Cd and Pb from the soil.

6.17 Conclusion and Future Prospects

Microbial land remediation holds tremendous future potential. Endophytic microbes show traits that influence their exhibit for plant growth promoting activities. The plant–endophyte association also shows different mechanisms for pollutant removal and management. It is largely governed by the pollutant origin, concentration and fate. In the present scenario, besides the conventional methods of land remediation, application of endophytes is very promising because of its feasibility, non-harmful nature and cost-effectiveness. There is a vast field of endophytic population yet to be explored. Utilization of endophytic biofertilizer not only reduces the amount of chemical fertilizer, but also improves soil quality and agricultural productivity.

Phytoremediation techniques assisted by endophytes are environment friendly and sustainable in nature. Owing to its compatible nature, this symbiotic association is gaining popularity in the scientific community. Several researchers have been working on in order to understand the mechanism behind a successful endophyte–plant combination. At the same time, genetically engineered bacterial introduction can improve the efficacy of transgenic plants with regard to metal remediation and faster POP degradation. Recent development in the field of omics has enabled to maximize such understanding, harness beneficial traits and improve quality. However, challenges remain due to the diversity of endophytes. Screening for the most efficient and competent genera is cumbersome and tedious. Moreover, their population cannot be limited to *in-vitro* conditions. Main challenge remains with fact that how they respond in natural environment. Permission for introduction of transgenic plants and genetically engineered endophyte for field study is a matter of concern, however, it opens up the door to explore ideas and limitations of such studies. Researchers in the near future can work on developing field realistic variables for endophyte–plant partnership to execute and apply. The mechanisms also need an in-depth investigation, so that endophyte–plant potential can be realized, applied for an improved soil environment.

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