Plant Growth Promoting Rhizobacteria (PGPR)-Assisted Phytoremediation of Contaminated Soils

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Abstract

The unprecedented augment in the concentration of diverse contaminants in the environment has grim impacts on the ecological balance of our ecosystem. Soil, being a major sink, holds up the maximum load of environmental contaminants. Heavy metals and petroleum hydrocarbons are the most common pollutants present in the soil. Plant growth promoting rhizobacteria (PGPR)-assisted phytoremediation is one of the competent methods for removal of pollutants, which has proven its efficiency in reclamation of contaminated soils. PGPR are bacteria that reside in close association with plant roots and facilitate growth and development of plants by influencing their physiological and metabolic activity. Rhizobacteria are known to amplify the effectiveness of phytoremediation by modulating contaminants transportability and accessibility to the plant via acidification, chelating agents, solubilization of phosphate, and redox changes. This chapter aims to explore the role of rhizomicrobiome in the phytoremediation of heavy metal- and petroleum-contaminated soils, the successful commercialization of PGPR, and the insights into the recent advances in PGPR research.

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A. K. Singh et al. (eds.), *Bacterial Endophytes for Sustainable Agriculture and Environmental Management*, https://doi.org/10.1007/978-981-16-4497-9_4

Keywords

Abiotic stress · Heavy metals · Plant growth promoting rhizobacteria · Petroleum · Phytoremediation

4.1 Introduction

The persistence of heavy metals and petroleum hydrocarbons is one of the serious environmental concerns demanding attention of researchers throughout the world. The plants which can accumulate metal or tolerate metals stress have been used for phytoremediation in metal-polluted soils. Phytoremediation is the use of green plants for removal of both inorganic and organic pollutants from air, water, and soil (McCutcheon and Jorgensen 2008). The name phytoremediation is obtained from the Greek word *phyto*, i.e. plant, and the Latin word *remedium*, i.e. cure. Its usage in scientific literature for the first time has been traced to paper written by Cunningham and Berti in 1993 (Novo et al. 2018). Speight (2020) rightly states that the description of phytoremediation can be redefined to include the utilization of green plants and the allied microorganisms, along with appropriate soil amendments and agronomic methods to either contain, eliminate, or render lethal environmental pollutants undamaging.

Many phytoremediation projects have been carried out worldwide to mitigate contaminants like pesticides, metals, crude oil, and explosives. The phytoremediation practice utilizes specific plants with roots that can absorb contaminants over time. Many plants such as hemp, mustard, pigweed, and alpine pennycress have the ability to hyper-accumulate contaminants from polluted sites (Speight 2020). Phytoremediation has immense potential as a natural, low cost, in situ approach driven by solar energy to moderately treat polluted sites spreading over large areas. However, the plants have to be cautiously selected depending on the type contaminants (Schwitzguébel 2017). The added advantage of phytoremediation over other technologies is that various kinds of nutrients, organic materials, and oxygen are supplemented to the soil through metabolic processes of plant and microbes. This enhances the value and consistency of remediated sites, stabilizes soil, and checks wind and water erosion as well (Schwitzguébel 2017). It can be concluded that this is an esthetically pleasing technology which helps in reducing erosion, increasing biodiversity, and fixing atmospheric carbon dioxide (Cunningham and Berti 1993). Phytoremediation techniques should, however, avoid the use of food crops for cleanup. The use of ornamental plants reduces the possibility of metals passing into the food chain along with the extra benefit of improving the environment's esthetics and producing extra earnings, together with added job opportunities from cut-flower trading and/or travel industry (Nakbanpote et al. 2016).

A survey of recent literature brings to light the numerous advantages of plant growth–promoting rhizobacteria (PGPR) to environment, agriculture, landscaping,

etc. PGPRs are also known to have the potential to enhance phytoremediation processes (Jing et al. 2007). The application of PGPR for improving metal tolerance of plants is increasingly being utilized these days. Plant roots have restricted capacity to absorb metals from soil, chiefly because metals are not very soluble in the soil solution. Phytoremediation of metal-contaminated soil depends on the rate of uptake of the metal by plants. Also the phytoavailability of metal depends on soil properties and the associated PGPRs. In this chapter we attempt to outline the benefits of PGPR in phytoremediation by enhancing mitigation of petroleum and heavy metal pollutants in the environment. It also focuses on the recent developments that have taken place in decoding the genetics and genomics of PGPR.

4.2 Rhizomicrobiome

The evolution and colonization of terrestrial plants has apparently been antedated by microbiome relationships (Berg et al. 2014). A land plant does not exist individually in nature; rather a consortium of bacteria is generally associated with plants and constitutes a phytomicrobiome (Smith et al. 2017). The phytomicrobiome is of crucial importance in determining the existence of plants, or rather the holobiont. Certain plant–microbe associations (e.g., *Cycas*, *Azolla*) are so inseparable that they are known to be symbiotic ubiquitously. Members of phytomicrobiome even ascertain the survival efficiency of plants under conditions of stress. Though microbes are associated with all the major plant structures, microbes associated with rhizosphere constituting a rhizomicrobiome are most elaborated and populous (Backer et al. 2018). Venturi and Keel (2016) define the rhizosphere as a complex zone around the roots of plants with a large population of microorganisms including bacteria, protists, invertebrates, nematodes, and fungi. Bacterial communities in the rhizosphere are called the rhizobacteria. Rhizosphere has much greater amount of bacteria than the bulk soil due to the presence of root exudates such as amino acids and sugars, called rhizodeposition, which provides energy and nutrients for development (Novo et al. 2018). Rhizodeposition may comprise nearly 15% of plant total nitrogen and 10% of photosynthetically fixed carbon (Venturi and Keel 2016).

The complex composition and well-guarded regulation of rhizomicrobiome has assisted land plants against various stresses in due course of evolution (Lundberg et al. 2012; Smith et al. 2015; Zhang et al. 2017). Plant roots secrete exudates of various compositions and signal compounds in order to recruit preferential microbes (Chaparro et al. 2012; Smith et al. 2017). Apart from the considerably controlled regulation by plants, microbes do exhibit facets of self regulation (by virtue of quorum sensing in favorable conditions) depending upon the ecological conditions, which are reciprocated by plants as a mechanism for further regulation (Leach et al. 2017; Ortiz-Castro et al. 2009). This degree of regulation is directly dependent upon the affinity between roots and microbes, i.e., it is much higher for endophytes and rhizospheric bacteria (Backer et al. 2018). The co-evolution of plants and microbes has facilitated certain free-living bacteria such as PGPRs to become endophytes (Bulgarelli et al. 2013). Members of the rhizomicrobiome play pivotal roles in

enhancement of plant growth by aiding in nutrient acquisition and assimilation, improving soil texture and modulating the secretion of extracellular molecules that also influence plant stress responses (Backer et al. 2018).

4.2.1 Plant Growth Promoting Rhizobacteria

Rhizobacteria can be characterized as neutral, beneficial, and harmful depending on their outcome on plant growth and development (Huang et al. 2014). Many of them have been aptly called Plant Growth Promoting Rhizobacteria (PGPRs) (Kloepper and Schroth 1978). A bacterium is called a PGPR when it can induce a positive impact on the plant after inoculation. Around, 2–5% of rhizosphere bacteria qualify as PGPR (Goswami et al. 2016). Most PGPRs belong to genera *Acinetobacter, Agrobacterium, Arthobacter, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Frankia, Pseudomonads, Rhizobium, Serratia,* and *Thiobacillus* (Glick 1995; Vessey 2003). They are known to aid plant growth by assisting in acquisition of minerals such as nitrogen and phosphorus. They also promote plant growth and development by controlling the plant hormonal balance, eliciting immune responses, mobilizing nutrients, and protecting the plant against pathogens (Glick 2012). The plant-beneficial rhizobacteria can also help to lessen the reliance on harmful fertilizers (Ahemad and Kibret 2014).

Somers et al. (2004) have categorized PGPR according to their activities as:

1. **Biofertilizers**: ones expanding the accessibility of nutrients to the plant.
2. **Phytostimulators**: ones that promote plant growth and development by releasing plant growth regulators.
3. **Rhizoremediators**: ones that break down organic contaminants.
4. **Biopesticides**: ones that control diseases by producing antibiotics and antifungal metabolites.

Thus we can say that PGPR can directly and indirectly influence plant growth. The direct mechanism involves the synthesis and modulation of phytohormones or the acceleration of resource accumulation including nitrogen fixation, phosphorus bioavailability, and iron sequestration. Indirect mechanism includes biocontrol which is the reduction of the effects of phytopathogens by production of antibiotics and antifungal compounds (Glick 2012; Novo et al. 2018).

4.3 Role of PGPRs in Phytoremediation

The use of plants and allied microbes for elimination of metal pollutants and soil reclamation has both ecological and economic benefits. In general, plant-associated microbes utilize one of these mechanisms to alleviate metal stress to plants (a) bioaccumulation, (b) bioavailability by transformation of metals into soluble form, (c) production of extracellular polymeric substances (EPSs) for binding, and

(d) production of iron siderophores. Therefore, a strategy utilizing the combinatorial effect of metal-tolerant plant species along with metal-resistant plant–associated microorganisms will be more efficient. Such a novel in situ approach for bioremediation is known as rhizoremediation and the microorganisms are called heavy metal-tolerant-plant growth promoting (HMT-PGP) microbes (Mishra et al. 2017). It exploits the combined capacities of the roots of plants and allied microbial communities of rhizosphere to tackle heavy metal contamination in soils (Ullah et al. 2015). The knowledge that plants assisted microbes could prove more beneficial in enhancing metal tolerance in plants has opened up new avenues. PGPRs provide metal tolerance by either using one or multiple mechanisms such as bioaccumulation, bioavailability, and production of binding and chelating compounds, and also they enhance plant growth in such conditions by protecting against various types of abiotic and biotic stress. For instance, PGPRs produce a phytohormone indole acetic acid (IAA) which can augment the uptake of metals in the roots of plant (Khan et al. 2009; Tak et al. 2013). These microorganisms are found in abundance in plant rhizosphere, they help to diminish metal buildup in plant tissues and in addition assist in reducing metal bioavailability in soil through a variety of mechanisms. PGPRs release siderophores, organic acids, and plant growth regulators which increase the rate of phytoremediation (Tak et al. 2013). Kloepper et al. (1980) reported that some strains of the *Pseudomonas fluorescens-putida* act as PGPR by producing extracellular siderophores which are microbial iron transport agents. They probably deprive native microflora of iron and make it less available to them and significantly improve the yield of potato, radish, and sugar beet.

4.3.1 Role of Rhizobacteria in Phytoremediation of Contaminated Soil

4.3.1.1 Metal-Contaminated Soil

The mining and extraction of mineral resources is important for development but frequently causes great harm to neighboring ecosystems (Novo et al. 2018). Industrialization and modern life style have led to drastic pollution of biosphere. Different types of inorganic (heavy metals) and organic (hydrocarbons, volatile organic compounds, and solvents) contaminants are being incessantly released into the environment by mining and industrial activities (Manoj et al. 2020). Soils from mining areas are nutrient deficient and have reduced organic matter, pH, and cohesion and elevated concentration of metals (Novo et al. 2018). Mining waste leaches into aquifers and contaminates agricultural lands and accumulates in plants planted for food or livestock feed; hence can enter food chain (Mendez and Maier 2008). It causes damage to water and soil flora and has lethal impacts on human health because of the mutagenicity, cytotoxicity, and carcinogenicity. Release of untreated industrial waste containing precarious heavy metals such as mercury, arsenic, and cadmium into the water bodies and soil is another source of heavy metal pollution of surrounding soil and water (Nordberg et al. 2009). Aluminum (Al), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), and

Zinc (Zn) are the most widespread polluting toxic heavy metals. These heavy metals have been declared as "priority pollutants" by United States Environmental Protection Agency because of their mutagenic and carcinogenic nature. Presence of heavy metals in soil is noxious to most plants as heavy metals ions are absorbed by roots and translocated to shoot; leading to reduction in metabolism and growth of plants (Jing et al. 2007). Soils contaminated with high metal concentration led to reduction in activity of soil microbes and thereby soil fertility (Jing et al. 2007). For example, Cd is recurrently accumulated by chief agricultural crops and its high concentration affects nutrient uptake and inhibits root and shoot growth. Such crop plants with Cd affect the health of animals and humans and negatively affect biodiversity. Cd pollution affects the activity of soil microbial communities, and the contaminated soil may eventually become unusable for crop production (Ma et al. 2011a). Therefore, research on remediation of heavy metals contaminated soils is now the topmost priority for scientists.

Few methods like thermal treatment, acid leaching, excavation and land fill, and electro-reclamation were explored for cleanup of contaminants, but they are time- and cost-consuming (Zubair et al. 2016). Currently, phytoremediation is being accepted as in situ eco-friendly technology (Abou-Shanab et al. 2019). Phytoremediation depends on the fact that numerous plant species have the capability to accrue large quantities of metals in their vegetative as well as reproductive organs. Depending upon the metal accumulating skills and tolerance, plants can be metal sensitive (excluders), having poor metal uptake and transport (indicators) or those with higher uptake efficiency (hyperaccumulators) (Khan et al. 2009). Hyperaccumulator plants have the capacity to endure high level of noxious heavy metal concentration (Ma et al. 2011a). The potential for use of a particular plant for phytoremediation depends on the BCF i.e. bioconcentration factor and the TF i.e. translocation factor. The BCF specify the capability of a plant to take up the contaminant and its accumulation in its tissues. The TF specify the capacity of the plant to transfer contaminants from the root to its aboveground parts (Novo et al. 2018). The efficiency of such plants to uptake and accumulate heavy metals also depends on edaphic factors like soil, temperature, redox potential, cation exchange capacity of the soil particles (CEC), metal bioavailability, pH, aeration, and amount of organic matter and water (Eliana Andrea et al. 2019). Also, plants chosen for the purpose of phytoremediation ought to be fast growing with high biomass production, widespread root system, ability to accumulate the pollutants, and preferably hardy, native species (Manoj et al. 2020). However, high levels of pollutants are also toxic to the plants used for reclamation of affected soil, and phytoremediation by plants alone is a very slow process. Still, this process can be accelerated by the synergistic action of plant and microbes. Plant-Microbe association improves plant development by enabling the sequestration of noxious heavy metals especially by phytostabilization and phytoextraction (Ma et al. 2011a). Rhizobacteria improve adaptation of host plants to altering environment by altering plant cell metabolism, so they can withstand exposure to high concentrations of metals (Welbaum et al. 2010).

The synergistic interaction between rhizobacteria and plants is now being investigated by many workers because of its potential to enhance plant growth, metal uptake, and tolerance during environmental stresses (Ma et al. 2011a). Rhizobacteria not only improve fertility of polluted soil but also enhance the growth and development of plants by exuding plant growth hormones (Zubair et al. 2016). Many plants like *Alyssum lesbiacum* and *Arabidopsis halleri* have been documented for phytoremediation as hyperaccumulators of Nickel (Ni) and Zn (Cluis 2004; McNair et al. 2000). Ni and Zn are known as most accumulated metals by different hyperaccumulator species (Pandey and Bajpai 2019). Khan and Bano (2018) have also emphasized the role of PGPRs in remediation of heavy metals by affecting heavy metal portability and accessibility to the plant through acidification, phosphate solubilization, and release of chelating agents. PGPRs used for phytoremediation of metal-contaminated soil in various laboratory and field experiments have been compiled in Table 4.1.

4.3.1.2 Petroleum-Contaminated Soil

Petroleum hydrocarbons symbolize the biggest group of organic pollutants (Hawrot-Paw and Nowak 2012). Phytoremediation of petroleum is proving to be a low-cost and sustainable approach for sustainable waste management technology. This strategy can be useful in petroleum-contaminated soils where other techniques have been unsuccessful. Attempts of remediation of soils polluted with petroleum products with various herbaceous plants including *Cynodon dactylon*, *Digitaria sanguinalis, Cyperus rotundus*, *Chloris babata*, and *Pasparlum vaginatum* have also been reported (Borowik et al. 2019). However, PGPR-associated plants are much better in withstanding the pressure of growing in the crude oil contaminated soils as compared to the plants without allied PGPR (Gurska et al. 2009). Afzal et al. (2012) also stated the importance of plants and associated microorganisms to remediate petroleum hydrocarbons-contaminated soils. Gurska et al. (2009) have reported the successful establishment of a system using PGPR to enhance phytoremediation of soil contaminated with total petroleum hydrocarbons. Addition of PGPRs to soil supports an active rhizosphere, minimizes plant stress in contaminated soils, causes an increase in root biomass, and promotes degradation of oil contaminants by the plants. They also noted a noteworthy boost in fresh weight and length of shoots in experimental PGPR-associated plants.

Afzal et al. (2012) reported that not only the strains used for inoculum purpose but also the inoculation strategy (seed imbibement and soil inoculation) employed determines bacterial colonization, plant growth advancement, and degradation of hydrocarbon. When the soil contaminated with diesel, where Italian ryegrass was planted, was inoculated with amalgamation of three alkane-degrading strains namely *Pantoea* species ITSI10, *Pantoea* species BTRH79, and *Pseudomonas* species MixRI75, maximum hydrocarbon degradation was achieved as compared to soil where single strain was used. Also soil inoculation method gave better results than seed imbibement method. PGPRs utilized for phytoremediation of petroleum-contaminated soil in various laboratory and field experiments have been compiled in Table 4.2.

Table 4.1 List of plant growth promoting rhizobacteria (PGPR)-assisted phytoremediation studies

PGPR	Host plant	Contaminant	Type of study/scale	Effect of PGPR	References
Pseudomonas aeruginosa, Burkholderia gladioli	*Lycopersicon esculentum*	Cd	In vitro	Decrease in Cd uptake	Khanna et al. (2019)
Bacillus subtilis	*Oryza sativa*	Cd		Decrease in Cd uptake	Treesubsuntorn et al. (2018)
Enterobacter sp.	*Oryza sativa*	Cd	In vitro	Decrease in Cd uptake	Mitra et al. (2018)
Pseudomonas moraviensis	*Triticum aestivum*	Cd, Cr, Cu Mn, and Ni	Field study	Decrease in accumulation and translocation	Hassan et al. (2017)
Bradyrhizobium sp. Per 3.61	*Glycine max*	As	Pot study, lab scale	Decrease in translocation factor	Bianucci et al. (2018)
Bacteroidetes bacterium, Variovorax sp.	*Brassica napus*	Cd, Zn	Pot study	Increase in accumulation	Dąbrowska et al. (2017)
Trichoderma sp.	*Cicer arietinum*	As	Greenhouse study	biotransformation of As and ameliorates stress	Tripathi et al. (2017)
Rhizobium sullae, Pseudomonas sp.	*Sulla conoraria*	Cu, Zn, Pb	In situ (field scale)	Increase in Zn phytostabilization	Saadani et al. (2016)
Pseudomonas putida (ATCC 39213)	*Eruca sativa*	Cd	Pot study	Increase in Cd uptake	Kamran et al. (2015)
Arthrobacter sp. TISTR 2220	*Ocimum gratissimum*	Cd	Field study	Increase in cadmium accumulation and translocation	Prapagdee and Khonsue (2015)
Sinorhizobium meliloti CCNWSX0020	*Medicago lupulina*	Cu	In vitro	Increase in plant growth and tolerance to Cu	Kong et al. (2015)
Sinorhizobium meliloti	*Medicago sativa*	Cd	Pot study, lab scale	Increase in Cd phytoextraction	Ghnaya et al. (2015)
Pseudomonas sp. A3R3	*Alyssum serpyllifolium*	Ni	Pot study	Increase in Ni accumulation	Ma et al. (2011b)
Bradyrhizobium sp. 750, *Pseudomonas* sp., *Ochrobactrum cytisi*	*Lupinus luteus*	Cu, Cd, Pb	In situ (Field scale)	Increase in phytostabilization	Dary et al. (2010)

Table 4.2 List of some PGPRs that assist in phytoremediation of petroleum-contaminated soil

PGPR	Plant	Type of study	References
Klebsiella D5A	*Festuca arundinacea* L.	Pot experiment after isolation of strain	Liu et al. (2014)
Pseudomonas strains, UW3 and UW4	*Festuca arundinacea*, var. Inferno, *Secale cereale*, *Hordeum vulgare*	Field study, seeds treated with inoculum	Gurska et al. (2009)
Pseudomonas sp. AJ15	*Withania somnifera*	Seed priming with biosurfactant	Das and Kumar (2016)
Bacillus circulans, Enterobacter intermedius and *Staphylococcus carnosus*	*Zea mays*	In vitro studies, seeds treated with inoculum	Ajuzieogu et al. (2015)
Serratia liquefaciens, Pseudomonas aeruginosa, Bradyrhizobium japonicum and *Flavobacterium* sp.	*Vicia faba*	In vitro studies on nodule	Radwan et al. (2007)
Pseudomonas aeruginosa strains AS 03 and NA 108	Tea (TV1 type)	Pot experiments in greenhouse using 1 year old tea plants TV1 type	Roy et al. (2013)
Azospirillum brasilense strain SR80	*Triticum aestivum L.* Saratovskaya 29	In vitro studies using both solid and liquid medium	Muratova et al. (2005)
Proteobacteria	*Cajanus cajan*	Pot experiments	Allamin et al. (2020)
γ-proteobacteria and Bacteroidetes	*Festuca arundinacea* L.	In vitro studies	Hou et al. (2015)

4.4 From the Lab to the Field and Commercialization

Certain PGPR mechanisms i.e., nitrogen fixation, phytohormone synthesis, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, siderophore production, antibiosis, and phosphate solubilization are the basis of laboratory screening assays designed to develop new PGPR inocula. Under laboratory conditions, these mechanisms are difficult to screen for because of complexity of the mechanisms along with gaps in understanding. Henceforth, results obtained in laboratory conditions are not always replicated under field conditions and vice versa. Consequently, promising strains are rejected due to underperformance at classical laboratory screening scale (Cardinale et al. 2015).

PGPR formulations propound to green alternatives over conventional agrochemicals as they promote plant growth, aid in soil fertility, and suppress

phytopathogens without contaminating the environment (Arora et al. 2016). The development of bioformulations includes (Backer et al. 2018):

(a) Isolation of PGPR from rhizospheric soil.
(b) Laboratory screening of traits.
(c) Field trial under different conditions (crop varieties, soil types, seasons, locations) and management practices (use of agrochemicals, etc.)
(d) Assessment of synergistic effects of possible PGPR combinations.
(e) Confirmation of safety against ecotoxicology.
(f) Refining PGPR formulation and knowledge about its texture and storage conditions.
(g) Product registration and approval by regulatory agency of country.
(h) Commercialization.

All these steps are time-consuming, laborious, and costly to perform. To facilitate this process, collaborations among industries, research institutes, and government organizations can play a vital role. Development of bioformulations is a business of intellectual property. Though living creatures and natural products can no longer be patented, formulations and their applications are patentable (Matthews and Cuchiara 2014).

Patenting holds a prominent place between discovery and commercialization of promising PGPR in the field of environmental management. *Variovorax paradoxus* JHP31 strain (EP2578675A1) was patented by Koga and Masuda (2015) for assisting Cd phytostabilization in the plants of Brassicaceae, Chenopodiaceae, Compositae, Gramineae, Leguminosae, Liliaceae, Polygonaceae, and Solanaceae families. PGPRs such as *Achromobacter piechaudii*, *Agrobacterium tumefaciens, Delftia acidovorans*, and *Stenotrophomonas maltophilia* were patented (Banerjee and Yesmin 2011) for their ability to oxidize elemental sulfur and in turn enhancing plant growth.

Some other PGPRs that have been patented are *Microbacterium arabinogalactanolyticum*, *Microbacterium liquefaciens*, and *Sphingomonas macrogoltabidus* for assisting phytoextraction ability of *Alyssum murale* (US7214516B2) (Angle et al. 2007).

Highly potent microbes possessing long shelf-life and good colonization rates present a major challenge to commercialization. Colonization rates are largely affected by inoculation and field conditions. PGPR inoculated without a suitable carrier or in amount not enough to compete with native soil microbes are the major challenges to successful rhizosphere colonization (Backer et al. 2018). Additionally, fumigation of soils with broad-spectrum biocidal fumigants during cultivation of high-value crops alters the soil ecology by affecting microflora and their interactions with plants in aiding nutrient acquisition and mobilization (Dangi et al. 2017). Many underlying issues should be tended to for substantial commercialization of PGPR strains such as the following:

(a) Identification of PGPR responsively suitable for particular soil conditions and overcome environmental constraints.
(b) Choosing ideal rhizoinoculation techniques depending upon cultivation conditions (i.e., greenhouse vs. field) and training farmers to apply them efficiently.
(c) Selection of desirable traits-possessing strains.
(d) Uniformity among regulatory agencies of different countries regarding safety and use of PGPB strains.
(e) Knowledge of potential interactions among PGPR and native microflora (other bacteria, algae, and fungi) and the advantages of using them over others.

With view of all above points, PGPR strains that have been commercialized have been enlisted in Table 4.3 (Glick 2012). Progressing from research lab and greenhouse analysis to field assessments and commercialization involves development of new processes to inoculate, formulate, store, and ship these strains. Necessary instructions will need to be imparted to the users of these formulations.

4.5 Genetics and Genomics of Heavy-Metal Resistance in PGPRs

Many plant-associated microorganisms mainly bacteria and fungi are well-known to display plant-growth advancing qualities under heavy metal stress by means of various direct and indirect mechanisms e.g. *Pseudomonas* sp., *Bacillus* sp., *Arthrobacter, Streptromyces, Methylobacterium*, and filamentous fungi such as *Trichoderma, Aspergillus, and Fusarium*. There have been many genetic studies to evaluate if the heavy metal-resistance and plant growth promoter-producing bacteria found in soils would support phytoremediation. Much of the studies were done in symbiotic rhizobia and has been reviewed by Fagorzi et al. (2018). In *Sinorhizobium meliloti* CCNWSX0020 genetic mechanisms responsible for Cu resistance were elucidated through transposon mutagenesis combined with RTPCR (Li et al. 2014). The transcriptional analysis of *Rhizobium etli* revealed the increase in the levels of defense-related genes namely PvWRKY33, PvERF6, and PvPAL2 as well as ABA-synthesis-related gene PvAAO3 following infection with the pathogen (Díaz-Valle et al. 2019). Genetic screening of a cosmid genomic library of *Mesorhizobium metallidurans* for Cd or Zn endurance revealed the presence of a gene encoding PIB-type ATPase homologous to CadA (Maynaud et al. 2014). The mechanism of arsenite [As(III)] resistance *via* methylation and successive volatization was characterized by Qin et al. (2006) and the enzyme for this function was encoded by the As(III) *S*-adenosylmethionine methyltransferase (arsM) genes. *Rhizobium leguminosarum bv trifolii,* which lacks an endogenous arsM gene, was genetically engineered by using an algal As(III) methyltransferase gene (CrarsM) for arsenic bioremediation and it was able to successfully methylate arsenic reducing toxicity. In *Mesorhizobium amorphae* genetic mechanism of Cu resistance was investigated by transposon mutagenesis, and CopA was found to be the major

Table 4.3 List of commercialized PGPR and their effects

PGPR	Application	Intended crop	References
Agrobacterium tumafaciens strain K-84 (formerly *A. radiobacter)*	Biocontrol of crown gall disease	Commercial and ornamental plants	Bhattacharyya and Jha (2012)
Azospirillum brasilense	Nitrogen fixation, promotes plant growth via synthesis of phytohormones, provides resistance against biotic and abiotic stress	Turf grass and forage crops	Fukami et al. (2018)
Azospirillum lipoferum	Promotes growth, ameliorates drought stress	Corn, wheat, rice, vegetables, and turf grass	Bashan and de Bashan (2005)
Azotobacter chroococcum	Potential biofertilizer, nitrogen fixation, P and K solubilizer, promotes plant growth via synthesis of phytohormones	Wheat, barley, oats, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee, and coconuts	Wani et al. (2013)
Bacillus firmus	Phosphate solubilization, nitrogen fixation, promotes plant growth via synthesis of phytohormones, provides protection against nematodes	Maize, Cotton, Tomato	Mendis et al. (2018)
Bacillus licheniformis	Potential biofertilizer, phosphate solubilization, nitrogen fixation produces auxins, siderophores, and antifungal cellulases, induces tolerance to both biotic and abiotic stress	Vegetable and grain crops	Mahdi et al. (2020), Lim and Kim (2013)
Bacillus megaterium	Phosphate solubilization, produces auxins, promotes plant growth	Wheat, maize, rice, and cotton	Tabassum et al. (2017)
Bacillus mucilaginous	Phosphate and potassium solubilization, nitrogen fixation	Sorghum, wheat	Bhattacharyya et al. (2016), Wu et al. (2005)
Bacillus pumilus	Phosphate solubilization, produces auxins, induces systemic resistance against wilt, molds, mildews, blights, rusts	Millets, Soybean, oak trees, and green house crops	Tabassum et al. (2017)
Bacillus subtilis	Phosphate solubilization, biocontrol agent against soil-borne pathogens such as *Fusarium* and *Rhizoctonia*	cotton, peanut, soya bean, corn, vegetables, and small grain crops	Nakkeeran et al. (2005)

(continued)

Table 4.3 (continued)

PGPR	Application	Intended crop	References
Burkholderia cepacia	Phosphate solubilization, antifungal in nature (provides protection against *Pythium, Fusarium*)	Alfalfa, Barley, Beans, Clover, Cotton, Maize, Peas, Sorghum, Vegetable crops, and Wheat	Zhao et al. (2014)
Delfitia acidovorans	S-oxidizing PGPR, promotes growth	Canola	Banerjee and Yesmin (2002)
Paenobacillus polymyxa	Nitrogen fixation, promotes growth	French beans, lodgepole pine	Chauhan and Bagyaraj (2015), Anand et al. (2013)
Pantoea agglomerans	Nitrogen fixation, synthesize auxins	French beans	Chauhan and Bagyaraj (2015)
Pseudomonas aureofaciens	Biocontrol against *Pseudomonas tolassi* (Dollar spot, Anthracnose)	Mushrooms Turf and other crops	Tabassum et al. (2017)
Pseudomonas chlororaphis	Biocontrol against *Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum*	Vegetables and ornamental plants	Tabassum et al. (2017)
Pseudomonas fluorescens	Biocontrol agent against major diseases	Edible, oil, cash, and ornamental crops	Ganeshan and Kumar (2005)
Pseudomonas syringae	Biocontrol agent against *Botrytis cinerea, Penicillium* spp., *Geotrichum candidum*	Pome fruit, citrus, cherries, and potato	Bhattacharyya and Jha (2012)
Rhizobium spp.	Nitrogen fixation, induction of plant stress resistance, synthesis of auxins, siderophore production	Legumes	Vejan et al. (2016)
Streptomyces griseoviridis K61	Biocontrol against fungal phytopathogens	Field, vegetables and ornamental plants	Bhattacharyya and Jha (2012)
Streptomyces lydicus	Resistance against soil-borne diseases (mildews)	Fruits and vegetables	Tabassum et al. (2017)

determinant (Hao et al. 2015). *Sinorhizobium melilotinia* was shown express a P1B-5-ATPase in the nodule and its expression is activated by the presence of $Ni^{2+}$and Fe^{2+}ions (Zielazinski et al. 2013). Genomic analysis of the role of the plant-beneficial function contributing genes (PBFC genes) was probed utilizing the genomes of 25 PGPR species, and it showed favored associations among certain genes engaged in phytobeneficial qualities (Bruto et al. 2014).

Currently, use of a novel phytobacterial strategy that uses genetically engineered plant growth promoting bacteria along with plants seems to be promising approach to mitigate heavy metal stress in plants (Gupta and Singh 2017; Ullah et al. 2015;

Ashraf et al. 2017, Tiwari and Lata 2018). Many genes belonging to metal uptake and its regulation, metabolic enzymes, metal chelators, and metal homeostasis can be used as potential target genes for such manipulation. Undoubtedly, these genetically modified microorganisms have better remediation prospective, yet their effect on biomes needs to be studied in detail. *Pseudomonas aeruginosa* strain Psew-MT, which was genetically modified by expressing metallothioneins to capture Cd^{2+}, showed tolerance along with plant growth-advancing properties (Huang et al. 2016). Similarly, enhanced Cd, Hg, and Silver (Ag) resistance and accumulation were shown by genetically modified *Pseudomonas putida KT244* (Yong et al. 2014).

Rhizobium–legume associations have been studied for various reasons in the past and they provide an excellent strategy which can be exploited in reclamation of heavy metal-polluted soils (Pajuelo et al. 2011; Ahemad 2012). For example, a genetically engineered *Ensifer medicae* MA11 strain having copAB gene from *Pseudomonas fluorescens* was analyzed for enhanced Cu resistance and reducing toxic effect of Cu in *Medicago truncatula* (Perez-Palacios et al. 2017; Delgadillos et al. 2015). Four different reports are available for the use of transgenic *Meshorhizobium huakuii* subsp. rengei strain B3 for Cd bioremediation in association with different plants (Ike et al. 2007, 2008; Sriprang et al. 2002, 2003). Wu et al. (2006) reported the use of genetically engineered *Pseudomonas putida strain* 06909 for enhanced Cd tolerance in alliance with the host plant *Helianthus annuus*. In another study, Weyens et al. (2013) analyzed the phytoremediation prospective of willow and its genetically engineered allied bacteria in Cd- and toluene-contaminated soils. In an independent study, the impact of genetically engineered *Burkholderia pyrrocinia* JK-SH007E1 on microbial communities of soil in the poplar rhizosphere during long-term use as biological control was analyzed (He et al. 2018). Whole genome sequence analysis has also been used to characterize the genetic basis of the PGPR and plant interactions. *P. fluorescens Pf-5* is a remarkable organism widely recognized for its use in PGPR for its rhizosphere competence and production of broad range of secondary metabolites and antibiotics. The genome of *Pseudomonas fluorescens Pf-5* was sequenced to identify the genetic features and molecular determinants responsible for biocontrol (Paulsen et al. 2005). The genome sequence *Pseudomonas psychrotolerans* CS51 was determined to understand the plant growth-promoting characteristics under multiple heavy metal stress (Cd, Cu, and Zn), and the existence of genes accountable for cobalt-Cd-Zn resistance, transportation of Ni, and Cu homeostasis was confirmed in the *P. psychrotolerans* CS51 genome. Genomes of other PGPR strains including the *Serratia fonticola* strain AU-P3, and *Bacillus* sp. strain JS, *Sinorhizobium meliloti* CCNWSX0020 have been sequenced, which is serving to comprehend the correlation among genes and PGPR activities (Devi et al. 2013; Song et al. 2012; Li et al. 2012).

There are few studies that have focused on the role of bacterial consortium in PGPR-mediated beneficial effects (Zolla et al. 2013). A synthetic microbial consortium containing seven 2,4-DNT-degrading microbes affiliated to *Bacillus, Burkholderia, Pseudomonas, Ralstonia*, and *Variovorax* species was found to augment root length of *Arabidopsis* under 2,4-DNT stress (Thijs et al. 2014). In another

study, phytoremediation potential of *Lupinus luteus* was improved when it was inoculated with a PGPR conglomerate inclusive of *Bradyrhizobium* sp. and two metal resistant bacteria including *Ochrobactrum cytisi* and *Pseudomonas* sp. (Dary et al. 2010).

4.5.1 Genetically Engineered PGPRs

There have been numerous attempts to understand the molecular features that define PGPR. But, it has remained largely unsuccessful due the ability of PGPR to occupy different habits, to display alternative/selective ecological niches. Moreover, the genes that are implicated in plant-beneficial functions are also involved in the essential primary metabolism like phosphate solubilization, nif (nitrogen fixation), and phl (phloroglucinol synthesis) or in the secondary metabolic functions like pqq (pyrroloquinoline quinone synthesis). So the role of PGPR in producing plant beneficial properties needs to be experimentally verified under controlled conditions and that too in isolation for each species. That has made the process of identification of plant-beneficial traits and their corresponding genes in PGPR a relatively difficult task.

In the last few decades, there have been various reports on introduction of specific genes accountable for the expression of certain enzymes from microbial species lineally into crop plants, but very few studies have been reported on genetic manipulation in the PGPR for enhancing plant productivity under environmental stress or metal stress (Ullah et al. 2015; Saxena et al. 2019). The transgenic techniques are used to either overexpress or knock down genes playing a crucial function in metal detoxification and tolerance to metal stress like genes encoding metal binding, transport, and chelation (Dhankher et al. 2011; Ullah et al. 2015; Sarwar et al. 2017; Saxena et al. 2019).

The enzyme ACC deaminase, encoded by AcdS gene, is common in bacterial and fungal species in soil. It breaks down ACC, precursor of the plant hormone ethylene, to α-ketobutyrate and ammonium. ACC deaminase enzyme has been recognized in soil bacteria and has been anticipated to play an important function in microbe–plant association by decreasing the harmful impacts of biotic and abiotic stress to plants. The activity of ACC deaminase is one of the most widespread qualities among PGPRs (Glick 2014). ACC deaminase microbes aid allied plants in phytoremediation by biotransformation of poisonous components, rhizodegradation facilitated by root exudates, as well as detoxification of heavy metals that let host plants to sustain under unfavorable conditions. The bacterial AcdS gene has been utilized to generate transgenic plants to improve their tolerance to abiotic and biotic stress. Many genetically modified plants with foreign AcdS gene have been generated to lessen the harmful ethylene levels in plants as reviewed by Saleem et al. (2007). When *Mesorhizobium ciceri* was exogenously transformed with *acdS* gene, it showed improved plant performance under salinity stress by enhancing nodulation suggesting the significant function of ACC deaminase in assisting symbiotic interaction under salinity stress (Conforte et al. 2010; Nascimento et al. 2012;

Brıgido et al. 2013). However, there is limited information on performance of these transgenic plants under farm conditions due to the environmental risks associated with them.

Thus biotechnological interventions can prove to be promising alternatives for enhancing agricultural productivity through PGPR-mediated beneficial effects. However, since the genetically engineered plant-associated microbes are mainly distributed in the rhizosphere, a detailed experimental validation is required for their use in field conditions. Currently, efforts are underway to understand the beneficial effects of root microbiome on plant productivity and stress endurance. Many differentially regulated genes were recognized in PGPR-treated roots of rice plants through microarray technique (Agarwal et al. 2019). Transgenic plants overexpressing OsASR6 (ABA STRESS RIPENING 6) showed a significant result on the growth of plant and root architecture, which could be the main reason for the positive impact of PGPRs in rice.

4.6 Conclusion

PGPRs play significant roles in assisting plant growth on soils polluted with diverse contaminants and in detoxification of soils. Microbial diversity and their interactions play an essential role in facilitating plant-based degradation of toxins. Therefore, microbiome analysis or a detailed rhiozbiome analysis in PGPR–plant interactions may provide more useful insights. The potential of "omics" technologies (such as genomics, transcriptomics, proteomics, metabolomics, and metagenomics) has to be utilized to get a clear holistic view of the role of various genetic, molecular, and regulatory mechanisms in microbe-assisted phytoremediation. For effectual utilization of genetically engineered PGPR for phytoremediation; well-structured, cost-efficient, and time-efficient tools for a trustworthy forecast of their effectiveness on contaminated sites and their repercussion on biomes required to be discoursed prior to commercialization. Also, assurance needs to be provided regarding safety upon large-scale release of strains, since public acceptance also comes into count.

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