Applied Water Science (2018) 8:154
https://doi.org/10.1007/s13201-018-0796-5

REVIEW ARTICLE

Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: a minireview

Edward Raja Chellaiah[1]

Received: 11 July 2017 / Accepted: 4 September 2018 / Published online: 11 September 2018

Abstract
Heavy metal pollution has become an issue of serious international concern. One of the heavy metal cadmium (Cd) is known to be a widespread environmental contaminant and a potent toxin that may adversely affect human health. Microbial remediation has been applied as an efficient strategy to remove or detoxify the heavy metals mainly from soil, water and sediments, etc. *Pseudomonas aeruginosa* is one of the most significant bacterium present in almost all contaminated sites. They are often resistant to antibiotics, heavy metals, detergents and organic solvents. This review concluded that *P. aeruginosa* is one of the versatile and high-tolerance cadmium-resistant bacteria isolated from different environment regimens. It can be used as suitable biosorbent for the removal of cadmium and other heavy metals from solution, contaminated waste, water and soil. Apart from this characteristic, *P. aeruginosa*, used as potent bioinoculant, express Plant growth promoting rhizobacteria (PGPR) activity, biofilm and biosurfactant production, and comprise key role in metal phytoextraction process.

Keywords Bioremediation · Cadmium · *Pseudomonas aeruginosa* · Resistance bacteria · Mechanisms

Introduction

Environmental pollution is the presence of a pollutant in the environment: air, water and soil, which may be lethal or toxic and will cause harmful to living things in the polluted environment (Duruibe et al. 2007). Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rainwater (Vijayaraghavan and Yun 2008).

Cadmium (Cd) is identified as major pollutant, non-essential metals, and it is harmful to organisms at relatively low concentrations about 0.001–0.1 mg/L (Alkorta et al. 2004; Tang et al. 2006). Cd is widely applied in many industries like chlor-alkali, paints, electroplating, and copper alloys, pulp and paper, alkaline batteries, and mining, fertilizer and zinc refining (USEPA 2000). Cd enters into human and animal body through food chain that can cause severe diseases (Zeng et al.2009). Cadmium is not involved in any known biological processes, and it is known to disturb enzyme activities, to inhibit the DNA-mediated transformation in microorganisms, to interfere in the symbiosis between microbes and plants, as well as to increase plant predisposition to fungal invasion (Kabata-Pendias and Pendias 2001). The accumulation of Cd in plants may cause several physiological, biochemical and structural changes (Khan et al. 2007; Feng et al. 2010) such as alters mineral nutrients uptake (Hossain et al. 2010), disturbs the Calvin cycle enzymes, photosynthesis and carbohydrate metabolism (Mobin and Khan 2007; Hossain et al. 2010) changes the antioxidant metabolism (Khan et al. 2009), and lowers the crop productivity (di Toppi and Gabbrielli 1999).

Heavy metals removal methods

Many conventional methods have been applied in order to remove heavy metals from aqueous streams. Among the most commonly used techniques are chemical precipitation, chemical oxidation and reduction, ion-exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery and solvent extraction (Xia and Liyuan 2002). These conventional techniques offered several problems such

✉ Edward Raja Chellaiah
edwardrajac@gmail.com; edwardrajac@rediffmail.com

[1] DST-Ramanujan Fellow, Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India

as unpredictable metal ions removal and generation of toxic sludge (Xia and Liyuan 2002).

Bioremediation is an alternative option to use of natural and recombinant microorganisms for the removal/reduction of toxic pollutants. It is considered as cost-effective and environment friendly approach (Brar et al. 2006). The living and dead biomass of microbes have been used for the efficient removal of metal ions through biosorption and bioaccumulation process (Chojnacka 2010; Joutey et al. 2015). Bioaccumulation is a dependent, active and partially reversible process that needs energy and requires respiration. In contrast, biosorption is an independent, revisable process that does not require energy/respiration (Vijayaraghavan and Yun 2008; Velásquez and Dussan 2009). The major advantage of biosorption is low operating cost, high ability, possibility of metal recovery and potent biosorbent revival (Volesky 2001; Göksungur et al. 2005).

Review bacterium *Pseudomonas aeruginosa*

Pseudomonas species is ubiquitous in soil, water ecosystems and are capable of metabolizing a wide range of organic and inorganic compounds. In addition, *Pseudomonas* was well-studied and showed high resistance to antibiotics, heavy metals and detergents and organic solvents (Pardo et al. 2003; Ansari and Malik 2007; Haritash and Kaushik 2009). *P. aeruginosa* is a gram-negative, rod-shaped bacterium. It is found in desert, agricultural, grassland, and forest soils, water and humans, plants, sewage and hospitals (Green et al. 1974; Lederberg 2000; Drees 2004) as well as in riverine ecosystems (Pellet et al. 1983) and metal-contaminated sites (Bodour et al. 2003). The application of *P. aeruginosa* in cadmium bioremediation is shown in Fig. 1.

Cadmium resistance mechanism of *P. aeruginosa*

Microbial Cd resistance was exhibited in at least six different ways. These include deposition of the toxic metal in the cell wall, altered accumulation of the toxic compound and alternation of the cell wall plasma membrane complex (Mitra and Bernstein 1977). Cd can enter bacterial cells through divalent cation uptake systems such as Mn^{2+} (Tynecka et al. 1981) or Zn^{2+} (Laddaga and Silver 1985), gene amplification (Beach and Palmiter 1981), active Cd efflux (Tynecka et al. 1981) and enhanced transcription of metallothionein genes (McEntee et al. 1986).

At first, one of the best characterized bacterial Cd resistance mechanisms was identified in gram-positive bacteria determined by the Cd-transporting ATPase (Silver and Phung 1996). In gram-negative bacteria, Cd is detoxified by RND-driven systems like Czc, which is mainly a zinc exporter (Nies and Silver 1989). A well characterized

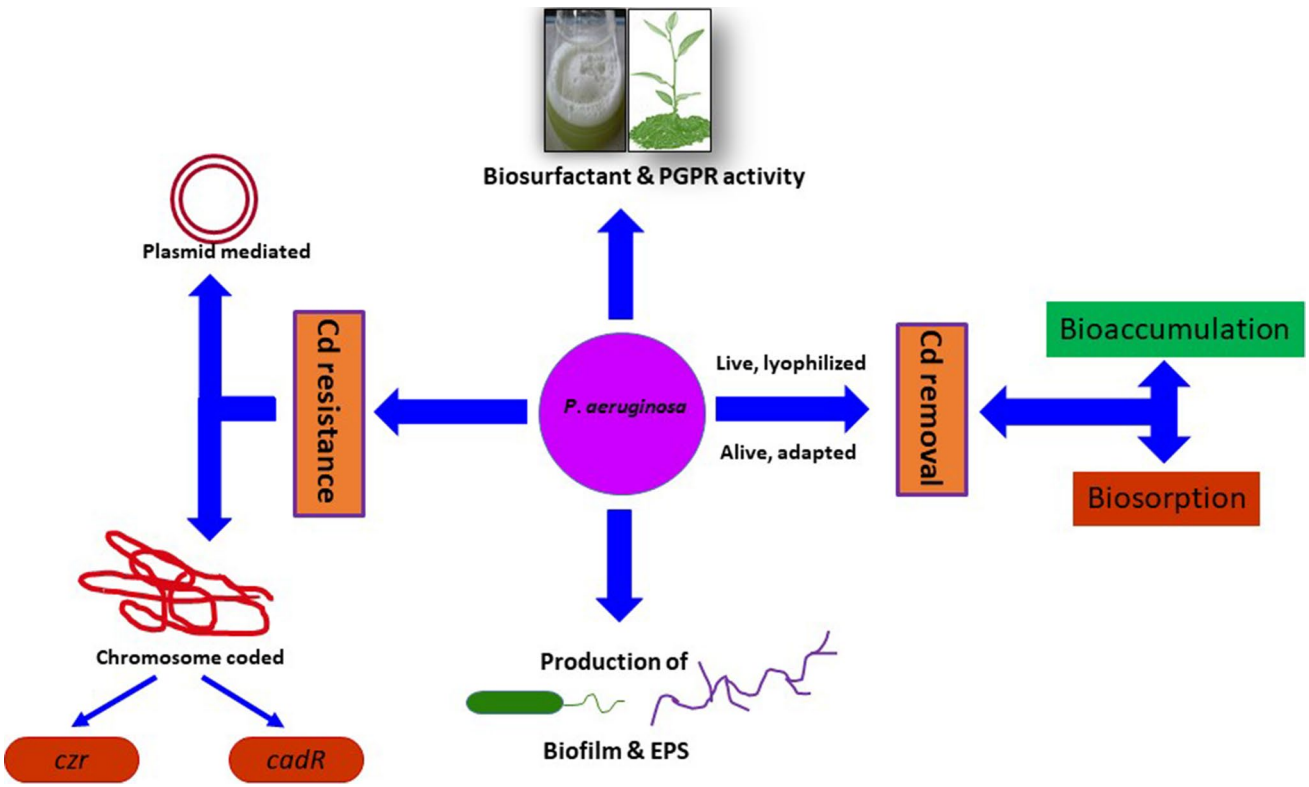

Fig. 1 Schematic representation of *P. aeruginosa* used in cadmium bioremediation

Cd-resistant system was identified in *Alcaligenes eutrophus* CH34. The gram-negative bacterium showed Cd, Zn and Co resistance was plasmid encoded with the *czc* gene cluster (Nies et al. 1989). The homologous of gene cluster (*czc*) called *czr* was identified in the chromosome of *P. aeruginosa* CMG103. The predicted CzrC, CzrB and CzrA proteins encoded by the CMG103 czrCBA genes show significant similarities with the proteins encoded by *A. eutrophus* CH34 and also czcCBA which determines a cation-antiporter efflux system for metal resistance. The establishments of the *czc* and *czr* gene clusters regarding regulatory modules are different. Moreover, in *A. eutrophus*, CH34 and related strains *czc* were plasmid borne, whereas *czr* was chromosomal coded resistance in *P. aeruginosa* CMG103 (Hassan et al. 1999). The amplification of the gene by using the chromosomal DNA indicates the presence of the cadmium-resistant gene on the chromosomal DNA and sequencing of the fragment confirmed the presence of czcA gene responsible for cadmium resistance (efflux pump) in EP-Cd1 (Muneer et al. 2016).

Two contrary transcribed genes *cadA* and *cadR* were identified in the chromosome of *P. putida* 06909. *CadA* was similar to Cd-transporting ATPases that is mainly from gram-positive bacteria, and to *ZntA*, Pb, Zn and Cd-transporting ATPase from *E. coli cadR* (Lee et al. 2001). *CadR* from *P. aeruginosa* encodes a transcriptional regulatory protein which responds Cd(II) ≫ Zn(II) > Hg(II) at its cognate promoter PcadA. *CadR* will also act to induce transcription at the *E. coli* ZntR cognate promoter PzntA (Brocklehurst et al. 2003). In addition, *cadR* was also found in the chromosome of environmental isolate *P. aeruginosa* BC15. Multiple nucleotide sequence alignments of *cadR* showed high homology with *P. aeruginosa* FLH033011 (100%), *P. aeruginosa* PAO1 (99%), and *P. aeruginosa* UCBPP-PA14 (98%), respectively (Edward Raja and Selvam 2012).

Plasmid-mediated cadmium resistance of *P. aeruginosa*

Microbial survival in the polluted environments depends on inherent biochemical, structural properties, physiological and/or genetic adaptation (Wuertz and Mergeay 1997). Various studies have been published of different heavy metal-resistant mechanisms found in the genus *Pseudomonas*, as well as reports of chromosome and plasmid-encoded genetic determinants for resistance to heavy metals (Coral et al. 2006).

Pseudomonas aeruginosa RA65 (~9.5 kb) plasmid-encoded resistance to Cd, Zn and Pb was confirmed by agarose gel and transformation analysis (Mohamed and Abo-Amer 2012). This finding was similar to the results reported that 100% of plasmid-mediated, resistant *P. aeruginosa* strains were isolated from heavy metal-contaminated regions (Pacheco et al. 1995). Likewise, El-Sayed et al. (2008) reported that plasmid (27.491 Kb)-mediated Cd-resistant *Pseudomonas* species was isolated from Sohag Governorate, Egypt. Also (60 kb) plasmid-mediated heavy metals resistance including Cd was identified in *P. aeruginosa* AA301 isolated Egyptian soil (Abo-Amer and Mohamed 2006). Cadmium-resistant *P. aeruginosa* EP-Cd1 harboured 23 kb plasmids (Muneer et al. 2016). The same size plasmid DNA was detected in Pb-, Cr-, Cd- and Ti-resistant *P. aeruginosa* (Kassab and Roane 2006; Park et al. 2006). *P. aeruginosa* tested for its multiple metal resistances was found to be plasmid mediated evidenced by Hassen et al. (2008) and Edward Raja and Selvam (2009). In general, the incidence of plasmid carrying bacteria is higher in metal polluted sites than in the unpolluted regions (Malik and Jaiswal 2004).

Pseudomonas aeruginosa as cadmium resistant

The importance of Pseudomonads in nutrient cycling and their ability to quickly adapt to the contaminated environments make them preferential choice for eco-friendly studies. The first concept was supported by reports of the key role of Pseudomonads in the heterotrophic mineralization of organic carbon and in denitrification (Bollag and Barabasz 1979). Then latter was supported by the tendency of this genus to develop novel enzymatic pathways which may contribute to its adaptability to harsh environments (Clarke and Ornston 1975). Therefore, Pseudomonads are abundant in a variety of contaminated environments including Cd- and Hg-polluted water and sediments (Houba and Remacle 1980).

Many researchers have reported that *P. aeruginosa* as Cd-resistant bacteria was isolated from different environment regions (Table 1). Microorganisms resistant to antibiotics and metals appear to be the result of exposure to metal-contaminated environments that cause coincidental selection of resistance factors for both antibiotics and heavy metals (Spain 2003). Cd-tolerant *P. aeruginosa* was resistant to a wide array of antibiotics, and heavy metal resistance is shown in Table 2. *P. aeruginosa* S6 with a relatively high minimum inhibitory concentration (MIC) for metals and a large spectrum antibiotic resistance appears to be a bacterial model for ecotoxicological studies (Hassen et al. 1998). The ability of biochemical and molecular methods was used to identify and characterize natural culturable bacterial community screened from polluted environment and then applied for potential exploitation of metal-resistant bacterial strains in bioremediation process (Chovanova et al. 2004).

Cadmium stress protein

Exposure of bacteria to heavy metals leads to altered expression of genes involved in metal transport as well

Table 1 Cadmium-resistant *P. aeruginosa* isolated from different environment regions

Strains/isolates	MIC of Cd (mM/mg/L)	Isolation site	Bioremediation characteristics	References
Clinical strain	2.18	Clinical lesions	–	Nakahara et al. (1997)
Strain G-1	4.3	Activated sludge	–	Hiroyuki and Haruyasu (1980)
ATCC 27853	1, 0.5	–	–	Gelmi et al. (1994)
Strain CW961	5	Deep sea vent	Biosorption	Wang et al. (1997)
Strain PU21	–	Hospital Sewage	Biosorption	Chang et al. (1997)
P. aeruginosa	2	Sewage	–	Filali et al. (2000)
P. aeruginosa	1.09	Irrigated agricultural soil	–	Ansari and Malik (2007)
Strain BC15	6	Oil mill-treated wastewater	Biosorption	Edward Raja et al. (2008)
Strain E1	18	Cd contaminated soil	Biosorption	Zeng et al. (2009)
Strain JP-11	6.8	Marine	Bioremoval	Chakraborty and Das (2014)
Strains SN1, SN3	9.2, 9.8	Contaminated soil	–	Nath et al. (2014)
P. aeruginosa	5.18	Hospital wastewater	–	Yamina et al. (2014)
P. aeruginosa	0.5	Mine tailing	PGPR/bioinoculant/Phytoextraction	Aka and Babalola (2016)
P. aeruginosa	12	Cd contaminated rice field	Bioaccumulation Bioadsorption	Lin et al. (2016)
P. aeruginosa	900 mg/L	Soil	Adsorption	Ghaima et al. (2017)
P. aeruginosa	–	Contaminated soil		Karimpour et al. (2018)

MIC Minimal inhibitory concentration

as other stress responses such as heat shock and oxidative stress (Blom et al. 1992). In general, exposure of bacteria to low levels of one stress can induce a consequent increase in resistance to the same (adaptive) or unrelated (cross-protection) stress (Mongkolsuk et al. 1997). The induction studies of *P. aeruginosa* BC15 with sub-lethal concentrations of Cd induced adaptive resistance to lethal doses of Cd. Cd-induced cells also showed cross-resistance to lethal concentration of zinc (Edward Raja et al. 2008). Upon growth in Cd and Pb supplemented plates, *Pseudomonas* S8A exhibited both exopolymer and biosurfactant production. They also displayed two morphologically distinct colony subtypes such as small and round or large and flat. The large morphotype produced greater amounts of surfactant than the small morphotype. It suggests that a unique subpopulation response to Cd toxicity and an unidentified 28 kDa protein was expressed when exposure to > 10 mg/L Cd (Kassab and Roane 2006). Under Cd stress, *Pseudomonas* sp. M3 expressed 25 kDa protein (Abbas et al. 2014), and in contrast, three high molecular weights proteins (208, 78 and 33.5 KD) were lost, while low molecular weight protein (2.5 KD) was induced for *Pseudomonas* isolates screened from Egyptian soil (El-Sayed et al. 2008). Under Cd stress, different molecular weight proteins were detected in supernatant as well as in the cell lysate of *P. aeruginosa* EP-Cd1 (Muneer et al. 2016).

Cadmium bioremoval

Several studies have been reported the ability of gram-negative bacteria to resist and accumulate Cd ions (Higham et al. 1984; Macaskie et al. 1987; Beveridge 1989; Gelmi et al. 1994; Wang et al. 1997). *P. aeruginosa* PU21 biomass appears an effective bioadsorbent for the removal and recovery of Cd, Cu and Pb from polluted water (Chang et al. 1997). Likewise, dead cell biomass of *P. aeruginosa* has a high ability to adsorption of Cd and Pb in aqueous solutions (Karimpour et al. 2018). In contrast, live cells of *Pseudomonas* BC15 was also capable of biosorbing Cd along with other metals such as Pb, Ni and Cr in a medium (Edward Raja et al. 2006). Zeng et al. (2009) also concluded that *P. aeruginosa* E1 living cell has performed better biosorption of Cd than non-living cells. The lyophilized cells of *P. aeruginosa* PAO1 adsorbed Cd from aqueous solution was estimated at acidic pH 5–6 (Peter et al. 2014). In another study, *P. aeruginosa* isolated from active sludge could efficiently remove 94.7% Cd from solution within 60 min (Kermani et al. 2010). During biosorption studies, adapted cells of *P. aeruginosa* strain JCM 5962 and genetically engineered (GE) *P. aeruginosa* also able to remove Cd (Bojorquez et al. 2016; Tang et al. 2018). Recently published strain *Pseudomonas aeruginosa* san ai is a promising candidate for cadmium bioremediation because of its large biosorption potential (Zivkovic et al. 2018).

Table 2 Co selection of heavy metals and antibiotic resistances of *P. aeruginosa*

Cadmium and other metal resistances	Antibiotic resistance	References
Cd, As, Hg	Chloramphenicol, dibekacin, gentamycin, kanamycin, streptomycin, tetracycline	Nakahara et al. (1997)
Cd, Cr, Cu, Hg	–	Hiroyuki and Haruyasu (1980)
Cd, Cr, Ni, Pb, Zn	Ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin, tetracycline	Edward Raja et al. (2006)
Cd, Cu, Pb	–	Chang et al. (1997)
Cd	–	Wang et al. (1997)
Cd, Co, Cu, Ba, Ag, Hg, La, Li	–	Filali et al. (2000)
Cd	–	Kassab and Roane (2006)
Cd, Cu, Ni, Pb, Zn, Hg	–	Ansari and Malik (2007)
Cd, Cr, Co, Cu, Ni, Pb, Zn	Ceftazidime, gentamycin, neomycin, norfloxacin, ofloxacin, vancomycin	Hassen et al. (2008)
Cd, Co, Cu, Mn, Pb, Zn	Amikacin, ampicillin, chloramphenicol, erythromycin, kitasamycin, nalidixic acid, neomycin, novobiocin, penicillin, polymyxin, streptomycin, tetracycline	Zeng et al. (2009)
Cd, Cr, Co, Cu, Mn, Ni, Zn	–	Sinha and Mukherjee (2009)
Cd, Co, Cu, Ni	–	Choudhary and Sar (2009)
Cd, Cu, Pb	–	Chang et al. (1997)
Cd	–	Kermani et al. (2010)
Cd, Te	–	Chien et al. (2011)
Cd, Cr, Cu, Pb, Ni, Zn	Amoxicillin, cephalexin, erythromycin, penicillin, streptomycin	Chien et al. (2013)
Cd	–	Abbas et al. (2014)
Cd, Cu, Zn	Amikacin, ampicillin, amoxicillin, ciproflaxin, neomycin, tetracycline, vancomycin	Chen et al. (2014)
Cd, Cr, As, Hg, Pb, Ni, Zn	–	Chakraborty and Das (2014)
Cd, Pb	Kanamycin, oxacillin, nalidixic acid, Sulfonamides	Nath et al. (2014)
Cd, Hg, Zn	–	Yamina et al. (2014)
Cd, Cr, Ni	–	Aka and Babola (2016)
Cd, Pb, Zn	–	Lin et al. (2016)
Cd, Cu, Zn	–	Chen et al. (2016)

Biofilm, EPS production by *P. aeruginosa*

Biofilm EPS is a rich matrix of polysaccharides, proteins and nucleic acids (Mangwani et al. 2014). In addition, biofilm and extracellular polymeric substances (EPS) production is important for the bacterial resistance against heavy metals. Biosorption or bioaccumulation of heavy metals by EPS production from *Pseudomonas* also can contribute to bacterial heavy metal resistance (Kilic and Dönmez 2008). Also, recently published high Cd-resistant (7.2 mM) *P. aeruginosa* san ai previously used in various environmental studies like heavy metal removal, rhamnolipid and exopolysaccharide (EPS) production (Zivkovic et al. 2018). *Pseudomonas* sp. EJ01 was able to tolerate Cd in growth medium and exhibits aggregated and forming biofilm at 2 mM Cd and above concentration. This phenomenon might be related to the bacterium's ability to produce exopolysaccharides (Chien et al. 2013). In another study, biofilm producing marine bacterium *P. aeruginosa* JP-11 showed resistance to Cd up to 1000 ppm in aerobic conditions. The strain possesses czcABC genes for cadmium resistance as reported (Chakraborty and Das 2014) in other *P. aeruginosa* strain E1 to efflux out the Cd (Zeng et al. 2012). This gene encodes proteins expressing ion transporters by which metal ions can be pumped out of cytoplasm (Nies 1992). Chakraborty and Das (2014) proved the Cd resistance though efflux mechanism as well as removal of Cd by binding to its biofilm EPS. The czcABC gene is known for encoding proteins showing resistance to Cd, Co and Zn (Nies 1992).

P. aeruginosa act as biosurfactant

Biosurfactants are surfactants produced or secreted by living organisms such as microbes. Many studies have been reported that biosurfactants are able to complex and remediate heavy metals such as Cd, Pb and Zn (Maier and Soberón–Chávez 2000; Mulligan 2005). Rhamnolipids are a class of biosurfactant produced by *P. aeruginosa* and (Maier

and Soberón–Chávez 2000) potential applications in industry and as additives for environmental remediation (Müller et al. 2012). An attempt was made to evaluate potential relationships between rhamnolipid production and the presence of heavy metals. In this finding, the influence of Cd on rhamnolipid (RL) synthesis was produced by *P. aeruginosa*. Cd-induced *rhlB* expression was observed in mid-stationary phase (53 h) and sustained production of rhamnolipid completed in 96-h late stationary growth phase. But Neilson et al. (2010) found that most significant was an observed increase in the ratio of RL2 to RL1 congeners produced by *P. aeruginosa* cultures grown in the presence of Cd. These data combined with previously published work documenting strong complexation constants between rhamnolipid and various heavy metals (Ochoa-Loza et al. 2001; Neilson et al. 2003).

P. aeruginosa as PGPR and bioinoculant

Plant growth-promoting rhizobacteria (PGPR) are beneficial, naturally occurring and free living bacteria that colonize the plant rhizosphere (Kloepper et al. 2001; Bullied et al. 2002). Many fluorescent *Pseudomonas* strains, for example, *P. aeruginosa* which colonize the rhizosphere exert a protective effect on the roots through the production of in situ antibiotic compounds that promote growth and inhibit microbial infections (Jenni et al. 1989; Wackett 2000). Bioaugmentation is a promising method for assisting phytoextraction of heavy metals from contaminated soil. In addition, phytoextraction is cheap and environmentally compatible process. PGPR characteristics of *P. aeruginosa* are shown in Table 3. In several studies, metal tolerant bacteria including *Pseudomonas* species can be isolated and selected for their potential to promote plant growth and heavy metal accumulation by plants (Sheng et al. 2008; Rajkumar and Freitas 2008; Aka and Babalola 2016). They can develop metal bioavailability and provide protection to plants against the toxic effects of heavy metals using a variety of processes with biosorption, bioaccumulation and biotransformation (Yang et al. 2005). Aka and Babalola (2016) revealed that inoculation with metal tolerant bacteria not only protects plants against the toxic effects of heavy metals, but also increases growth and metal accumulation of plants significantly. In another study, *S. nigrum* combined with Cd-tolerant *P. aeruginosa* strains ZGKD5 and ZGKD2 might have the potential to improve Cd phytoextraction efficiency in farmland soils contaminated with low levels of Cd (Shi et al. 2016). Besides, the inoculation of Cd-polluted soil with genetically engineered Pse-w-MT significantly elevated the shoot and root biomass and leaf chlorophyll content. Likewise, plants inoculated with Pse-w-MT proved significantly lower Cd accumulation in the root and shoot system (Huang et al. 2016).

Conclusion

In conclusion, *P. aeruginosa* expresses its attitudes like Cd-resistant, bioremoval (biosorption, bioaccumulation) characteristics, heavy metal and antibiotic resistances, adaptive and cross-resistance, PGPR activity and biofilm formation, biosurfactant and EPS production, bioinoculant and phytoextraction process. The above-mentioned features were confirming and proved that *P. aeruginosa* is one of the most suitable bacterium or more appropriate choice for cadmium bioremediation.

In addition, the problems and prospects in the practical application and research of *P. aeruginosa* are a typical example of dual debt in a scientific environment, and it is one of the most substantial opportunistic pathogen and is responsible for the majority of nosocomial infections.

Table 3 PGPR characteristics of Cd-resistant *Pseudomonas aeruginosa*

Cd-resistant PGPR	PGPR traits	Plants	References
P. aeruginosa	–	*Abelmoschus esculentus* L. (okra), *Lycopersicon esculentum* L. (tomato), *Amaranthus* sp. (African spinach)	Adesemoye et al. (2008), Adesemoye and Ugoji (2009)
P. aeruginosa	Siderophores	–	Braud et al. (2009)
P. aeruginosa 4EA	Siderophores	–	Naik and Dubey (2011)
P. aeruginosa	IAA, HCN, NH_4, siderophores, exopolysaccharides, PSB	*Vigna radiata*	Ahemad and Khan (2010)
P. aeruginosa ZGKD5, ZGKD2	IAA, siderophores, NH_4, biosurfactant, PSB, N_2 fixation activity	*S. nigrum*	Shi et al. (2016)
P. aeruginosa (Pse-w)	IAA, siderophore PSB	*Pisum sativum* L.	Huang et al. (2016)

IAA indole acetic acid, *PSB* phosphate solubilizing bacteria

Nevertheless, in the field of environmental protection, the human health concerns of this bacterium are not predictable, and the strains of this species are commonly used for bioremediation resolutions.

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