Cadmium resistant bacteria mediated cadmium removal: a systematic review on resistance, mechanism and bioremediation approaches

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Cadmium resistant bacteria mediated cadmium removal: a systematic review on resistance, mechanism and bioremediation approaches

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Abstract. Cadmium-resistant bacteria that are used to remove cadmium (Cd) are becoming increasingly of the most important and hygienic method. Resistant mechanisms are involved in different ways, and some of them which can be used in cadmium removal techniques based on their molecular mechanisms and minimum inhibitory concentration (MIC). This review summarises recent improvements in understanding the mechanisms by which bacteria are either intrinsically resistant or acquire resistance to cadmium to be used as a way for cadmium removal.

Keywords: Bioremediation, cadmium-resistant bacteria, resistant mechanisms, Minimum inhibitory concentration.

Introduction

Some metals are essential for living organisms in trace amounts, such as cobalt, copper, iron, manganese, molybdenum, and vanadium. In contrast, others, such as cadmium, chromium, mercury, lead, arsenic and antimony, are non-essential. Both essential and non-essential metals cause toxic effects, depending on the dose an organism is experiencing (Bernard, 2008) and its speciation and availability in environments (Jebril *et al*., 2021a). Cadmium is a non-essential element for life, and it is toxic to *Homo Sapiens*. The United States Environmental Protection Agency (US EPA) and the International Agency for Research on Cancer (IARC) classified some elements, including Cd, As, Hg, and Pb, as human carcinogens (ATSDR, 2012). Furthermore, chronic exposure to low levels of Cd is associated with several diseases, such as deranged blood pressure regulation, osteoporosis, early onset of diabetic renal complications, and end-stage renal failure. Humans may be exposed to Cd by drinking polluted water, ingesting contaminated food, and inhaling polluted air, which causes diseases. For example, end-stage renal disease was reported in a Swedish population, living near a Cd battery plant, and using renal replacement therapy (Hellström *et al.*, 2001). Skin exposure to Cd is rare, and it occurs mainly as an occupational hazard through contact with Cd-contaminated workplaces (ATSDR *et al.*, 2012). After absorption, the blood transfers Cd into different organs, such as the liver, kidney, testis, lungs, heart, prostate, and bone, potentially with harmful consequences. One of the biggest concerns about the presence and accumulation of Cd in the human organs is that the Cd can persist for many years, as it has a long biological half-life of 30 years (ATSDR *et al.*, 2012). Standard and guidelines for Cd in various media include (ATSDR *et al.*, 2012): Soils with concentrations of > 1 mg/kg Cd are considered contaminated with Cd; Drinking water standard guideline concentration is 3 µg/L (WHO, 2011); Daily intake through food of Cd is 7 µg Cd/kg body; Air pollution exposure is > 5 ng/mL. The mechanism of Cd toxicity is associated with reactive oxygen species (ROS), despite the Cd not being observed as a Fenton metal. Cd is capable of protein depletion and glutathione-bound sulfhydryl groups, resulting in an oxidant, such as the hydroxyl radical species, in cellular environments. These ROS lead to the peroxidation of the lipid to evolve until the DNA is damaged (Waisberg *et al*., 2003). Animal, molecular, and epidemiological models have shown that exposure to Cd causes remarkable health effects upon humans. Interestingly, the depletion of glutathione, which is the dominant form of Cd-induced hepatic toxicity, was observed in

the liver of a rat (Liu *et al*., 2009). Cd toxicity is usually determined by measuring its concentration and its biologically effective dose at the target organ. A study on Australian people between the ages of 41 to 50 years, who died of accidental causes, showed Cd concentrations in the kidney cortex and liver were 26 and 1 μg Cd/g wet weight, respectively (Satarug *et al.*, 2002). The use of a biomarker for exposure is the best way to determine the biologically effective dose when it is impossible to decide the dosage of Cd at the target tissue. Commonly, the biomarker of exposure to Cd is determined by quantifying Cd levels in urine or blood. The level of Cd in urine reflects the accumulation of Cd in the body or, more commonly, the kidney load of Cd, while the level of Cd in blood indicates possible recent exposure to Cd (Wittman, 2002). Most epidemiological studies for Cd exposure in humans showed that the main target organs for Cd toxicity are the liver and the kidney, due to having a high concentration of metallothionein protein (Waalkes and Klaassen, 1985). Biomarker studies for assessing Cd-induced kidney damage in the general population have provided indications of the association between Cd and critical renal impairment concentrations between 100 and 200 μg/g Cd, which links to a urinary threshold limit of 5 − 10 μg Cd/g creatinine (Satarug *et al*., 2009). The Food and Agriculture Organization/World Health Organization (FAO/WHO, 2010) guideline for a provisional tolerable daily intake of Cd is 1 μg Cd/kg body. Several studies have shown differing kidney effects at urinary Cd levels below the urinary threshold limit of 5-10 μg Cd/g creatinine. The elevation of protein markers, beta-2 macroglobulin, albumin, and retinol-binding protein, in a population with urinary Cd contamination, living in an area near a zinc smelter, have presented with > 2 μg Cd/g creatinine (Trzcinka-Ochocka *et al.*, 2004). Women living in an area contaminated with low Cd also revealed a positive association between Cd concentration and renal damage, which showed that the protein marker at a lower concentration (0.67 μg Cd/g creatinine, Åkesson *et al.*, 2005). Several studies have reported an increase in lung cancer (increased excretion of creatinine) from chronic Cd exposure (0.01 μg Cd/m^3 ≥ 1 year). A very small study in a silver soldering company reported that 90% of ten workers who were exposed to Cd contaminated air from 0.006 to 0.015 mg/m^3, were found to have urinary Cd contamination, 63.0 μg/g creatinine, having urine protein (beta coefficient 10.27) (Choi *et al*., 2018). Therefore, due to the high association between Cd intake and carcinogenic effects, the standard guideline concentration for Cd in drinking water is 3 μg/L (WHO, 2011). The ecotoxicity of Cd has been established for different organisms. Pavlaki *et al.* (2016) studied acute toxicity (lethal concentration, LC_{50}) in relation to different marine species. *Daphnea magna*, *Lemna minor,* and fish eggs (*Solea seegalensis*) were found to be most sensitive to Cd, for a day with LC_{50} values around 0.03 mg Cd/L (Clément and Lamonica, 2018; Pavlaki *et al.* 2016; Santos *et al.*, 2018). The cyanobacteria *Synechocystis* sp. PCC6803 is more tolerant to Cd for 5 h with a mean LC_{50} of 8.2 mg Cd/L (Du *et al.*, 2019). It is well-known that Cd inhibits soil microalga, for example, *Chlorococcum* sp. MM11 showed a mean LC_{50} of 2.85 mg Cd/L for 4 days (Subashchandrabose *et al.*, 2015). More Cd tolerant organisms include the earthworm, *Eisenia fetida*, for seven days with a mean LC_{50} value of 129.4 (91.6-202.8) mg Cd/L (Du *et al.*, 2014). Because of the toxicity of cadmium, this review summarizing the possible using of bacteria in cadmium removal from environments.

1. Transporters of cadmium to a cell and bacterial toxicity

Although some elements are essential for cellular metabolism in bacteria, high concentrations of metals can have fatal effects on bacteria. Some elements present in nature at low concentrations, such as Cd, are not necessary for bacteria (Schirawski *et al.*, 2002). Nevertheless, organisms, including bacteria, have mechanisms to take up Cd into their cells (Palmer and Skaar, 2016). Mainly, there are two main carriers categories of Cd in a cell: Firstly, the proteins, which are involved in the absorption of essential micronutrients, such as Zn^{2+} and Mn^{2+}, can also transfer Cd across the cell wall. Cd is the structural equivalent of Zn^{2+} and Mn^{2+} and is handled by Zn^{2+} or Mn^{2+} carriers in most bacteria (Palmer and Skaar, 2016). Two transmitters, the inner membrane ATP-ABC (ATP-binding cassette) ZnuACB and ZIP (Zrt, Irt-like Protein, ZRT/IRT), facilitate the absorption of Zn^{2+} in the cell. ZnuACB shows the carrier for Zn^{2+} absorption in *Yersinia pseud otuberculosis* (Figure1A). ZIP carriers are the dominant carrier, which allows Cd to enter a cell (Binet and Poole, 2000). ABC carriers facilitate the transport of Mn^{2+} across the outer membrane. The SitABCD vector (an example of ABC transport protein) is predominantly a Mn^{2+} transporter (Figure 1B) and also facilitates the

entrance of Cd into cells (Kehres *et al.*, 2002). Secondly, specific ligands, such as siderophores, located on the cell surface, can transfer Cd to a cell. Siderophore is a protein with low molecular weight and a significant affinity to Fe^{2+}. The proteins FecA, FepA, and FhuA, mediate the absorption of iron, resulting in ferrisiderophore. The protein substrate is ferrous like FhuD, located in the space between the cell wall membranes, which carries a ferrisiderophore complex from the outer layer to the internal membrane (Figure 1C; Palmer and Skaar, 2016). Similarly, the ferrisiderophore appears to contribute to the uptake of Cd^{2+} in the cell (Winkelmann, 2002). After Cd entered the cell, its toxicity appears mainly due to oxidation reactions with biological macromolecules. Fenton`s reaction summarises the oxidative stress of elements, generally, on the bacterial cells and the production of reactive oxygen species (ROS) as shown in the reactions below (Fenton, 1894):

$M^{(n)} + O_2^{\cdot-} \; M^{(n-1)} + O_2$

$2O_2^{\cdot-} + 2H^+ \; H_2O_2 + O_2$

$M^{(n-1)} + H_2O_2 \; M^{(n)} + HO^{\cdot} + OH^-$

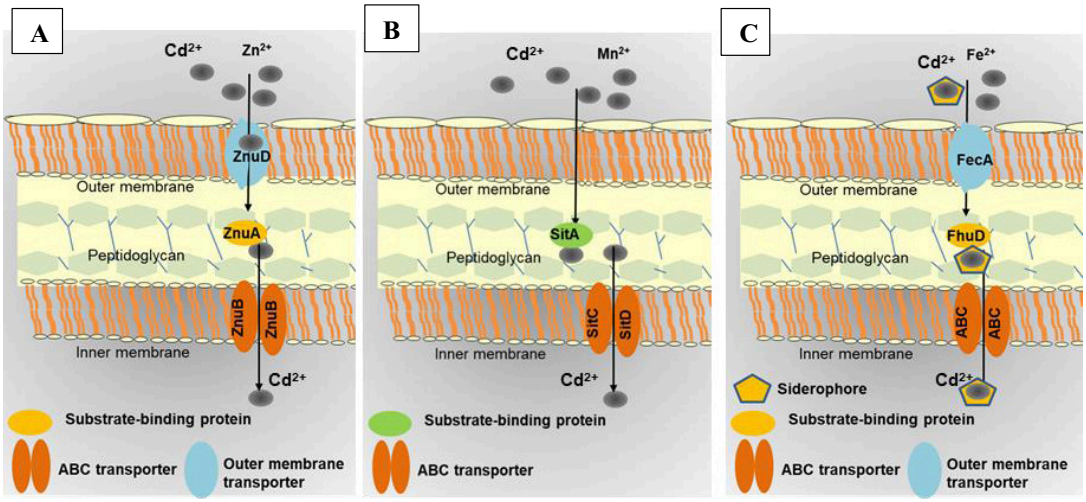

Figure 1. Schematic representation of Cd transposition into a cell (Palmer and Skaar, 2016). **(A)**Transposition of Zn^{2+} in the bacterial cell. Bacteria translocate Zn^{2+} from the extracellular spaces to the cytoplasm by ZnuACB transporters. This Figure is based on the structure of ABC transporter to transport Zn^{2+} into *Yersinia pseudotuberculosis*. **(B)**Transposition of Mn^{2+} in Gram-stain-negative bacteria by SitABCD transporters. **(C)** Fe^{2+} transporter by ABC transporter via siderophore. This structure is based on the structure of ABC transporter to transport Zn^{2+} into *Pseudomonas aeruginosa*.

Hydrogen peroxide (H_2O_2) can be reduced in the presence of element, thus creating one of the most powerful oxidising reagent, the hydroxyl radical ($OH^{\cdot}$). Although Cd does not appear to be working immediately on ROS production, it has been speculated that Cd generates ROS, leading to cell damage (Stohs and Bagchi, 1995). Several other mechanisms enable Cd to damage bacteria (Nies, 2013). The first mechanism is replacing Cd with other ions that occur in functional groups, which are located on the surface of an organism. This substitution is mainly performed with sulfhydryl groups, which are a part of the proteins, or with hydroxyl groups, the main groups of phospholipids, leading to protein damage and lipid oxidation (Chrestensen *et al.*, 2000). Therefore, the toxicity of Cd on the cell is dependent on fatty acids and protein compositions of its cell wall, in addition to the Cd concentration. The second mechanism involves Cd binding with cellular thiols groups, which are part of the proteins, leading to the oxidation of thiols. This oxidation sequentially reduces ATP and NADPH, leading to cell destruction. The third mechanism is DNA damage through $OH^{\cdot}$ oxidation, which occurs when $OH^{\cdot}$ attacks DNA, causing DNA to have permanent reductions in its structure and function. The destruction of the DNA leads to the inhibition of gene expression to proteins and prevents the growth of the cell. The high affinity of Cd with thiol groups is the main chemical basis of Cd toxicity. The superfamily proteins thioredoxin, which has Cys-Gty-Pro-Cys linkage in an active position, such as Dsb (disulfide bond proteins), are placed in the periplasm (Missiakas and Raina, 1997). Most organisms encode mechanisms that protect the proteins from the oxidative damage, and

the thioredoxin protein plays an important role in this process (Khairnar *et al*., 2013). During severe conditions of oxidative stress, these thioredoxin proteins become depleted or overloaded, so the cysteine residues become susceptible to oxidation. The proteins DsbA (disulfide bond A) and DsbB (disulfide bond B) were studied in *E. coli* (Rensing *et al*., 1997; Stafford *et al*., 1999) under severe conditions of oxidative stress.

2. Mechanisms of cadmium resistance in bacteria

Some bacteria have a variety of protection mechanisms that increase their tolerance to very high concentrations of Cd without any effect on their growth and metabolism. The protective mechanisms include efflux of the Cd out of the cell, extracellular sequestration, biosorption, precipitation, and bioaccumulation within cells (Bruins *et al*., 2000, Jebril, 2020a and 2020b). Generally, Cd resistance is defined by the minimum inhibitory concentration (MIC), which is the lowest concentration of a chemical that inhibits the visible growth of a bacterium (Jebril *et al*., 2022a). The MIC of bacteria is determined by incubating cultures with increasing concentrations of the chemical solution of interest on agar (well-known agar dilution) or in broth (well-known broth dilution) and respectively, evaluating the results by observing the number of bacterial colonies or by determining the biomass of the bacterial growth (Wiegand *et al*., 2008). Microbes are assumed to be tolerant to Cd if their MIC values exceed 0.8 mM Cd (Matyar *et al*., 2008). Table 1 illustrates some cadmium strains with their classifications and their natural MIC resistance levels. According to this table, the bacterial phyla *Actinobacteria*, as well as *Firmicute* and *Acidobacteria*, can resist Cd. In addition, strains that belong to Proteobacteria phylum have been reported to have a promising Cd-resistance compared to the other phyla (200 - 700 mM Cd, Ghosh *et al*., 1997; Mahapatra and Banerjee, 1996). *Proteobacteria* is a major phylum of Gram-stain-negative bacteria, while *Firmicutes* and *Actinobacteria* phyla are the Gram-stain-positive bacteria. Gram-stain-negative bacteria are commonly known to be more resistant to metals compared to Gram-stain-positive bacteria, due to their cell wall composition. Gram-stain-negative bacteria have a compound wall layer, consisting of the outer membrane, the plasma membrane, and the peptidoglycan layer. The outer membrane is a wall through which undesirable elements move from the inside to the outside the cell. The outer membrane consists of an exclusive molecule, LPS (lipopolysaccharide), proteins, lipoproteins, and porins. The plasma membrane, which forms up to 40% of the cell wall, is the high region, separating the outer membrane and the peptidoglycan layer. The peptidoglycan layer is a thin layer of about 3 nm and comprises 10% of the cell wall. In comparison, Gram-stained-positive bacteria contain a single wall layer, consisting of 30 - 90% of the peptidoglycan layer with an estimated thickness of 25 - 30 nm. In addition, secondary polymers, such as teichoic and lipoteichoic acids are formed from cell wall components. Gram-stain-negative bacteria occur in metal-rich environments, and it is reported that, under similar conditions, absorbs less than 10% of the amount of metals that Gram-stain-positive bacteria absorb (Konhauser, 2009). This low absorption rate is due to the metal associated with the functional groups of the outer membrane of Gram-stain-negative bacteria, LPS. Carboxyl groups in the LPS of the cell wall provide binding sites of sufficient electrical charge to bind metal cations to the cell surface effectively. LPS provides enough electrical charge, which can respectively bind with more minerals to the cell surface. LPS consists of three sections: lipid A, a core oligosaccharide, and an O-antigen polysaccharide. Lipid A is the most hydrophobic section, which is composed of glucosamine associated with acyl chains (fatty acids). Chemically, glucosamine and fatty acids are abundant with the carboxylic groups. The second part of LPS is the core, which is linked to the lipid contribution, contains the distinctive sugars of 3-deoxy- *D*-mannooctulosonate and *L*-glycerol-D-mannoheptose, in addition to *N*-acetylglucosamine, galactose and a number of additional sugars, which vary between the bacterial species. These acids and sugars provide large numbers of carboxyl groups, which are the main groups in LPS cores, providing a negative charge for COOH-Cd assembly. The O-antigen polysaccharide is a glycan polymer containing monosaccharides, resulting in a large number of carboxyl groups (Raetz and Whitfield, 2002).

Table 1. Minimum inhibitory concentration of some cadmium resistant strains with their classifications. *No strain code established

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
Phylum: "*Acidobacteria"*						
Acidimicrobiia	*Actinomycetales*	*Actinomycineae*	*Actinomyces turicensis*	AL36Cd	10	Oyetibo *et al*., 2010
Phylum: "*Actinobacteria"*						
Actinobacteria	*Micrococcales*	*Micrococcaceae*	*Arthrobacter ramosus*	AY509238	2.5	Abou-Shanab *et al*., 2007
			A.rhombi	AY509239	5.0	
			Arthrobacter sp.	D9	0.6	Aiking *et al*., 1985
		Microbacteriaceae	*Clavibacter xyli*	AY509235	5.0	Abou Shanab *et al*., 2007
			C.xyli	AY509236	5.0	
			C.xyli	AY509237	5.0	
			Microbacterium arabinogalactanolyticum	AY509224	2.5	

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
			M.arabinogalactanolyticum	AY509226	2.5	
			M.arabinogalactanolyticum	AY509220	0.5	
			M.arabinogalactanolyticum	AY509219	1.0	
			M.arabinogalactanolyticum	AY509221	2.5	
			M.arabinogalactanolyticum	AY509222	5.0	
			M.arabinogalactanolyticum	AY509223	1.0	
	Actinomycetales	*Corynebacteriaceae*	*Corynebacterium ulcerans*	CA56Co	2.0	Oyetibo *et al.,* 2010
			C.kutscheri	FL108Hg	10	
	Actinomycetales	*Streptomyceetaceae*	*Streptomyces*	CdTB01	50	Zhou *et al.*, 2016
Phylum: *"Firmicutes"*						
Bacilli	*Bacillales*	*Bacillaceae*	*Bacillus circulans*	ATCC11778	1.0	Baligarx, 2012
			B. cereus	ATCC11778	1.0	
			B. cereus	S5	10	Wu *et al.*, 2016

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
			B.subtilis	BNi11	2.5	Ndeddy and Babalola, 2017
			B. cereus	BCr26	0.2	
			B.cereus	BNi12	0.1	
			B.cereus	BNi22	2.5	
			B. pumulis	BCd2	5.0	
			B. safensis	BCr7	0.5	
			B.thuringiensis	DM55	0.25	El-Helow *et al.*,2000
			B.cereus	RC-1	0.98	Huang *et al.*, 2013
			B.flexus	AY509229	5.0	Abou-Shanab
			B.niacini	AY509227	0.1	*et al.*, 2007
			B. psychrosaccharolyticus	AY509230	5.0	
			B. megaterium	H3	0.9	Li *et al.*, 2017
		Paenibacillaceae	*Brevibacillus agri*	C15	16 ± 0.7	Jebril *et al.*, 2021b

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
			B. agri	C15 Cd^R	21 ± 0.4	Jebril *et al*., 2021c
			Geobacillus stearothermophilus	DSM 6453		Hetzer
			G.thermocatenulatus	DSM 5507	0.6	*et al*., 2006
					0.05	
		Moraxellaceae	*Acinetobacter junni*	CA109Cr	1.0	Oyetibo
						et al., 2010
Phylum "*Proteobacteria*"						
Alphaproteobacteria	*Rhodobacterales*	*Acetobacteraceae*	*Acidiphilium cryptum*	Lhet 2	700	Mahapatra and Banerjee, 1996
			A.symbioticum	KM2	700	
			A.symbioticum	H8	700	
			A.multivorum	JCM	700	
			A.multivoram	GS19h	400	Ghosh
			Acidocella facilis	*	200	*et al*., 1997

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
	Rhizobiales	*Rhizobiaceae*	*Rhizobium etli*	AY509210	0.1	Abou-Shanab
			R. etli	AY460185	5.0	*et al.*, 2007
			R.galegae	AY509213	0.1	
			R. galegae	AY509214	2.5	
			R. galegae	AY509216	0.1	
			R. galegae	AY509215	0.1	
			R. gallicum	AY509211	0.1	
			R.mongolense	AY509212	0.1	
			R.mongolense	AY509209	0.1	
			Sinorhizobium fredii	AY509217	2.5	
	Sphingomonadles	*Sphingomonadaceae*	*Sphingomonas alaskensis*	AY509242	0.1	Abou-Shanab
			S.asaccharolytica			*et al.*, 2007
			S.macrogoltabidus	AY509241	0.1	
				AY509243	0.5	

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
	Parvularculaceae	*Caulobacteraceae*	*Caulobacter crescentus*	AY512823	0.1	Abou-Shanab *et al*., 2007
Betaproteobacteria			*Alcaligenes feacalis*	BCd33	7.5	Ndeddy Aka and Babalola , 2017
			A. feacalis	BCr32	2.0	
			A.eutrophus	X58441	10	Abou-Shanab *et al*., 2007
		Comamondaceae	*Acidovorax avenae*	AY512827	2.5	Abou-Shanab *et al*., 2007
			A.delafieldii	AY512826	0.1	
			Variovorax paradoxus	AY512828	5.0	
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas putida*	KT2440	0.8	Miller *et al*., 2009
			P.aeruginosa	CA207Ni	10	Oyetibo *et al*., 2010
			P. aeruginosa	AL80Ni	2.0	
			P.aeruginosa	B237	8.0	Baligarx, 2012

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
			P. aeruginosa	BCr3	1.5	Ndeddy and Babalola, 2017
			P.aeruginosa	EP-Cd1	0.45	Muneer *et al.*, 2016
			P.riboflavina	AY512822	5.0	Abou-Shanab *et al.*, 2007
			P.stutzeri	MTCC101	0.7	Halder and Basu, 2016
			P. aeruginosa	CW-96-1	5.0	Wang *et al.*, 1997
	Betaproteobacteria	*Burkholderiales*	*Cupriavidus metallidurans*	DSM 2839T	4.5	Jebril *et al.*, 2022
		Lysobacterales	*Stenotrophomonas minatitlanensis*	AY512829	0.5	Abou-Shanab *et al.*, 2007
		Enterobacteriales	*Enterobacter cloacae*	CMCB-Cd1	1.90	Haq *et al.*, 1999

Class	Order	Family	Genus and species	Strain code	MIC (mM)	Reference
			Escherichia coli	ATCC25922	1.0	Baligarx, 2012
			Klebsiella aerogenes	NCTC 418	0.6	Aiking *et al*., 1985
			Klebsiella sp.	CMBL-Cd2	0.97	Haq
			Klebsiella sp.	CMBL-Cd3	1.97	*et al*., 1999
			K. yangling	12	1.51	Hou *et al*., 2015
			K. planticola	Cd-1	15	Sharma *et al*., 2000
			Proteus mirabilis	BNi6	2.5	Ndeddy and Babalola, 2017
			Salmonella enterica	43C	13.3	Khan *et al*., 2016
Acidothiobacillia	*Acidothiobacilales*	*Acidothiobacillaceae*	*Acidithiobacillus*	DDSM583	500	Baillet *et al*., 1997

The production of extracellular polymeric substance (EPS) by Gram-satin positive/negative bacteria allows the binding of metal cations to anionic carboxyl groups. The types of EPS vary in terms of the nature of their association with the cells, including C-EPS (capsular), S-EPS (slime), LB-EPS (loosely bound), and TB-EPS (tightly bound) (Żur *et al*., 2016). EPS consists of carbohydrates, homopolysaccharides, such as either cellulose and dextran or heteropolysaccharides (xanthan, gellan, and alginate) or proteins. Similarly, Poly-*L*-Lysine and poly-gamma-glutamate act as proteins in EPS (More *et al.*, 2014). These carbohydrates and EPS proteins provide sufficient hydroxyl groups for binding Cd, preventing Cd from cytotoxicity and increasing the cell resistance to Cd (Jebril, 2020c, Jebril *et al*. 2021d). Bacteria utilise a range of effective survival mechanisms to assist them in stressful conditions. The mechanisms of bacterial resistance to Cd are shown in Figure 2 A.

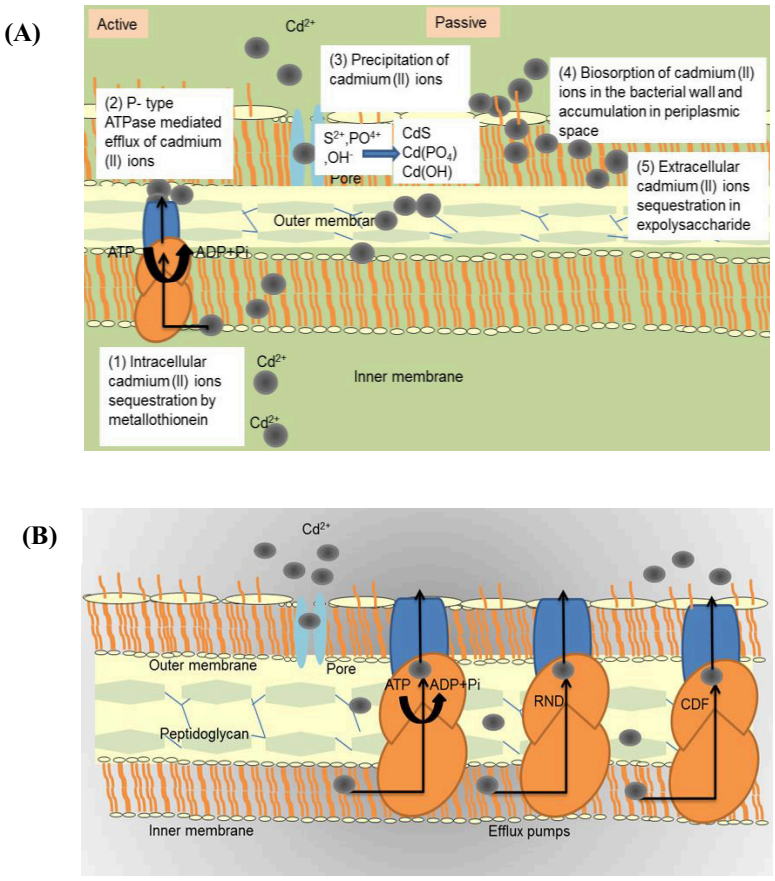

Figure 2. Schematic representation of the bacterial resistance mechanisms to Cd. **(A)** Bacterial defence against Cd. **(1)** Bacteria can deactivate Cd through binding with metallothionein for facilitating the sequestration. (2) The transport of Cd from the cytoplasm to extracellular spaces by efflux pumps. **(3)** Cd can be deactivated via binding with either S, OH, or PO_4 for facilitating precipitation of Cd or **(4)** extracellular substances outside cells for facilitating adsorption of Cd on the bacterial wall (ion exchange, complexation, and physical adsorption). **(5)** Extracellular polymeric substance serves the binding of Cd. The structure was adopted and redrawn according to Prasad *et al*. (2006); Sochor *et al*. (2011); Das and Dash (2017); and Abbas *et al*. (2018). **(B)** Efflux-mediated Cd resistance in bacteria. Cd enters the cell through a transporter. Upon the presence of efflux transporters: P-type ATPase, RND, and CDF, transporters start to pump Cd ions to the out cell. Abbreviations: ATPase (Adenosine triphosphate), RND (resistance-nodulation-cell division) and CDF (cation-diffusion facilitator). The scheme was adopted and redrawn, according to Nies (2003); Rensing and Mitra (2007) and Hynninen (2010).

2.1. Surface biosorption mechanism

P-type ATPase, RND, and CDF, transporters start to pump Cd ions to the out cell. Abbreviations: ATPase (Adenosine triphosphate), RND (resistance-nodulation-cell division) and CDF (cation-diffusion facilitator). The scheme was adopted and redrawn, according to Nies (2003); Rensing and Mitra (2007) and Hynninen (2010). Surface biosorption is an independent metabolism process. Several mechanisms are mediated by surface biosorption such, chelation, complexation, and surface precipitation (Vieira and Volesky, 2000). These mechanisms involve the binding of cadmium to functional groups in the microbial cell wall via electrostatic interactions. As mentioned earlier, the cell walls of the bacteria consist of peptidoglycan, which contains two molecules of NAG (*N*-acetylglucosamine) and NAM (*N*-Acetylmuramic acid), LPS (lipopolysaccharide) and proteins, lipoproteins, porins, and EPS. These components provide several functional groups such as -OH (hydroxyl), -COOH (carboxyl), $-PO_3$ (phosphate), $-NH_2$ (ammonia) and -SH (sulfhydryl), which generally have a negative charge, and concurrently a great binding attraction to Cd cation via van der Waals force or covalent bonding (chemisorption). In the adsorption mechanism (Bruins *et al*., 2000), Cd coordinates with the functional groups, forming cadmium complex, sorbed on the bacterial surface. For example, the reaction of Cd with sulphur bonding is

$Cd + S + 2e^- \rightarrow CdS$ (Xiao *et al*., 2020).

Following this process of forming CdS, the chelation process could occur (Bruins *et al*., 2000). As the cells are resistant to Cd exposure, they have evolved their chelation mechanism, such as the extracellular production of substances that can complex with Cd, and eventually, cover its occurrence. The chelation (also well-known as complexation) is a firm binding of Cd with a ligand such as EPS, resulting in a stable cadmium complex. The cadmium chelation is the formation of the coordinate bonds, and it is distinguished from a ligand, which forms a complex compound between the ligand and a single central atom of cadmium. EPSs in bacterial cells are related to their roles in the initial attachment of a cell to different unwanted molecules and Cd biosorption. The carboxyl groups of EPS contribute significantly to the Cd binding site at the bacterial wall of Gram-stain-positive bacteria; whereas, the phosphate groups, which are in the form phospholipids, contribute to Gram-stain-negative bacteria. Recently CadR binding protein, which is responsible for the biosorption of Cd, was found in *E.coli* BL21 (DE3), and the *capB* gene, responsible for Cd adsorption, was present in this strain (Qin *et al*., 2017).

2.2. Precipitation of cadmium on the bacterial cell surface

The positive charge at cell surfaces resulting from protonation of functional groups at low pH leads to a limitation of Cd cation sorption and their ensuing precipitation on the cell surface, and cadmium remains in solution. Conversely, at a high pH, the functional groups of the cell walls are associated with anions, permitting the approach of Cd cation, resulting in an adequate bond force for sorption to take place. Cell surface precipitation independent of metabolism involves the production of certain compounds when the cells are exposed to Cd, resulting in Cd precipitation. Bacterial cells produce a variety of compounds that react with Cd ions, forming cadmium compounds with different properties. Some of these cadmium compounds have low solubility constants. Some of the theoretical solubility constant in the water at 25 °C are 2.53 $X10^{-33}$ for $Cd_3(PO_4)_2$, 1 $X10^{-28}$ for CdS, 7.2 $X10^{-15}$ for $Cd(OH)_2$, 1.0 $X10^{-12}$ for $CdCO_3$, 2.6 $X10^{-4}$ for CdO and 4.3 $X10^{-1}$ for $CdSO_4$ (Rumble, 2018). With reduced solubility of Cd, the bioavailability and toxicity of the metal are reduced, and precipitation occurs. Macaskie and Dean (1982) studied the first biological precipitation of Cd by *Citrobacteria* sp. with phosphate, which was mediated by phosphatase to accumulate Cd as $CdHPO_4$. Whereas later, Cunningham and Lundie (1993) suggested the precipitation of Cd in *Clostridium thernoaceticum* as

CdS, which was formed by active metabolism of the cells that were able to produce sulfide in a growth culture containing EDTA. It has been reported that sulfides can be produced biologically by several mechanisms, for example, the engineered *E. coli* produced sulfide by heterologous expression of the thiosulfate reductase gene (Bang *et al*., 2000a). The gene responsible for the surface precipitation of Cd is the thiosulfate reductase gene (*phsABC*), which represents the production of hydrogen sulphide, in *Salmonella enterica* (Bang *et al*., 2000b).

2-3. Bioaccumulation of cadmium within the cells

Bioaccumulation is a complex detoxification process that involves either the localisation of metal within specific organelles or the enzymatic transformation of the metal within specific enzymes (Bruins *et al*., 2000). The bioaccumulation of Cd involves the intracellular accumulation of Cd, rather than including the enzymatic pathway, which is the possible transformation for the transmission of metals such as Hg, Se, and Cr. A metal-binding protein, such as the metallothionein can bind with cadmium ions, thereby aiding the accumulation of free-cadmium ions. Metallothioneins are cysteine-rich proteins that are capable of forming stable complexes with different metal cations, such as Cd, Zn, and Cu, to the thiol groups of cysteine (R-SH). Cadmium-metallothioneins sequester Cd within the cell, leading to bioaccumulation and facilitating Cd detoxification by prevention the interaction of Cd with other crucial proteins of cellular metabolism. The first report of bacterial cadmium metallothionein (in *Pseudomonas putida*) was in the early 1980s (Higham *et al*., 1984), and metallothionein was found to be responsible for the sequestration of Cd in *Synechococcus* spp (Li *et al*., 2020). The bioaccumulation of Cd in *Arthrobacter* sp. and *Pseudomonas* sp. has been documented by Scott and Palmer (1990) and Velásquez and Dussan (2009) reported that more ions accumulated in living cells than dead ones, due to two processes occurring: (1) the adhering of Cd onto the cell surface and (2) the accumulation of Cd inside the cell. Heterologous protein, LamB, which was obtained from gene cloning of *E. coli*, enables binding with Cd (Kotrba, 1999). Proteomic approaches showed that SBP`s (selenium binding protein) overexpression protects *Arabidopsis* from the toxic effects of Cd (Hugouvieux *et al*., 2009).

2.4. Efflux pumps

The transport of Cd from the outer membrane to the cytoplasm of the cells is a dynamic process. The transporters (either proteins or siderophores) are present at the cell surface, transferring the Cd and concentrating it within the cell. The basic strategy for reducing Cd concentrations within the cell is to pump Cd outside the cell (Wood and Wang, 1983). The efflux pumps are the central detoxification systems of divalent metal ions, including Cd. The P-type ATPase transporter is the primary efflux pump transporter, which is required for Cd resistant microbes to export Cd from their cytoplasm to periplasm. P-type ATPase transporter belongs to the transmembrane transporter family, the superfamily 1B-2 subgroup (Tsai and Linet, 1993). Three cadmium resistant transporters for the efflux pumps are shown in Figure 2 B: the resistance-nodulation-cell division (RND), the cation-diffusion facilitator (CDF), and the ATP-binding cassettes (ABC), (Leedjärv *et al*., 2008). These transporters can be divided further into two classes, CzcCBA, and Czn systems, which are categorised depending on the mechanism they use to pump Cd out of the cell (Stähler *et al*., 2006). Cd transport in Gram-stain-positive bacteria is similar to those of the Gram-negative bacteria, but their transport functions vary. The cation-proton antiporter in the Gram-stain-negative bacteria CDF is a transporter rather than a cation-transporting ATPase, which is the primary transporter in Gram-stain-positive bacteria (Nies, 2003). The proteins CDF, DmeF, and FieF in *Wautersia metallidurans* CH34 (Munkelt *et al*., 2004), and FrnE protein in *Deinococcus radiodurans* (Khairnar *et al.*, 2013) were

found to be the efflux proteins for Cd. RND proteins are the leading Cd exporters in Gram-stain-negative bacteria, as it has been found in *Ralstonia metallidurans* CH34 (Nies, 2003).

3. Molecular mechanisms of cadmium regulation in bacteria

Gesturing proteins, transcriptional regulators, and metabolites are the main mediators of the cell`s response to cadmium. Glutathione, glutamyl- cysteinyl-glycine (GSH) is the significant ligand of Cd ions in *E. coli* K-12. GSH contributed to gain resistance against Cd by using a GSH biosynthetic pathway (Suzuki *et al*., 2005). The activation of methanogenesis studied in *Methanosarcina acetivorans* under cadmium stress found an alternative process that increases ATP yield, which can be used as a biofuel (Lira-Silva *et al.*, 2012). Advancing proteomic analysis has identified the essential proteins to understand the mechanisms that defend microbes against Cd stress. For example, some bacteria resist Cd have been thoroughly studied, and their related genes have been identified. Zhai *et al.* (2017) demonstrated that *L. plantarum* CCFM8610 had prophage P2b 18, CadA, mntA, and lp –3327 proteins, as investigated by the proteomic analysis, and these proteins were associated with the genes: *cadA* and *cadC*. Some proteins have been identified as regulating active Cd translocation between cytoplasm and periplasm of the bacterial cells. These proteins mediate the transporter systems and regulate the Cd efflux, such as *OxyR*, and *OhrR* regulons in *Xanthomonas campestris* (Banjerdkij *et al*., 2005), and *czrA* regulon in *Caulobacter crescentus* (Valencia *et al*., 2013). Also, other types of proteins have been identified as regulating the active Cd uptake amongst the members of ZIP transporter, which are mainly involved in the Cd translocation across the cellular membranes. In *E. coli* GG48, two genes (*zntA* and *zitB*) were recognised to encode for ZIP transporter (Grass *et al.*, 2002). Some studies indicate the ability of ZIP transporters to carry and uptake Cd, for example, the transporters ZntA, and RcnA contribute to the Cd uptake of *E. coli* ECA580 (Taudte and Grass, 2010). It has been demonstrated that the *zntA* gene of *E. coli* K-12, encodes the Cd transporter, P-type ATPase ZntA, which belongs to the MerR transcriptional regulator family (Binet and Poole, 2000). Many genes such as *cadA*, *cadB cadC*, *cadD* and *cadX*, and many operons such as *cadA* and *cadC* mediate the efflux system (Busenlehner *et al*., 2002). The *cadA* operon has been reported in many Cd resistant bacteria such as: *S. aureus* (Nucifora *et al*., 1989), *Bacillus subtilis* (Tsai *et al*., 1992), *Listeria monocytogenes* (Lebrun *et al*., 1994), *Helicobacter pylori* (Herrmann *et al.*, 1999), *Stenotrophomonas maltophilia* (Alonso *et a*l., 2000) and *Pseudomonas putida* (Lee *et al*., 2001). The cadmium operon consists of two genes, the *cadA* gene, which acts as a P-type ATPase transporter, and the *cadC* gene, which acts as a control gene for the *cadA* gene. Figure 3 shows the *cadA* operon and regulation of the expression of Cd resistance in the *S. aureus* pl258 (Nucifora *et al*., 1989). When *S. aureus* pl258 is exposed to low concentrations of Cd, the Cd occupancy decreases it's binding to the regulatory binding protein of the *Rcad* promoter region, which inhibits the translation of mRNA. While, when *S. aureus* pl258 is exposed to a high concentration of Cd, the high occupation of Cd increases the binding of Cd to the *Rcad* promoter region, allowing the translation of mRNA and the expression of *cadA* gene, which confers the resistance to Cd. The genes, which are responsible for Cd resistance in bacteria, can be located on either plasmids or chromosomes. It is reported that the plasmids can encode a reduced susceptibility to any pollutant, which was firstly involved in the toxic metals (Bruins *et al*., 2001). However, it has been suggested that the efflux system, which avoids any internal accumulation of Cd, is due to the plasmid-dependent cadmium resistance. The plasmid-dependent cadmium resistance, the operons *cadA*, and *cadB* have been described in *S. aureus* (Kuroda *et al*., 2001).

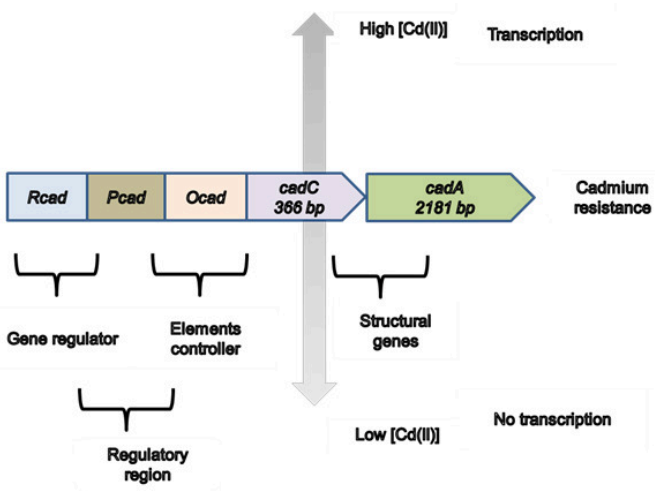

Figure 3. Molecular regulating of cadmium detoxification systems as an example of *cadA* operon structure in *Staphylococcus aureus* pl258 (Nucifora *et al*., 1989). This model was assumed and redrawn according to the regulation model of the expression of cadmium resistance in *Streptococcus thermophilus* 4134 (Schirawski *et al.*, 2002). The regulatory genes and levels of Cd can relieve mRNA-transcription and control the gene expression of *cadA*. The non-existence of binding protein with Cd at low Cd concentrations causes the *Rcad* promoter region to lose the ability of mRNA transcription. Stimulation of the binding of Cd to the *Rcad* promoter region at high Cd concentration causes the *Rcad* promoter region to gain the ability of the mRNA transcription and increases the gene expression of *cadA*.

In addition, the plasmid-dependent cadmium resistance of the transporter zinc-cobalt, *czcNICBADRS* has been demonstrated in plasmid pMOL30 of *R. metallidurans* CH3 (Nies, 1995). *czcNICBADRS* is an inducible non-ATPase cation efflux Cd, Zn, and Co system. However, the essential Cd resistance systems are usually chromosome-based and are more complex than plasmid-based resistance systems (Khan *et al*., 2015). Chromosomal mediation of the bacterial resistance to Cd could occur in the gene, gaining the genetic elements overexpression of the cadmium detoxification systems (Bruins *et al*., 2001). The chromosome of *P. putida* KT2440 includes the genes: *cadA1* and *cadA2* for P-type ATPase and the two CBA transporters are CzcCBA1 and CzcCBA2 (Cánovas *et al*., 2003). The possibility of the gene expression of the transporters: *cadA1* (P-type ATPase) and *czcCBA1* (CBA) is very low, while the expression of the gene *cadA2* (P-type ATPase) is very high (Leedjärv *et al*., 2008).

4. Bacterial mechanisms for cadmium bioremediation

The two resistance mechanisms that are capable of achieving cadmium bioremediation are biosorption and bioaccumulation. These mechanisms are physiochemical and/or catabolic processes. Physiochemical processes are non-biological, not dependent on metabolism, passive, and include biosorption (Jebril *et al*., 2022b). Catabolic processes are biological, active, and defined as the metabolic breakdown of molecules into smaller units, thus strictly dependent on cell metabolism and include bioaccumulation (Kim *et al*., 2016). However, both metabolic and non-metabolic processes can be responsible for the precipitation of Cd. The bacterial cell wall consists of peptidoglycan, teichoic acids, phospholipids, and lipopolysaccharides, which are the first useful elements for adsorbing Cd due to having anionic functional groups. Therefore, bacterial bioremediation can be divided into three main steps (Bruins *et al*., 2000): (1) Biosorption is a physicochemical process, in which Cd is passively adsorbed on to the surface of the bacterial cells. The bacteria cell wall consists of peptidoglycan ($C_9H_{17}NO_7$) that has sufficient anionic functional groups that enable binding with Cd. Therefore, biosorption occurs on the surface of either living or non-living bacterial cells, as the functional groups are available in both forms of the bacterial cells. The availability and types of these

functional groups control the process, which is also dependent on the species and concentration of Cd, which is determined by the pH and ion strength of the solution. (2) Bacterial cells act as the precipitation surfaces for cadmium, in a process independent of metabolism, resulting in cadmium accumulation. The adherence of Cd on the functional groups of the cell walls through different biosorption mechanisms is followed by the formation of Cd compounds with reagents produced by the cell that has low solubility and hence are prone to precipitation on the cell surface. The potential advantages of the precipitation of Cd as CdS compound over other compounds is the low solubility product compared to hydroxide, carbonate, and phosphate precipitation, leading to a fast precipitation rate and the re-use of sulfide. Several bacterial strains such as *Cupriavidus taiwanensis* KKU2500-3 and *P. aeruginosa* KKU2500-8 (Siripornadulsil and Siripornadulsil, 2013) can precipitate cadmium sulfide and generate sulphide under strict anaerobic or aerobic conditions. These strains can precipitate Cd as CdS due to having a cysteine desulfhydrase that has D-cysteine, which contains hydrogen sulfide (H_2S) (Sakimoto *et al*., 2016). This dissolved sulfide can then be complexed with Cd to form insoluble CdS precipitates. The precipitation of CdS is typically associated with the bacterial cell wall and occurs predominantly in the periplasmic space. Marusak *et al*. (2016) provided evidence for this process in engineered *E. coli*, from which they harvested, and purified nanoparticles (NP), and NP formed CdS. (3) Following the surface interactions (adsorption and precipitation), Cd-bioaccumulation as an intracellular interaction of cadmium ions with the cell components may occur. As Cd bioaccumulation is more efficient in live cells (Velásquez and Dussan, 2009), using live bacterial cells in bioaccumulation is desirable, and this requires adjustments of nutrients and other environmental conditions during bioremediation.

4.1. Phytoremediation of cadmium

Cd-bioaccumulation is well-applied in the remediation of Cd by rhizobia, diazotrophic bacteria to improve the accumulation of cadmium by plants in phytoremediation (Ike *et al*., 2007). Phytoremediation has also been used for the treatment of soil contamination with metals. The use of phytoremediation for cadmium and other compounds were first reported in the 1970s (Henry, 2000). Some plants have developed different mechanisms of selective phytoremediation of cadmium from soils. Most Cd phytoremediation in the root and shoot systems occurs via the process called phytoextraction, or through rhizofiltration, the adsorption and precipitation of cadmium in the plant`s roots. Another mechanism of phytoremediation is phytostabilisation, which is the process of reducing the migration of contaminants by limiting their mobility (Kuppusamy *et al*., 2016).

4.2. Bioremediation of cadmium involving Eextracellular Ppolymeric Ssubstance (EPS)

Bioremediation of Cd may be facilitated by cadmium uptake through the production of EPS. EPS can be used in bioremediation as mixed culture EPS, single-cell EPS, live, dead, or immobilised EPS. EPS contains polysaccharides, proteins, and lipids, which have functional groups such as sulfhydryl, amino, carboxylic, and phosphate groups that enable binding to metals. The accumulation of the metal ions onto the cell wall by EPS is used widely in the bioremediation of Cd and other metals, as listed in Table 2. The removal capacities for metals vary, depending on the reduction potential, atomic weight and the ionic size of the metal. Table 2 shows the variation in the removal capacities for certain metals that are related to the properties of the bacterium, such as active functional groups of EPS. Gupta and Diwan (2017) reviewed the advantages and disadvantages of using EPS in metal bioremediation: EPS is a cost-effective material and can be recycled for the adsorption and the recovery of metal ions. However, EPS shows low abilities for biosorption and requires maintaining the viability of live cells during the biosorption process. In addition to the contribution of EPS of bacterial cells in the detoxification system of Cd, its role in the bioremediation of Cd has been well-studied (Zhou *et al*., 2009).

Table 2. The removal capacities of some of the bacterial species derived EPS used previously for the bioremediation of some metals.

Bacterial species derived EPS	Removal capacity (nmol /g EPS)	Reference
Gloeocapsa gelatinosa	396 Pb	Raungsomboon *et al*., 2006
Calothrix marchican	314 Pb	Ruangsomboon *et al*., 2007
Lactobacillus plantarump	1333 Pb	Feng *et al*., 2012
Wangia profunda SM-A87	535 Cd	Zhou *et al*., 2009
Paenibacillus jamilaen	1449 Pb	Morillo *et al*., 2006
	187 Cd	
Azotobacter chroococcumn	161 Pb	Rasulov *et al*., 2013
	190 Hg	
Chryseomonas luteola	7.5 Cu	Ozdemir *et al*., 2005a
	20 Ni	
Chryseomonas luteola	570 Cd	Ozdemir *et al*., 2005b
Ochrobactrum anthropic	1117 Cr	Ozdemir *et al*., 2003
	412 Cu	
	263 Cd	

5. Conclusions

Bioremediation of cadmium from water based on natural microbes with resistance to Cd that are isolated in order to augment their resistance then, to further enhance their application in the bioremediation of Cd. Recent developments in the cadmium bioremediation process have increased the need for greater development on three key issues (1) Microbial resistance capability based on microbial relationships and cadmium concentration; (2) Lack of procedural knowledge needed to provide strategic guidance for environmental biotechnology, and (3) Lack of improvement in the bioremediation process.

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