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Bacterial resistance to lead: Chemical basis and environmental relevance

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ABSTRACT

Natural bacterial isolates from heavily contaminated sites may evolve diverse tolerance strategies, including biosorption, efflux mechanism, and intracellular precipitation under the continually increased stress of toxic lead (Pb) from anthropogenic activities. These strategies utilize a large variety of functional groups in biological macromolecules (e.g., exopolysaccharides (EPSs) and metalloproteins) and inorganic ligands, including carboxyl, phosphate and amide groups, for capturing Pb. The amount and type of binding sites carried by biologically originated materials essentially determines their performance and potential for Pb removal and remediation. Many factors, e.g., metal ion radius, electronegativity, the shape of the cell surface sheath, temperature and pH, are thought to exert significant influences on the abovementioned interactions with Pb. Conclusively, understanding the chemical basis of Pb-binding in these bacteria can allow for the development of effective microbial Pb remediation technologies and further elucidation of Pb cycling in the environment.

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Introduction

Heavy metals are generally referred to metals with densities greater than 4.5 g/cm^3. These metals can usually exert detrimental effects on the metabolism and growth of living organisms (Etesami, 2018; Li et al., 2019). Nonessential Pb is identified as a notorious representative of the heavy metals due to its high toxicity. The U.S. Environmental Protection Agency has included Pb in the list of hazardous inorganic wastes. The pollution level of Pb in the environment has been increased 1000 times over the past three centuries mainly as a consequence of rapid industrialization, which includes smelting, mining, the electroplating industry, sewage sludge, and waste incineration plants (Naik and Dubey, 2013).

Pb is able to accumulate and biomagnify along food chains and can therefore exceed its safety threshold in organisms (Luo et al., 2019; Naik and Dubey, 2013). Once absorbed, Pb is difficult to eliminate from living bodies; e.g., the Pb half-life in the blood of adult humans is approximately 30 days, and the excretory half-life for bone-associated Pb is as long as 16 years (Börjesson and Mattsson, 1995; Rabinowitz et al., 1976). Excessive Pb exposure in humans raises global health concerns, as this exposure can cause various deleterious effects, e.g., neurodegeneration, renal failure, reproductive damage and cancer. Molecular mechanisms responsible for these deleterious effects include altered conformation of nucleic acids and proteins, inhibition of enzyme activities, disruption of membrane functions and oxidative phosphorylation, and interference with homeostasis. Overall, significant anthropogenic input of Pb has posed serious threats to both environmental ecosystems and humans (Hu et al., 2019).

Pb contamination is also a great challenge to the survival and growth of primitive bacteria in human-impacted environments. Remarkably, bacteria may evolve diverse ways to tolerate Pb (e.g., extracellular immobilization, surface absorption, efflux mechanisms, intracellular precipitation, etc.) without any detrimental impacts on their growth and regular metabolism. Interestingly, these resistance mechanisms are often found in a large variety of bacterial strains with a wide taxonomic range; e.g., the strong absorption ability toward Pb, as reported in recent studies, is possessed by *Acidithiobacillus ferrooxidans* (Kim et al., 2012), *Citrobacter* sp. (Mohseni et al., 2014), *Bacillus pumilus* sp. (Sayyadi et al., 2017), *Pseudomonas aeruginosa* (Kang et al., 2008), and psychrotrophic strain *Pseudomonas* sp. I3 (Li et al., 2017b). Unlike organic contaminants, heavy metals are unable to be chemically or biological degraded and are only removed by changing the speciation and mobility of the heavy metals. Bacterial strains with unique characteristics of Pb tolerance

are undoubtedly important bio-resources for the development of bioremediation approaches for Pb-polluted environmental sites. Elucidating the chemical basis related to the above resistance mechanisms is substantial for improving the effectiveness and efficiency of Pb removal and elimination from environmental wastes.

Microbes play a pivotal role in metal cycling in the environment. Pb transformation and migration, mediated by microbes, are usually the byproduct processes associated with amelioration of Pb toxicity. The chemical basis of Pb resistance mechanisms adopted by microbes decides what Pb is converted into and how Pb is immobilized and mineralized in the environment. Therefore, elucidating chemical mechanisms related to bacterial resistance to Pb is of important environmental significance in Pb biogeochemical cycle. Phosphate solubilizing bacteria (e.g., *Pantoea* sp. and *Enterobacter* sp.) may release P from insoluble phosphorus-containing compounds, and simultaneously lead to the formation of Pb compounds with reduced solubility and bioavailability in the environment (Jin et al., 2011; Teng et al., 2019). It has also been reported that autochthonous bacteria (*Bacillus* sp. KK1) is able to increase Pb levels in the carbonate phase of bioaugmented soils through calcite precipitation, where $Pb(NO_3)_2$ is converted into PbS or $PbSiO_3$ with a decreasing toxicity (Govarthanan et al., 2013). Bacteria resistant to multiple metals (*Pseudomonas* spp. strain PG-12) enables plants (*Pteris vittata*) to rapidly grow and to significantly decrease Pb uptake in Pb-contaminated medium via extracellular sequestration, suggesting that bacteria can be a controlling factor in the transfer of Pb from external circumstance to plants (Manzoor et al., 2019).

Although many publications have reviewed Pb resistance of microbes (Das et al., 2016; Kushwaha et al., 2018; Naik and Dubey, 2013; Pan et al., 2017), we aim to discuss the chemical foundation on which bacterial resistance to Pb was developed. Insightful knowledge regarding the chemical basis of Pb resistance mechanism of bacteria and relevant important influencing factors are essential for understanding Pb-pollution microbiology and environmental chemistry, as well as establishing good Pb bioremediation methods.

1. Pb resistance mechanisms in bacteria

1.1. Extracellular immobilization of Pb(II)

The toxicities of heavy metals are highly dependent on their bioavailability. Soluble metals can more readily permeate the

cell membrane barrier, and thus, more readily cause adverse effects on bacterial growth or regular metabolism (Choi et al., 2018; Ferguson and Deisenhofer, 2004). Extracellular immobilization is considered one of the most effective Pb tolerance strategies employed by bacteria, which is achieved mainly via biologically secreted materials or ligands such as exopolysaccharides (EPSs) and phosphate.

Bacterial EPSs are identified as major components of the extracellular matrix and consist of polysaccharides, proteins, nucleic acids, lipids, uronic acid, glycoproteins and other nonpolymeric constituents (Sheng et al., 2010). EPSs are chemically diverse and contain a large variety of functional groups that serve as potential sites for sequestering heavy metals, including carboxyl, phosphate, and amide groups, and H-bonding partners (Vimalnath and Subramanian, 2018). The interactions of Pb and these functional groups were determined using Fourier-transform infrared spectroscopy analysis (FT-IR) (Oh et al., 2009). The carboxyl groups in these EPSs can approach metal ions along the C=O direction (Ibrahim, 2005), whereas amide groups mainly access metals through O atoms on the carbonyl group (Clement et al., 1998). Other components in EPSs (uronic and hexuronic acids) also contribute appreciably to bacterial biosorption of Pb and other metals (Morillo Perez et al., 2008). EPSs accounted for approximately 8% of the total Pb biosorption by the *Aeromonas caviae* strain KS-1 after exposure to 1.4 mmol/L $Pb(NO_3)_2$ (Shamim et al., 2013).

Many factors have significant influences on the interactions between bacterial EPSs and heavy metals, e.g., metal ion radius, electronegativity, the shape of the cell surface sheath, temperature, and pH. The biosorption capacity of *P. aeruginosa* ASU 6a biomass was higher for Pb than for Ni (Pb(II), 1.20 Å and Ni(II), 0.9 Å) (Gabr et al., 2008). The higher electronegativity of metals indicates a stronger attraction to negative organic groups, which may address the difference in the biosorption capacity of EPSs among metals. Metal biosorption of a marine algal biomass (*Sargassum* sp.) was in the order of Pb > Cu > Cd, which was in good accordance with their electronegativity (Pb(II) (2.33) > Cu(II) (1.90) > Cd(II) (1.69)) (Ping et al., 2007). It was also demonstrated that the biosorption capacity of capsulated cells (*K. pneumoniae*) for heavy metals was apparently higher than that of decapsulated cells (*C. freundii*) regardless of pH, initial concentration and contact time, suggesting that the capsule played an important role in Pb biosorption efficiency (Al-Garni, 2005). In general, the optimized pH for Pb biosorption was in the range of 4–7 (Al-Garni, 2005; Pardo et al., 2003). Under acidic conditions, EPSs are positively charged with a large abundance of H^+; thus, the interactions of functional groups with metal ions are partially blocked. EPSs appear to gradually become negatively charged as the pH is increased to their optimal value, which consequently facilitates the biosorption of metal cations by potential sites such as carboxyl, phosphate and amino groups (Pardo et al., 2003). When the pH is higher than 7, the metals are present mainly as insoluble hydroxides and are not accessible for biosorption by EPSs (Zhang et al., 1998).

Extracellular immobilization by Pb biosorption is a common microbial remediation strategy. The biosorption capacities of Pb(II), Cd(II) and Cu(II) by lyophilized cells of *Pseudomonas stutzeri* in aqueous solution were 142.0, 43.5 and 36.2 mg/g dry mass, respectively (Oh et al., 2009). EPSs of *Paenibacillus jamilae* exhibited high biosorption capacities for multiple metals, and Pb biosorption (303.0 mg/g) was approximately 10 times greater than those of the other metals (Morillo Perez et al., 2008). Addressing the chemical basis of extracellular immobilization will allow us to promote the biosorption capacity of Pb. Greater contents of uronic and hexuronic acids in bacterial EPSs correspond with better biosorption capacities of bacteria for heavy metals, e.g., the EPSs of *Bacillus firmus* contained uronic (38.0%) and pyruvic acids (6.3%) and its maximal Pb biosorption was 1103 mg/g (Salehizadeh and Shojaosadati, 2003). High abundance of acidic groups (i.e., S_1 (COOH), S_2 (NH_2) and $S_3PO_4^{3-}$) might enhance the Pb biosorption capacity of *Pseudomonas aeruginosa* ASU 6a that was isolated from metal-contaminated water (123 mg/g dry weight by dead cells and 79 mg/g dry weight by living cells) (Gabr et al., 2008). EPSs, in combination with citrate and oxalate, were used to significantly change the surface properties of an acidic Ultisol and effectively controlled the Pb mobilization in soils (Nkoh et al., 2019). Following *Rhodococcus opacus* EPSs being immobilized on synthetic microspheres, the monolayer capacity toward Pb (II) in aqueous solution was increased by 47% (Dobrowolski et al., 2019). Obviously, EPSs are considered one of the best options to remove and remediate Pb from the contaminated environmental samples.

Extracellular immobilization is also performed via the formation of insoluble Pb forms using inorganic ligands (e.g., phosphates, sulfides and oxides). Jin et al. (2011) reported that the dissolution of rock phosphate enhanced Pb immobilization by 8.3%–13.7% and 14.7%–26.4% for *Pantoea* sp. and *Enterobacter* sp., respectively. *Burkholderia cepacia* was able to precipitate Pb(II) as pyromorphite ($Pb_5(PO_4)_3Cl$) adjacent to the outer membrane (Templeton et al., 2003). The *Providencia alcalifaciens* strain 2EA isolated from battery-contaminated soil may be resistant to Pb concentration as high as 1.4 mmol/L by extracellular conversion of Pb to an insoluble solid ($Pb_9(PO_4)_6$), and this process may be catalyzed by a phosphatase enzyme (Naik et al., 2013). A red and red-brown pigment (Pb_3O_4) is able to fortify Pb tolerance of *Pseudomonas vesiculari* and *Streptomyces* sp. (up to 1300 ppm $Pb(NO_3)_2$) (Zanardini et al., 1997). Obviously, the above bacterial strains are able to transform toxic Pb into stable and immobilized inorganic minerals, which may be the main Pb storage form in polluted sites. This in situ conversion can substantially reduce the mobility and risks of Pb to living organisms.

1.2. Surface adsorption

Surface adsorption usually occurs on the surface or in cell wall structures of bacterial cells, and is considered a barrier for preventing toxic metals from entering cells and an important location for metal accumulation. The cell surfaces of bacteria are a series of concentric circles, including an ion layer, a micro gripping film, a cell aggregation layer, and cell walls. Metal surface adsorption is involved in the processes of ion exchange, precipitation, complexation, etc. The ion layer is located at the outermost layer of bacterial cells, where toxic metal ions are immobilized by replacing ions on this layer (Bai et al., 2014). Analysis using Scanning electron microscopy

coupled with energy dispersive spectrometry showed that ion exchange was the main mechanism for Pb removal from aqueous solutions using *Bacillus pumilus* sp. AS1 (Sayyadi et al., 2017). It was also suggested that the Pb biosorption capacity of *Pseudomonas putida* I3 was as high as 62.89 mg/g via ion exchange (Xu et al., 2017). Normally, Pb surface adsorption was performed via the synergism of different mechanisms. It was estimated that the total Pb sequestration of *Bacillus subtilis* DBM was collectively achieved by ion exchange (43.3%), intracellular precipitation (38.5%), immobilization in the cell wall (8.5%), and interactions of functional groups or precipitation on the cell surface (9.7%) (Bai et al., 2014).

The bacterial cell wall contains ample supply of binding sites for metals, e.g., carboxyl, phosphoryl, carbonyl and sulfhydryl groups on the cell wall, and N, P and O atoms on structural proteins. FT-IR analysis demonstrated that *Bacillus cereus* could immobilize Pb and Cu via the above groups on the cell wall (Pan et al., 2007). Phosphoric acid, as one of the main components in the cell wall of Gram-positive bacteria, is rich in carboxyl and phosphoryl groups that serve as metal binding sites. With respect to Gram-negative bacteria, lipopolysaccharides contain carboxyl groups that function as potential binding sites for metals (Beveridge, 1989). Our recent study first reported that a Pb-binding protein (flagellin), abundant with carbonyl-containing amino acids (aspartic acid, asparagine and glutamine), was found in prokaryotic species, and the stoichiometric amount of Pb incorporated into one single flagellin was estimated to be at least 16 (Chen et al., 2018). Heterogeneous expression of this Pb-binding flagellin considerably enhanced Pb accumulation in the cells of *E. coli.*, which confers this recombinant strain a great potential in the application of Pb remediation. Evaluation of metal adsorption on these cell surfaces using a chemical equilibrium modality demonstrated that the stability constants for metal–carboxyl complexes varied among metals, with log values of 3.4 for Cd, 4.2 for Pb, 4.3 for Cu, and 5.0 for Al, suggesting that these metal–surface complexes are stable enough for bacterial immobilization of metals in an aqueous solution (Fein et al., 1997).

Surface adsorption may be utilized for both Pb removal from waste samples and Pb detoxification in animals. The biomass of dead *Arthrobacter viscosus* was able to remove 97% of 100 mg/L Pb(II) from aqueous solution through adsorption (Hlihor et al., 2017). A novel biosorbent synthesized using endogenous bacterium *Bacillus licheniformis* may remove 98% of Pb from waste water with a maximum capacity of 113.84 mg/g via surface adsorption (Wen et al., 2018). *Lactobacillus bulgaricus* KLDS1.0207 has a remarkable Pb binding capacity and Pb tolerance, and its cell surface is covered with Pb as observed using scanning electron microscopy and energy dispersive spectroscopy analysis. This strain can be evaluated as a potential probiotic to ameliorate acute Pb toxicity via reducing Pb enrichment in the tissues of rats (Li et al., 2017a). The similar phenomenon and potential application was also found for the mice administrated with lactic acid bacteria (LAB) (Yi et al., 2017).

1.3. Intracellular precipitation

Once Pb permeates through the membrane barrier and enters the bacterial cells, intracellular precipitation may be adopted by bacteria. Intracellular precipitation is an important way to relieve the toxicity of absorbed Pb, during which insoluble Pb-containing compounds are produced similar to what occurs during extracellular immobilization (Table 1). (Levinson et al., 1996) explained that intracellular precipitation of Pb could be divided into 3 processes: (1) binding to the cell surface, (2) uptake of cellular ions, and (3) formation of crystalline Pb phosphates. Bacteria are so adaptable that they can select Pb resistance strategies according to the environmental conditions; e.g., a good paradigm is that *Pseudomonas fluorescens* employs intracellular accumulation of Pb in P-limited media instead of extracellular accumulation (Al-Aoukaty et al., 1991) (See Fig. 1.)

Pb may also be captured by macromolecular substances inside bacterial cells. Metallothioneins (MTs) are prominently able to capture a wide range of heavy metals within different parts of the cells – localizing heavy metals in order to prevent their toxicities (Murthy et al., 2011). Cysteine residues of MTs that exist in a reductive state exhibit great variability in their binding affinities for heavy metals. In particular, metal ions with a d^{10} electron configuration easily form metal-sulfhydryl clusters and conjugate to proteins (Peshkov and Khursan, 2017). MTs structurally contain α and β domains, which have varying combinations and stabilities in their binding to metal ions. In general, each MT can bind up to 7 divalent metal ions, where 3 of them bind with the β domain via 9 cysteines with a tetrahedral arrangement and the remaining ions are similarly held by 11 sulfhydryl groups in the α domain (Winge and Miklossy, 1982). A previous study demonstrated that ZmMT1 could coordinate with 6 Zn(II), 6 Cd(II), and 10 or greater Pb(II); the affinity of this protein to metal ions was as follows: Cd (II) > Pb(II) > Zn(II) (Duan et al., 2018). Bacteria can be modified

Table 1 – Intracellular Pb precipitates in bacteria.

| Intracellular Pb precipitation	Bacteria	References
PbS	Sulfate-reducing bacteria	Teekayuttasakul and Annachhatre (2008)
	Klebsiella aerogenes NCTC 418	Aiking et al. (1985)
	Acidithiobacillus ferrooxidans	Ye et al. (2017)
	P. aeruginosa CH07	De et al. (2008)
	B. pumilus S3	De et al. (2008)
	B. iodinium GP13	De et al. (2008)
	Rhodobacter sphaeroides	Li et al. (2016)
	Bacillus sp. KK1	Govarthanan et al. (2013)
$Pb_3(PO_4)_2$	*Enterobacter cloacae*	Park et al. (2011)
	Citrobacter freundii	Levinson and Mahler (1998)
	Staphylococcus aureus	Levinson and Mahler (1998)
	S. putrefaciens CN32	Smeaton et al. (2009)
$Pb_9(PO_4)_6$	*Vibrio harveyi*	Mire et al. (2004)
$PbSO_4$	*Rhodobacter sphaeroides*	Li et al. (2016)
$PbSiO_3$	*Bacillus* sp. KK1	Govarthanan et al. (2013)
$Ca_{2.5}Pb_{7.5}(OH)_2(PO_4)_6$	*Bacillus cereus* 12–2	Chen et al. (2016)
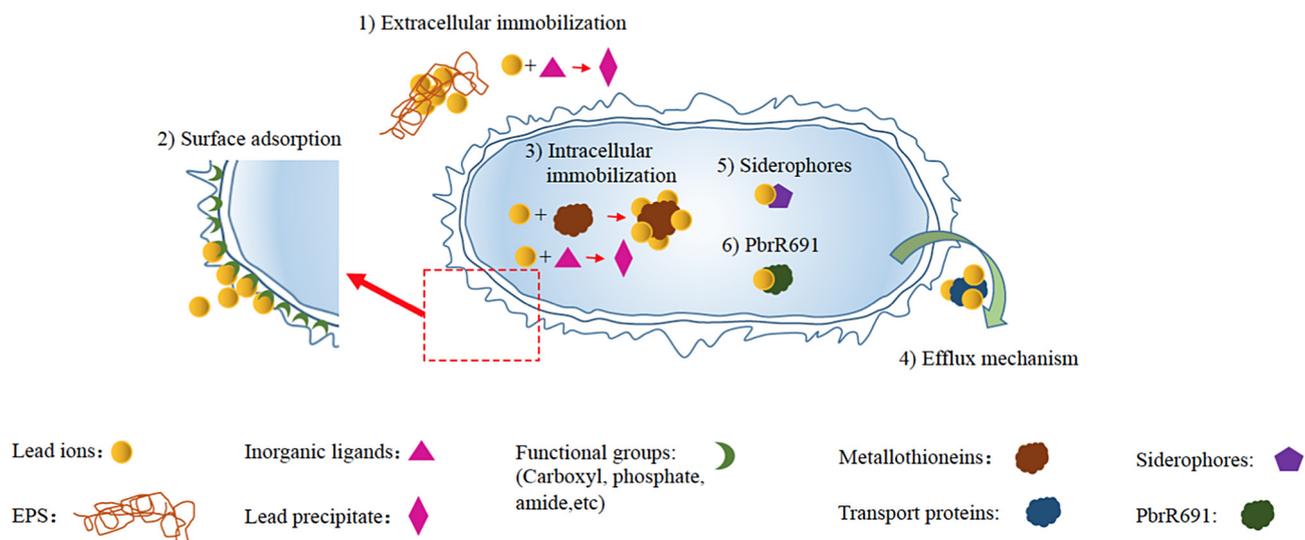
Fig. 1 – Schematic diagram showing Pb resistance mechanisms of bacteria.

by genetic engineering to induce high expression of specific Pb-binding proteins, improving their performance on Pb remediation. Many case studies have demonstrated that heterogeneous expressions of MTs in bacteria is beneficial for intracellular immobilization of Pb(II), e.g., *Tetrahymena thermophila* MTs (Zhou et al., 2018a), *Corynebacterium glutamicum* MT (Jafarian and Ghaffari, 2017), MBP-MT (Sauge-Merle et al., 2012), and *Bruguiera gymnorrhiza* BgMT2 (Huang et al., 2011). Moreover, bacterial MT product of the *bmt*A gene in *Pseudomonas aeruginosa* strain WI-1 significantly contributed to Pb bioaccumulation within the cells (26.5 mg Pb/g dry weight of cells) (Naik et al., 2012a) and MTs encoded by *smt*AB genes led to intracellular bioaccumulation of 19 and 22 mg lead per gram dry weight in *Salmonella choleraesuis* strain 4A and *Proteus penneri* strain GM10, respectively (Naik et al., 2012b). A comparative study further demonstrated that *E. coli* recombinantly expressing the type 2 metallothionein isoform (OsMTI-2b) could accumulate more Pb than when expressing OsMnTII-1b, OsMnTII-1a or OsMTI-3a, suggesting a distinct difference in Pb binding among MT isoforms (Pirzadeh and Shahpiri, 2016). These differences highlight that morphology of MTs may be a determinant for the effectiveness of genetically recombinant bacteria in Pb remediation.

1.4. Efflux mechanism

Expulsion of metals from cells is a common way for bacteria to fight against toxic metals. A large variety of bacterial strains possess efflux systems to effectively decrease the intracellular concentration of heavy metals. Such bacterial strains include *E. coli* (Fan and Rosen, 2002), *Enterococcus hirae* (Bissig et al., 2001), *Candida albicans* (Riggle and Kumamoto, 2000), and *Pseudomonas putida* (Adaikkalam and Swarup, 2002). The core engine in efflux systems is a family of proteins that are located at the cell membrane and function as ion pumps to transport metals across the bacterial membrane.

Metals may be expelled in active and/or passive modes: an active mechanism is when the efflux of metals is performed by transport proteins such as ABC transporters and P-type ATPases, accompanied by energy consumption; a family of resistance-nodulation-cell division (RND) proteins can utilize the proton gradient as the driving force for metal extrusion (a passive mechanism) (Ruggerone et al., 2013). The first layer for metal efflux is a family of RND proteins that export superfluous cations. Cation diffusion facilitators (members of the CDF family) may serve as secondary cation filters and make up the second layer for metal efflux. The third layer is a group of P-type ATPases (Nies, 2003). Additionally, there are other export systems, such as the CHR protein family, NreB and CnrT.

P-type ATPases can be categorized into two subgroups: (1) Cu(I)-translocating ATPases that are involved in the export of Cu(I) and Ag(I); and (2) Zn(II)-translocating P-type ATPases that extrude Zn(II), Cd(II) and Pb(II) out of microbial cells (Hou et al., 2001; Rensing et al., 1999). With respect to Pb transporters, PbtA (*Achromobacter xylosoxidans* A8) (Suman et al., 2014), ZntA (*E. coli*) and CadA (*Staphylococcus aureus*) (Rensing et al., 1998) all belong to the family of Zn(II)-translocating ATPases. Interestingly, elaborate collaboration of Zn(II)-translocating ATPase PbrA and undecaprenyl pyrophosphatase PbrB, with the former functioning to remove Pb(II) from the cytoplasm and the latter capturing Pb(II) in the form of insoluble phosphate (Hynninen et al., 2009), substantially contributes to the Pb tolerance of *Cupriavidus metallidurans* CH34.

The interaction of protein transporters with metals (e.g., the sites, affinity, and specificity or coverage of metal binding) is a prerequisite for metal efflux out of bacterial cells. P1-type ATPases usually contain a polar amino-terminal domain where a conserved motif (GXXCXXC) serves as a metal binding site. A comparison of the metal specificity between ZntA and ΔN-ZntA (lacking the N-terminal domain) demonstrated that the N-terminal domain of ZntA was not necessary to the resistance to Pb(II) (Hou et al., 2001), but increased the metal-

binding capacity and catalytic rate of ZntA (Mitra and Sharma, 2001). Liu et al. (2005) reported that motifs with a sequence of GXXCXXC could bind Cd(II) and Zn(II) instead of Pb(II); however, proteins containing the CCCDGAC sequence might act as good binding sites for Pb(II). This finding was in good accordance with another study in which cysteines in the CCCDGAC motif were mainly responsible for Pb-binding activity (Dutta et al., 2005). In other words, Pb efflux efficiency was highly related to the amount of CCCDGAC motif repeats present in the transport proteins. Identification and characterization of these Pb-binding motifs provide useful clues for designing and bio-synthesizing novel Pb-binding proteins with good affinity and capacity.

Generally, efflux mechanisms are not suitable for the development of Pb remediation approaches. However, transporter and regulation proteins related to these mechanisms are good materials to synthesize highly specific and sensitive Pb sensors due to their good affinities to Pb. The *cadC*-controlled T7 RNA transcription systems (*cadC*, a heavy metal-responsive transcription factor) were constructed to combine with a microfluidic platform for inventing heavy metal biosensor devices with increased sensitivity (Kim et al., 2016). A previous study utilized synthetic biology principles to develop plasmid-based whole-cell bacterial biosensors for detection of lead, which included *pbr*R gene as the core genetic element. The biosensors developed here could achieve a rapid, specific and sensitive monitoring for lead-contaminated water (Bereza-Malcolm et al., 2016). This group of Pb sensors could be a good tool for Pb monitoring due to their advantages regarding both detection performance and biological compatibility such as easily recombining into living systems.

1.5. Binding of Pb with siderophores

Bacteria, fungi and monocotyledonous plants can produce siderophores to capture Fe inside cells – the mechanism of which was developed in response to environmental stresses of heavy metals over a long-term evolution. Siderophores have low-molecular mass and are iron-chelating agents (0.2–2.0 kDa). They are characterized by an extremely high affinity for Fe(III). In an Fe-deficient environment, bacteria can synthesize iron receptor proteins on the extracellular membrane, which can specifically identify the Fe-siderophore complex and further transport Fe(III) into the cells (Rajkumar et al., 2010). Structurally, the coordination of three siderophores with one Fe(III) forms a stable octahedron complex (Boukhalfa and Crumbliss, 2002). It has been documented that siderophores of *P. aeruginosa* may chelate many toxic metals (e.g., Co, Cu, Ni, Pb, and Zn) in order to confer resistance against these metals (Braud et al., 2010). Siderophores (in particular, pyochelin and pyoverdine) secreted by *Pseudomonas aeruginosa* 4EA (Naik and Dubey, 2011) and *Pseudomonas putida* KNP9 (Tripathi et al., 2005) increased Pb tolerance of these bacteria. Siderophores have several binding sites specific for metals. For instance, azotobactin δ, which belongs to the fluorescent pyoverdine-like chromopeptidic hexadentate siderophores, possesses 3 Fe(III)-binding sites, viz. one hydroxamate group, one α-hydroxycarboxylic group and one catechol group (Palanché et al., 2004). The chelation ability of siderophores can be explained by the lone pair electrons in functional groups; e.g., the lone pair electrons of O and N atoms on the hydroxamate group may readily increase its metal-chelation ability, allowing for the formation of a stable polycyclic chelation of this group with metals.

1.6. Binding with Pb(II)-specific proteins

Specific proteins of the MerR family can also bind with Pb(II) in order to confer resistance: PbrR691 protein and its homologs expressed by *Cupriavidus metallidurans* CH34 are identified as the only proteins with a unique selectivity to Pb(II) found in nature. A fluorescent Pb(II) probe was designed by cloning and expressing the *pbr*R691 gene from *Cupriavidus metallidurans* CH34, and this probe exhibited almost a 1000-fold higher selectivity toward Pb in comparison to other metals (Hg, Cd, Zn, and Ni) (Chen et al., 2005). This highly specific combination was further analyzed to better understand the unique Pb coordination chemistry. It was earlier determined that the hybridization of *s* and *p* orbitals produces active lone pair electrons, and the repulsion force between lone electrons and bonded electrons prefers the adoption of a hemidirected geometry with a lower coordination number (2–5) rather than a holodirected structure (9–10) (Shimoni-Livny et al., 1998). A molecular mechanism of Pb-specific recognition by PbrR691 was proposed where the hemidirected geometry of this protein excluded the accessibility of other metals to the binding sites, with the exception of Pb(II) (Chen et al., 2007). Unique Pb selectivity of this probe implies its great potential in microbial monitoring and remediation of Pb pollution.

2. Theoretical chemical basis of Pb biosorption

The hard and soft acids and bases (HSAB) theory can currently be regarded as the main theoretical principle to understand the interactions between functional groups and metals (Pearson, 1963). The HSAB theory is used to understand the stabilities of chemical compounds and to predict the direction of chemical reactions. Basically, the most stable interactions are thought to be combinations of hard base–hard acid (ionic bonding) or soft base-soft acid (covalent bonding) (Minkin, 1999). According to the HSAB theory, ligands that contain O and N atoms are hard bases and thus prefer to bind with hard acids (e.g., IA, IIA, IIIA, IIIB, Ln^{3+}, Fe^{3+}, and Co^{3+}). In contrast, ligands with C, S and Se atoms are soft bases, and preferentially interact with soft acids (e.g., Ag^+, Hg^{2+} and Cd^{2+}). Metal ions, such as Pb^{2+} and Cu^{2+}, are identified as borderline acids. Pb(II) has a high polarizing power and forms a preferential interaction with soft bases (such as cysteine and histidine residues in proteins), which contain S, N and O atoms (Lippard and Berg, 1994). PbS is the most common and nontoxic inorganic form produced intracellularly or extracellularly by bacteria (Rensing et al., 1999).

Furthermore, the HSAB theory is helpful in addressing the competition for binding sites among different metals. Pb ions are also more efficiently bound to carboxyl groups than are Cd ions (soft acid) (Benettayeb et al., 2017). Sulfonic groups

exhibited a stronger chelating ability for Pb(II) than Cd(II) (Yipmantin et al., 2011). Competitive power is also relevant to electronegativity, and metal ions with higher electronegativity values always show a stronger ability to bind with negative groups (Al-Aoukaty et al., 1991).

3. Chemically modified biosorbents

Biosorbents can be chemically modified to increase their Pb adsorption using our current knowledge regarding composition and types of active sites for Pb binding. These chemical modification methods include acylation, alkylation, redox reactions, and substitution of hydroxyl, carboxyl, amino and thiol groups of biological molecules on aromatic rings, which may considerably alter the surface properties of living organisms and enhance their adsorption of heavy metals (Vijayaraghavan and Yun, 2008). Moreover, acidic pretreatment (e.g., amic acid) in order for enhancement, modification and polymerization of specific binding sites are common modification procedures adopted by many researchers (Yu et al., 2007). Waste textiles can be modified through grafting with acrylic acid via radical polymerization process using ceric ammonium nitrate as an initiator and microwave and/or UV irradiation as energy supply, and treated materials are able to remove approximately 95% of Pb(II) from the aqueous solution containing 10 mg/L at pH 6.0–8.0 (Zhou et al., 2018b). *Fucus vesiculosus* biomass was pretreated with different reagents, including HCl, $CaCl_2$, formaldehyde, Na_2CO_3 and NaOH, and the results showed that $CaCl_2$ was the most effective reagent for increasing the biomass sorption of Cu, Pb and Ni, where Pb absorption capacity in particular was enhanced by up to 20% (Rincón et al., 2005). The treatment of $CaCl_2$ may induce a cross-linking reaction of the alginate polymeric chains and form a new molecular structure with large cavities occupied by calcium ions. These calcium ions retained by alginate plays a significant role in metal biosorption via ion exchange. Crosslinked PEI was also introduced into *Penicillium chrysogenum* biomass via amine and carboxylate groups, and consequently, the sorption capacities of the modified biomass for Cu, Pb and Ni were greatly increased (Deng and Ting, 2005).

4. Conclusion and perspective

Bacteria have the ability to utilize the interactions between Pb and functional groups of biological materials for fighting against Pb toxicity. The stabilities of these Pb-containing complexes can be predicted and evaluated using theoretical chemical principles. The chemical structure and amount of functional groups in biomolecules (e.g., C=O-containing and phosphate groups), as well as environmental conditions such as pH and temperature, determine the binding affinities and capacities for Pb. These principles usually relate to coordination structure, radius and electronegativity. Understanding these chemical principles and how they allow bacteria to tolerate Pb may provide explicit clues on chemical or genetic modifications that can be harnessed for Pb bioremediation

and detoxification, as well as the exact roles of bacteria in environmental Pb cycle.

The identification and characterization of Pb-containing biomolecules is the initial step in elucidating Pb resistance mechanisms of bacteria and further developing effective microbial remediation approaches. Certainly, the rapid development of robust and sophisticated analytic separation and detection techniques will allow us for efficient discovery of novel Pb-containing biomolecules. Further characterization of these biomolecules will shed light on the abundance and diversity of Pb-interaction sites (e.g., carboxyl, phosphoric, amine, and hydroxyl groups). Structural morphology is also necessary to depict the precise three-dimensional position of interaction sites in biological macromolecules as some of these interaction sites are buried, impeding their accession to Pb.

State-of-the-art next-generation sequencing platforms and potent bioinformatics approaches can deliver a global analysis to further investigate the biological response of bacteria to Pb. These techniques may maximize the possibility of delicately parsing the entire process of achieving Pb tolerance in terms of transcriptomics and proteomics. These processes include Pb uptake and efflux, and signal transduction and biosynthesis of biological molecules to immobilize and inactivate Pb. With an explicit focus on newly identified Pb-containing biomolecules, bioinformatics analysis will provide accurate information on Pb-tolerance mechanisms, e.g., identification of DNA sequences from which to synthesize these molecules and investigation of intracellular regulation thereof.

Finally, standard genetic engineering is a useful tool that can be utilized to promote and/or combine the valuable features of Pb-resistant bacteria and develop effective remediation approaches. Bacterial strains can be genetically modified to increase production of Pb-interacting biomolecules and enhance their Pb biosorption. From a perspective of synthetic biology, new bacterial strains with optimized performance for metal removal can be expectably invented for the remediation of severe and complex metal pollution based on all of our gradually increasing knowledge regarding bacterial tolerance to metals. Agents with a good performance for Pb binding can also be designed and artificially synthesized for new Pb remediation methods.

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