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Metabolic reengineering invoked by microbial systems to decontaminate aluminum: Implications for bioremediation technologies

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ABSTRACT

As our reliance on aluminum (Al) increases, so too does its presence in the environment and living systems. Although generally recognized as safe, its interactions with most living systems have been nefarious. This review presents an overview of the noxious effects of Al and how a subset of microbes can rework their metabolic pathways in order to survive an Al-contaminated environment. For instance, in order to expulse the metal as an insoluble precipitate, *Pseudomonas fluorescens* shuttles metabolites toward the production of organic acids and lipids that play key roles in chelating, immobilizing and exuding Al. Further, the reconfiguration of metabolic modules enables the microorganism to combat the dearth of iron (Fe) and the excess of reactive oxygen species (ROS) promoted by Al toxicity. While in *Rhizobium* spp., exopolysaccharides have been invoked to sequester this metal, an ATPase is known to safeguard *Anoxybacillus gonensis* against the trivalent metal. Hydroxyl, carboxyl and phosphate moieties have also been exploited by microbes to trap Al. Hence, an understanding of the metabolic networks that are operative in microorganisms residing in polluted environments is critical in devising bioremediation technologies aimed at managing metal wastes. Metabolic engineering is essential in elaborating effective biotechnological processes to decontaminate metal-polluted surroundings.

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Contents

1. Introduction

Metal pollution is a threat that all organisms must contend with, particularly in an increasingly industrialized environment. The adverse effects of metal toxicity have been well documented. Heavy metals such as mercury, lead and cadmium as well as toxic metals like Al and chromium in addition to metalloids like arsenic are known to cause major damage to the ecosystem (Bernhoft, 2012; Godt et al.,

Abbreviations: ACN, aconitase; AGODH, acylating glyoxylate dehydrogenase; FUM, fumarase; G6PDH, glucose-6-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase; ICL, isocitrate lyase; KGDH, alpha-ketoglutarate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; NADK, NAD kinase; NOX, NADH oxidase; OCT, oxylate-CoA transferase; PC, pyruvate carboxylase; PE, phosphatidylethanolamine; PEPCK, phosphoenolpyruvate carboxykinase; ROS, reactive oxygen species; SCS, succinyl-CoA synthase; SDH, succinate dehydrogenase.

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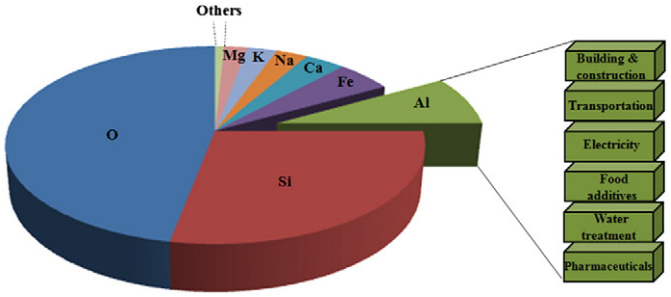

Fig. 1. Elemental composition of the earth's crust and the industrial applications of Al.

2006; Jomova et al., 2011; Patrick, 2006). Given its prevalence in most aspects of our lifestyle, the noxious effects of Al are now beginning to emerge. This trivalent metal is the most abundant metallic element in the earth's crust yet, despite its ubiquity, biological systems have seemingly circumvented the incorporation of this metal in their processes (Verstraeten et al., 2008). The apparent exclusion of Al from all living systems certainly raises questions about its toxicological significance. In spite of an expanding body of literature detailing the harmful consequences of overexposure to Al, there is still substantial debate regarding its safety and usage (Mailloux et al., 2011; Tomljenovic, 2011). Although Al is plentiful, the free metal is almost non-existent, and much of this element exists in complexes which make it inaccessible to most organisms. The increased bioavailability of this metal correlates well with increased anthropogenic activity and utilization (Verstraeten et al., 2008).

The desirability of Al lies in its chemical properties. Being a light, corrosion-resistant material, Al and its alloys have gained considerable traction in numerous fields of human progress (Fig. 1). As the most widely used non-ferrous metal, primary Al production in 2011 was 43.4 million tons, a number that is only predicted to increase in coming years (International Aluminum Institute, 2012). Transportation, particularly in the aerospace industry, benefits greatly from Al's high strength to weight ratio. Furthermore, Al's superconductive properties render it an ideal lightweight alternative to copper wiring. The increase in industrialization has brought about a higher frequency of acid rain, thus lowering the pH of soil and allowing Al to leach into groundwater. This leads to the bioaccumulation of the metal in vegetation and livestock (Piña and Cervantes, 1996). Al's industrial applications notwithstanding, the majority of the exposure to this metal for the average individual comes from diet and drinking water. Certain medications, such as antacid tablets, contain several hundred milligrams of aluminum hydroxide. Additionally, Al is a popular food additive in the form of sodium aluminum phosphates (SALPs). The latter is generally added as emulsifying salts to processed cheese and cheese spreads (López et al., 2002; Yokel et al., 2008). Al is often used as a coagulant in the form of aluminum sulfate ($Al_2(SO_4)_3$) or polyaluminum chloride (PACl). The application of either compound in drinking water treatment allows for the removal of particulate and dissolved contaminates. However, the end result of this process is usually a higher concentration of Al than was in the water initially (Soni et al., 2001). The average daily human intake of Al from all sources combined ranges between 6 and 14 mg, with variations stemming from age, sex, diet and location (López et al., 2002).

2. Toxicopathology of Al and adaptation to this trivalent metal

2.1. Al toxicity

Once in the body, Al is distributed and accumulated, particularly in the kidneys, liver and brain. Indeed, this trivalent metal has been linked to several disorders in human beings (Exley and Esiri, 2006; Exley et al., 2006; Lemire and Appanna, 2011; Mailloux et al., 2011; Nayak, 2002). While no single mechanism has been identified which accounts for Al's neurotoxic effects, there is a body of literature detailing its pro-oxidant properties in a number of species (Esparza et al., 2003; Exley, 2004). At concentrations exceeding 0.1 mg/mL in acidic waters (pH 5.0–5.5), Al is acutely toxic to aquatic fauna. Al ions accumulate on and subsequently clog the gill, thus bringing about respiratory dysfunction and accelerating cell necrosis in the gill epithelium (Exley et al., 1991). The induction of oxidative stress and disruption of antioxidant enzymes and neurotransmitter synthesis are apparent in the grass carp *Ctenopharyngodon idella* when exposed to an environment containing 0.1 mg/L Al (Fernández-Dávila et al., 2012). Furthermore, it has been observed that the bioaccumulation of Al in fish can potentially cause damaging effects higher in the food chain (Oberholster et al., 2012).

Research dealing with the exposure of Al in microorganisms has further elaborated on the mechanisms underlying the toxicity of this metal. Concentrations of Al lower than 3 mM have been shown to inhibit the growth of *Escherichia coli*. However, sensitivity to this metal increased with decreasing pH (Guida et al., 1991). Furthermore, the uptake of Al is known to utilize Fe transport systems, thus interfering with the ability of this microbe to capture this essential micronutrient (Davis et al., 1971; El Hage Chahine et al., 2012). This trivalent metal has also been shown to regulate bacterial motility, disrupt plant nodulation and perturb photosynthesis and nitrogen fixation in bacteria (Appanna, 1989; Guzzo et al., 1991; Munns, 1986; Pettersson et al., 1985). Although the molecular details underlying the toxicity of this metal have not been fully delineated, it is clear that the remediation of Al in polluted ecosystems is of paramount importance (Table 1).

2.2. Al-mediated disruption of cell function

While Al itself has no role in biological processes, it interferes with metals that do, such as Ca, Mg and Fe (Fig. 2). Al has been shown to increase the intracellular concentrations of Ca, thus perturbing Ca-dependent pathways and contributing to neurotoxicity (Mundy et al., 1997). Mg is known to stabilize phosphate groups in enzymatic reactions and on nucleic acids. Al has been shown to bind to ATP, a key binding partner of Mg, 10^7 times more tightly than Mg itself (Piña and Cervantes, 1996). Indeed, this complex with ATP has been implicated in the progression of neurodegenerative disease (Exley, 2012). The ability of the Al cation to compete with Mg promotes the loss of membrane fluidity and the disruption of replicative and transcriptional processes in the cell (Johnson and Wood, 1990). As Al and Fe have similar ionic properties, they compete for Fe binding sites in biomolecules. However, while Fe is redox active, Al is not, and the substitution inactivates the target molecule (Middaugh et al., 2005). As we shall see later, this perturbation disables central metabolism and pressures the organism to adapt if it is to survive. Once displaced, labile Fe becomes

Table 1
Examples of locations where the concentration of Al exceeds the maximum permissible limit as defined by regional guidelines.

Region	[All]	Maximum permissible [all] limit	Source
Gironde/Dordogne, France	Up to 0.514 mg/L	0.1 mg/L	Rondeau, V et al. 2009
Biga Peninsula, Turkey	Up to 15.7 mg/L	0.2 mg/L	Bakar, C et al. 2009
Xi'an city, China	Up to 2.773 mg/L	0.1 mg/L	Wang, D et al. 2012
Chimaliapan wetland, Mexico	Up to 24.82 mg/L	0.02 mg/L	Garcia, G et al. 2012
Maresh and Luda Yana rivers, Bulgaria	Up to 110 mg/L	0.1 mg/L	Rabadjieva, D et al. 2009
Ogun State, Nigeria	Up to 14 mg/L	0.2 mg/L	Odukoya, O et al. 2010

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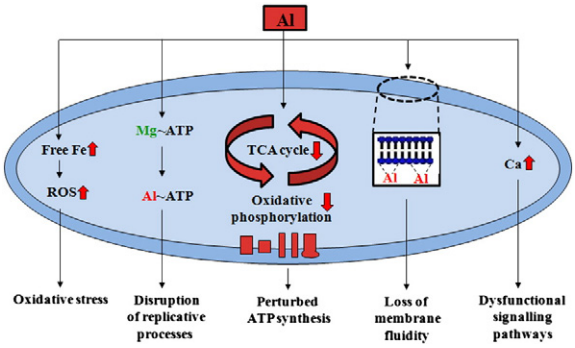

Fig. 2. An overview of the cellular processes affected by an Al-rich microenvironment.

a potent generator of hydroxyl radicals via the Fenton reaction, thus creating oxidative stress and exacerbating the burden brought about by Al (Mailloux et al., 2011). Additionally, this metal is known to interfere with protein kinase C, cAMP homeostasis and glutamate-nitric oxide synthase-cGMP signaling networks (Hartle et al., 1996; Lajeunesse et al., 1998; Quarles et al., 1994).

2.3. Adaptation to Al

To date, most studies on Al tolerance have been performed on plants, where the interaction of this metal with the root apex in acidic soils has been linked to the reduction of crop yields (Krill et al., 2010). The activation of anion transporters in the plasma membrane permit the plant to exude organic acids and phenolic compounds which chelate Al and negate its toxicity (Krill et al., 2010). Several species of fungi are known to adopt a similar strategy to dispose of this trivalent metal, utilizing negatively charged metabolites such as citrate and malate to chelate and reduce free Al (Klugh and Cumming, 2007). Indeed, such a strategy limits the bioavailability of the metal in the vicinity of plant roots, thus conferring resistance to Al's noxious effects (Kelly et al., 2005).

Mechanisms of tolerance tailored specifically to this metal are scarcely found in microbial systems. In general, chelation by organic acids, followed by exudation or compartmentalization is commonly utilized to control Al's dangerous effects. However, it has recently been discovered that the gene product of G2ALT from the thermophilic bacterium *Anoxybacillus gonensis* is a PP-loop type ATPase which plays a role in Al expulsion in the microbe. Indeed, G2ALT has an 87% similarity with alu1-p, a gene associated with the Al tolerance phenotype from *Actinomyces viscosus* (Beris et al., 2011). Reports of a community phylogenetically related to the dissimilatory-sulfate reducing bacteria *Desulfovibrio desulfuricans* describe their ability to remove 85% of Al in a 0.9 mM solution in the presence of the sulfate anion. X-ray diffraction (XRD) and transmission electron microscopy (TEM-EDS) were utilized to uncover the precipitation of this metal in an amorphous Al-hydroxide aggregate after 27 days of incubation (Martins et al., 2012).

The combination of clay and strain MAM-4 of *Providencia rettgeri* has been applied to the removal of Al, Cu and Co from wastewater. Indeed, the negatively charged clay in conjunction with the ability of *P. rettgeri* to withstand high concentrations (>18 mM) of Al permitted the removal of 87% of this trivalent metal. Fourier transform infrared spectroscopy (FTIR) delineated that hydroxyl, carboxyl and phosphate groups were applied by the microbe for the biosorption of these heavy metals (Abo-Amer et al., 2012). In another study, the generation of exopolysaccharides by nitrogen fixing *Rhizobium* strains correlates with resistance to concentrations of Al up to 1 mM in acidic conditions. Additional defense mechanisms in these microbes include the reduction of negative charges in the cell membrane and the formation of insoluble aluminum phosphate complexes (Avelar Ferreira et al., 2012).

Although a plethora of Al-resistant microorganisms have been uncovered, a detailed analysis of this metal's toxic effects on intracellular pathways has eluded researchers. Here, we elaborate on how the soil microbe *Pseudomonas fluorescens* utilizes its metabolic versatility to survive in Al-rich environments. The reengineering of metabolic networks aimed at eliminating Al and fending ROS is described. Furthermore, the significance of these Al-induced metabolic modules in bioremediation technologies is elaborated. It is critical that the molecular details conferring metal resistance be unraveled if microbial-mediated biotechnology aimed at waste metal decontamination is to be optimized.

3. *P. fluorescens*, an Al-resistant microbe

P. fluorescens derives its name from its ability to synthesize the fluorescent siderophore pyoverdin, and is predominantly a soil bacterium known for its ability to adapt to a great variety of nutrient sources (Paulsen et al., 2005). Metabolic flexibility has rendered this microbe suitable for a variety of biotechnological processes. While this bacterium can survive in temperatures as low as 0 °C, its optimal growth temperature is 25 °C. Its inability to grow at body temperature means *P. fluorescens* is considered non-pathogenic and its presence in the environment not deemed harmful to mammals (Srivasava, 2009). This microbe frequently colonizes the rhizosphere of crop plants where it displays antagonistic activity towards various plant pathogens. Fungi, such as *Alternaria cajani*, *Curvularia lunata* and *Fusarium sp.* which may cause disease and death in the target plants, are eradicated in the presence of *P. fluorescens*. To accomplish this, the organism produces a number of antifungal metabolites, such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, 2, 3-de-epoxy-2, 3-didehydra-rhizoxin and pyrrolnitrin. The latter has been used as the lead structure for the development of a novel agricultural fungicide. Additionally, *P. fluorescens* produces secondary metabolites such as siderophores, HCN and proteases that directly kill or scavenge nutrients from competing organisms. Indeed, the application of this microbe in biological control offers a safer alternative to chemical control, which engenders environmental concerns (Srivasava, 2009).

We have previously reported on the ability of *P. fluorescens* to withstand concentrations of Al up to 50 mM (Appanna and St. Pierre, 1994). The metal is chelated to citric acid, which translocates across the bacterial membrane as a complex which is then degraded intracellularly. After 80 h of grown, 65.1% of the soluble Al is sequestered when *P. fluorescens* is grown in media containing an Al concentration of 15 mM (Appanna and St. Pierre, 1996). Analysis of the soluble cell free extract following 75 h of growth showed that less than 1% of the Al in the media was found in this compartment (Appanna and St. Pierre, 1996). The ability of this bacterium to cope with such a high concentration of Al involves a detoxification strategy, which consists primarily of excluding it from the cell as an insoluble lipid containing precipitate tailored to alleviate the toxicity of this metal (Appanna and St. Pierre, 1996) (Fig. 3A,B). The nature of this gelatinous residue harboring Al was shown to be phosphatidylethanolamine (PE), a phospholipid commonly found in biological membranes (Appanna and St. Pierre, 1996).

Scanning electron microscopy and transmission electron microscopy (SEM and TEM) were utilized to ascertain the nature of this precipitate, and the percentage of Al found sequestered ranged from 38 to 80% in cultures with 1 to 30 mM Al (Appanna and St. Pierre, 1996). In addition, the amount of PE found in the spent fluid increased with increasing concentrations of Al, thus confirming that this phospholipid is intricately linked to the detoxification of this trivalent metal. Indeed, the addition of cerulenin, an inhibitor of lipid synthesis, greatly limits the amount of Al removed (Hamel and Appanna, 2003). Clearly, the exudation of Al is the primary stratagem by which the microbe copes with its toxicity. This is true of other metals, such as gallium (Ga) and indium (In), where disparate stratagems are deployed.

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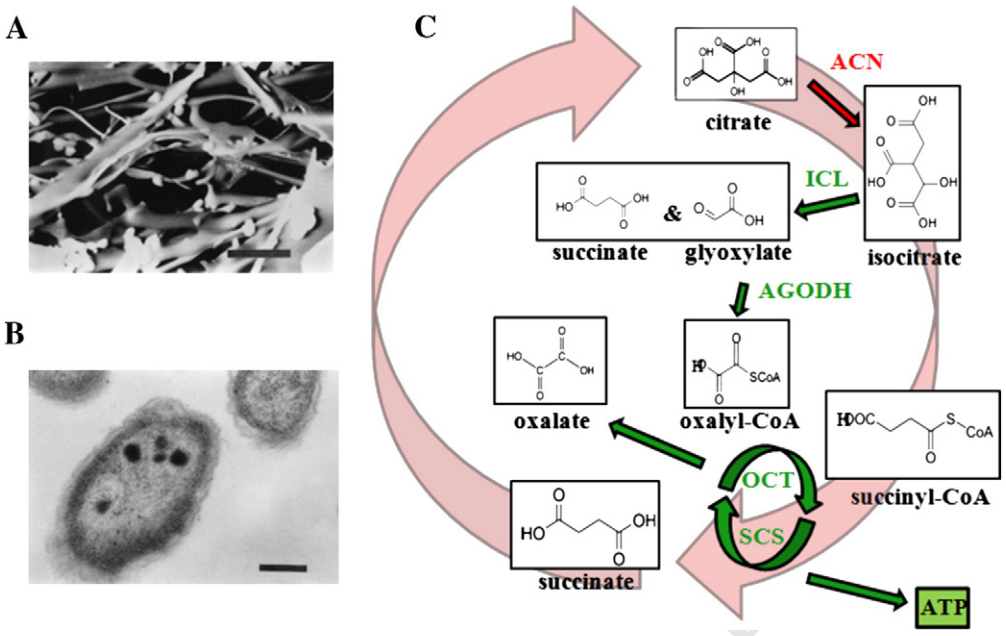

Fig. 3. Metabolic engineering and Al in *P. fluorescens*. A: Scanning electron micrograph of Al (round bodies) exuded from *P. fluorescens* in a phosphatidylethanolamine-containing precipitate. B: Transmission electron micrograph of *P. fluorescens* cultured in 15 mM Al for 45 h (note the aluminum inclusions). C: The up-regulation of ICL and a downstream network allows for the synthesis of ATP via substrate level phosphorylation in Al-treated *P. fluorescens*. ICL: isocitrate lyase; AGODH: acylating glyoxylate dehydrogenase; OCT: oxylate-CoA transferase; SCS: succinyl-CoA synthetase. ACN: aconitase. Green and red arrows are indicative of enzymes whose activities are up- and down-regulated, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Figures in panels A and B adapted from Appanna and St. Pierre, 1996. Figure in Panel C adapted from Singh et al., 2009.

However, *P. fluorescens* can only tolerate 1.0 and 0.5 mM concentrations of these metals, respectively (Anderson and Appanna, 1993; Bériault et al., 2007). While In is immobilized and expulsed from the cell as a phosphate residue devoid of lipids, Ga is removed as a soluble organic conjugate (Appanna et al., 1996). The elimination of Al in association with PE necessitates a major reconfiguration of metabolic pathways in order to generate all the ingredients for this process to occur efficiently.

3.1. A modified TCA cycle produces ATP under Al stress

Since Al interferes with Fe homeostasis, enzymes that depend on this metal for catalytic activity are easily disrupted. In particular, proteins of the tricarboxylic acid (TCA) cycle and electron transport chain which rely on iron–sulfur (Fe–S) clusters or heme groups are disabled (Lemire et al., 2010; Yamamoto et al., 2002). These include aconitase (ACN), fumarase (FUM), succinate dehydrogenase (SDH), complex I and complex IV (Lemire et al., 2010; Middaugh et al., 2005). The displacement of Fe in these enzymes has the effect of crippling the aerobic production of ATP via ATP synthase, thus bringing about an energy deficiency within the affected organism (Yamamoto et al., 2002). To compensate for this loss, organisms can up-regulate the production of ATP via substrate level phosphorylation (SLP). The two pathways in *P. fluorescens* which can produce ATP by SLP are glycolysis and a modified TCA cycle (Singh et al., 2009). For the latter, the microbe elects to express isocitrate lyase (ICL), which cleaves isocitrate to produce succinate and glyoxylate (Middaugh et al., 2005). When stressed with Al, the activity of ICL is nearly five times higher than in untreated conditions (Lemire et al., 2010). This, combined with the up-regulation of NADP-dependent isocitrate dehydrogenase (ICDH-NADP), allows for the continuous flow of metabolites through the TCA cycle, despite the lowered activity of ACN (Middaugh et al., 2005). Working in tandem with ICL are the enzymes acylating glyoxylate dehydrogenase (AGODH), succinyl-CoA synthetase (SCS) and oxylate-CoA transferase (OCT) (Singh et al., 2009). SCS produces ATP by SLP, thus fulfilling the organism's energy needs when oxidative phosphorylation is disrupted (Bochud-Allemann and Schneider, 2002). Completion of the TCA cycle is rendered possible by the switch from an iron-dependent isoform of fumarase (FUM A) to one which does not require a Fe–S cluster for catalytic activity (FUM C). If not for this metabolic reconfiguration, the TCA cycle would grind to a halt (Chenier et al., 2008). The metabolic network described here allows the bacterium to produce energy, generate critical metabolites and proliferate when stressed with high concentrations of Al (Fig. 3C).

3.2. Curtailing ROS in an Al-contaminated environment and NADPH homeostasis

While the production of ATP via a modified TCA cycle allows *P. fluorescens* to remain viable in an Al-rich environment, this feat would be impossible if the levels of reactive oxygen species (ROS) were not suppressed within the cell. The accumulation of free radicals, such as the hydroxyl radical generated by Fenton chemistry, is dangerous owing to their ability to react with and disable crucial biomolecules (Freinbichler et al., 2011). Classical enzymatic mechanisms to curb ROS levels, such as catalase, superoxide dismutase and glutathione peroxidase/reductase are at the bacterium's disposal. However, *P. fluorescens* makes several other adjustments to further negate ROS damage (Fig. 4A). As NADPH is required for the regeneration of ROS scavenging molecules such as glutathione, these bacteria up-regulate enzymes which produce this reducing agent. While the aforementioned ICDH-NADP helps accomplish this, other enzymes which participate in NADPH production, such as malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PDH) also show increased activity (Singh et al., 2008). A constant supply of NADP for these reactions is assured by increasing the expression of the NAD phosphorylating enzyme, NAD kinase (NADK) (Singh et al., 2008). Not only does NADK enhance NADPH production, it also diminishes the level of NADH, a pro-oxidant (Kussmaul and Hirst, 2006).

While NADPH promotes a reductive environment, NADH encourages the opposite. This moiety serves as substrate for aerobic respiration, which generates ROS when electrons leak from the ETC. (Grose et al., 2006). These complexes are disturbed by Al treatment. However, to

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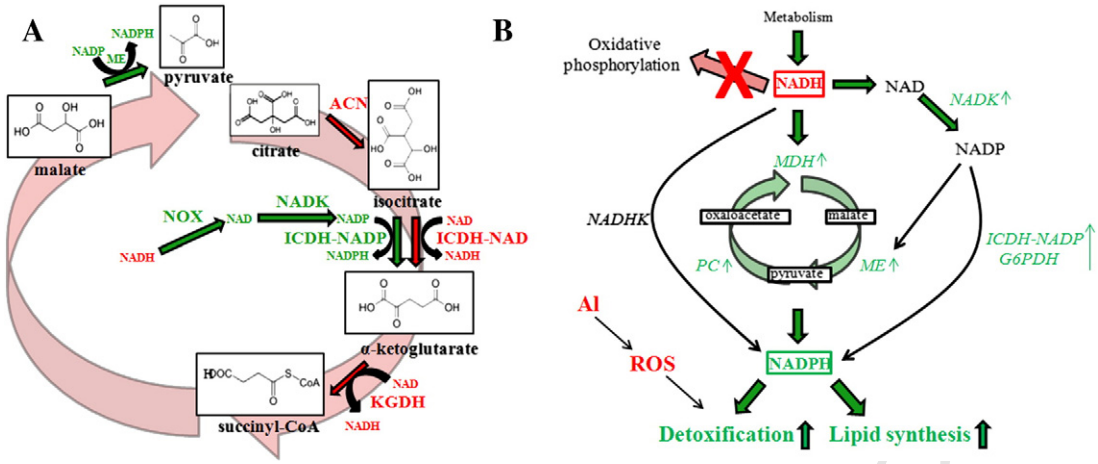

Fig. 4. Metabolic networks to decontaminate Al in *P. fluorescens*. A: Al triggers a metabolic shift that favors the synthesis of NADPH and curbs the intracellular concentration of the pro-oxidant NADH in *P. fluorescens*. B: Metabolic reengineering aimed at the conversion of NADH to NADPH allows for the detoxification of Al-derived ROS and the synthesis of lipid moieties for exudation of this trivalent metal. ICDH: isocitrate dehydrogenase; KGDH: α-ketoglutarate dehydrogenase; ME: malic enzyme; NOX: NADH oxidase; NADK: NAD kinase; MDH: malate dehydrogenase; PC: pyruvate carboxylase; ME: malic enzyme; NADHK: NADH kinase; G6PDH: glucose-6-phosphate dehydrogenase.
Q3 Adapted from Mailloux et al., 2008.

further limit their activity and maintain a reductive environment, *P. fluorescens* decreases the intracellular concentration of NADH. Two stratagems are invoked to accomplish this. Firstly, NADH producing enzymes, such as ICDH-NAD and α-ketoglutarate dehydrogenase (KGDH) are down-regulated (Mailloux et al., 2007). These enzymes are not required in the modified TCA cycle utilized by this microbe as they generate NADH (Singh et al., 2009). Additionally, the increased expression of ICDH-NADP accompanied by the lowered activity of KGDH builds a pool of α-ketoglutarate, a keto-acid which has been shown to scavenge ROS in situ (Varma and Hegde, 2004). Secondly, the direct consumption of NADH is encouraged by the microbe via the expression of H_2O-generating NADH oxidase (NOX) (Chenier et al., 2008). The product of this reaction, NAD, can be utilized to propel redox reactions or for NADP generation by NADK. The enhanced production of NADPH does not only help fend Al-induced oxidative stress, but it also provides the crucial reducing factor required for lipogenesis, an event that results in the synthesis of PE. The latter contributes to the extrusion of the contaminant from the microbial system. Furthermore, *P. fluorescens* elaborates a metabolic circuit that results in the conversion of NADH into NADPH. In this instance, oxaloacetate is converted to pyruvate with the concomitant production of NADPH. Malate dehydrogenase (MDH), ME and pyruvate carboxylase mediate this process (Fig. 4B).

3.3. Organic acids as potent aluminophores

While PE synthesis offers one route of Al expulsion, it is not the only moiety on which *P. fluorescens* relies for the elimination of this metal. Organic acids, owing to their negatively charged carboxylic acid groups, are well-suited for the chelation and removal of noxious heavy metals (Ma et al., 2001). In order to ameliorate the biogenesis of these aluminophores, *P. fluorescens* undergoes a complete overhaul of its metabolic networks. The first of these changes is the increased expression of OCT. This enzyme permits the formation of succinyl-CoA from oxalyl-CoA (Singh et al., 2009). While the former can be utilized to produce ATP from SLP as described earlier, the latter becomes oxalate, a dicarboxylic acid and potent chelator of Al (Hamel and Appanna, 2001). Thus, while generating energy, the microbe finds an effective route to the production of oxalate. This reengineered metabolic network allows the bacterium to produce both ATP and a chelator of Al.

A second metabolic reconfiguration occurs in order to generate another dicarboxylic acid, oxaloacetate, which is used as a precursor to a polycarboxylic aluminophore (Appanna et al., 2003; Lemire et al., 2008). The homeostasis of this metabolite is regulated by numerous enzymes. Malate dehydrogenase (MDH), a critical TCA cycle enzyme, appears to be up-regulated by the microbe in order to produce oxaloacetate at a greater rate when Al is present (Lemire et al., 2008). Pyruvate carboxylase (PC), which carboxylates pyruvate to form oxaloacetate, is also overexpressed by *P. fluorescens* treated with Al (Lemire et al., 2008). On the other hand, phosphoenolpyruvate carboxykinase (PEPCK), an enzyme that converts oxaloacetate to phosphoenolpyruvate shows lower expression (Lemire et al., 2008). Furthermore, the activity of nucleoside diphosphate kinase (NDPK), a critical enzyme for the generation of the PEPCK cofactor GTP, also shows lowered activity in the Al-treated bacteria (Lemire et al., 2008). PC and PEPCK are normally coupled in the gluconeogenic pathway, allowing the organism to produce intermediates for biosynthetic reactions (Owen et al., 2002). However, it appears as though Al toxicity disrupts this process.

These metabolic shifts aimed at the production and pooling of two dicarboxylic acids, oxaloacetate and oxalate, permit the microbe to expulse Al as a PE containing precipitate and continue to proliferate (Fig. 5). Hence, the metabolic reengineering invoked to neutralize the toxic effects of Al enables the production of NADPH, ATP, oxaloacetate, oxalate, and PE. While the latter three molecules are directly involved in the immobilization of the trivalent metal, NADPH provides the redox environment to sustain life in an oxidatively charged milieu and the reducing equivalent for lipid biosynthesis. Despite the diminished effectiveness of oxidative phosphorylation at fulfilling the need for high-energy phosphate under Al assault, the microbe devises a unique metabolic route to ATP and oxalate that is devoid of the demand of oxygen. These reengineered metabolic networks are keys to the survival of *P. fluorescens* in an Al-contaminated environment and in the elimination of the toxic metal. Thus, understanding these metabolic changes in Al-tolerant bacteria is essential in bioremediation technologies.

4. Proposed bioremediation device fabrication and applications

As high Al levels (≥0.1 mg/L) in drinking water have been associated with an elevated risk of dementia and Alzheimer's disease, the removal of this heavy metal in contaminated regions may alleviate this burden (Rondeau et al., 2000). Biotreatment of wastewater has been shown to efficiently remove a number of organic contaminants such as hydrocarbons (oil), polychlorinated biphenyls (PCBs) and pharmaceutical substances (Bao et al., 2012; Federici et al., 2012;

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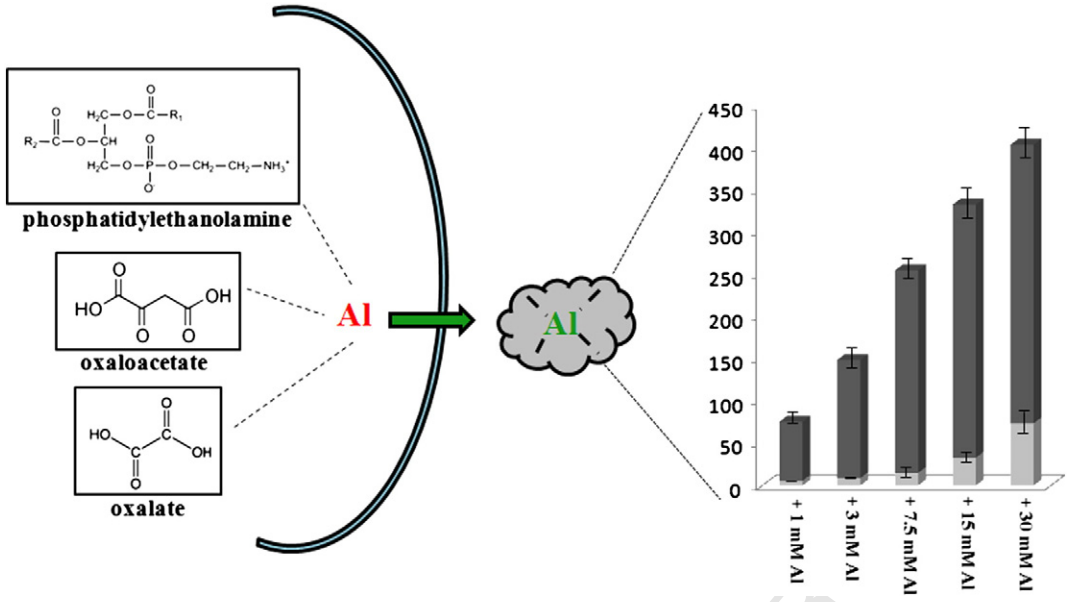

Fig. 5. Under Al stress, *P. fluorescens* favors the biosynthesis of phosphatidylethanolamine and dicarboxylic acids which bind to and expulse Al as an insoluble pellet. Pellet weight and Al content vary with the concentration of this metal in the medium. ▒; pellet weight (mg/mL culture). ■; aluminum (nmol/mg [wet wt] of pellet).

Mansour et al., 2012). However, inorganic waste such as metals cannot be degraded into benign compounds like H_2O and CO_2. The ability of microorganisms to alter the speciation of metals, leading to their bioprecipitation, volatilization, or biotransformation into innocuous forms has become a key tool in environmental remediation (Singh and Cameotra, 2004). Bioprecipitation concentrates the metals and thus reduces their bioavailability. Further, these biotransformed concentrates can be stored in backfills or by vitrification. Several physical and chemical methods are currently undertaken to remediate Al and other metals in polluted ecosystems. These include chemical precipitation or reduction, ion-exchange, activated charcoal, adsorption and reverse osmosis among others (Srivastava and Majumder, 2008). However, the cost of reagents and materials in addition to the possibility of secondary environmental pollution become limiting factors for these treatments (Malik, 2004).

Given that *P. fluorescens* can tolerate such high concentrations of Al while simultaneously exuding it as an insoluble precipitate, this microbe is well-suited for the removal of this metal from polluted ecosystems. Therefore, we propose that *P. fluorescens* be applied in the fabrication of a biofilter (Fig. 6). The latter may incorporate the fixing of the microorganism to a porous medium to decontaminate organic and inorganic contaminants from wastewater (Srivastava and Majumder, 2008). By immobilizing the growing biofilm in a rotating or fixed reactor, the waste stream containing dissolved Al can be treated and the metal subsequently filtered out. The vessel would function at ambient temperature and a pH ranging from mildly acidic to neutral. Rotation speed and flow rate are parameters which can be optimized for Al removal. A nutrient broth, supplied daily via a peristaltic pump may be utilized to support the viability of the microbe. Due to the metabolic versatility of *P. fluorescens*, multiple carbon sources can be utilized for sustenance. These Al-resistant bacteria can also be maintained in a defined mineral medium consisting of 6.0 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.8 g NH_4Cl, 0.2 g $MgSO_4$ and 4.0 g citrate per L of media. Trace metals consisting of 2 μM Fe, 1 μM Mn, 0.5 μM Zn, 1 μM Ca, 0.25 μM Co, 0.1 μM Cu and 0.1 μM Mo may help in the process. Precipitated Al is eliminated at the bottom of the receptacle via a filter while the decontaminated water is collected for use and/or released into the environment indeed after verifying the Al and microbial content. Although *P. fluorescens* is being exploited as a model microbe in the foregoing progress, other Al-resistant microbes can also be applied in the fabrication of biofilters, either alone or in the form of a mixed biofilm. Indeed, heterogeneous bacterial communities often remove the undesired contaminant more effectively than a single species (Srivastava and Majumder, 2008).

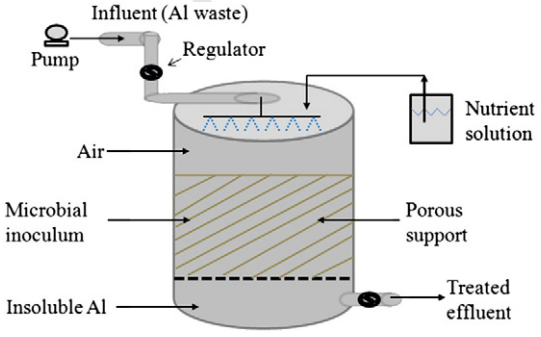

Fig. 6. Proposed bioreactor design. A trickling biofilter which feeds in the wastewater and a nutrient source for the microbe permits the bioprecipitation of Al, which is filtered out of the system. Valves controlling intake and outtake determine the flow rate, allowing for maximal removal of the trivalent metal.

Alternatively, these microbes can be utilized in bioaugmentation, as seeding organisms which precipitate aluminum from the soil. In effect, *Pseudomonas* inoculants have been applied with great success to protect plants from noxious chemicals and organisms (Ajithkumar et al., 1998; Li and Alexander, 1988). Inoculation of Al-contaminated soil with *P. fluorescens* may precipitate this metal, thus rendering it unavailable for uptake in plants and augmenting crop yield.

Although the pathogenesis underlying most neurodegenerative disorders is multifactorial, one cannot discount the increasing body of evidence that high concentrations of Al are associated with a variety of diseased states. A number of regions around the globe are laden with quantities of this metal which are beyond the maximum permissible limit (Table 1). The removal of Al from contaminated waters may has a positive effect on human health, crop viability and the balance of the ecosystem. While purification strategies do exist, many of these procedures are inaccessible due to economic reasons, particularly in less developed nations (Bakar et al., 2010). Bioremediation of wastewater is both cost-effective and benign, particularly when compared to alternatives such as chemical treatment or incineration which entail risks to the environment (Bitton and Koopman, 1992). *P. fluorescens*' resistance to Al renders it an ideal microorganism for

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bioremediation technologies. While it is feasible that genetic alterations would enhance the ability of this microorganism to dispose of Al, one of the primary advantages of this system is its innateness; i.e. no genetic manipulation is involved. Indeed, the introduction of genetically modified organisms into the environment has long since had a negative stigma attached to it (Marris, 2001).

5. Conclusion

Here we demonstrated how microorganisms can reengineer cellular processes to bioprecipitate Al, thus rendering it unavailable for biological uptake in other organisms. The metabolic modules that render this decontamination strategy feasible allow for the biotransformation and expulsion of Al without significantly impeding the survival of the organism. The natural resistance to high concentrations of Al demonstrated by certain microbes is a feature which can be harnessed to design bioreactors and the molecular details outlined may indeed help improve the efficiency of such processes. Metabolic engineering is pivotal in elaborating efficient biotechnological processes aimed at decontaminating metal pollutants.

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