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Research Paper

Proteomic and morphological insights into the exposure of *Cupriavidus metallidurans* CH34 planktonic cells and biofilms to aluminium

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Highlights

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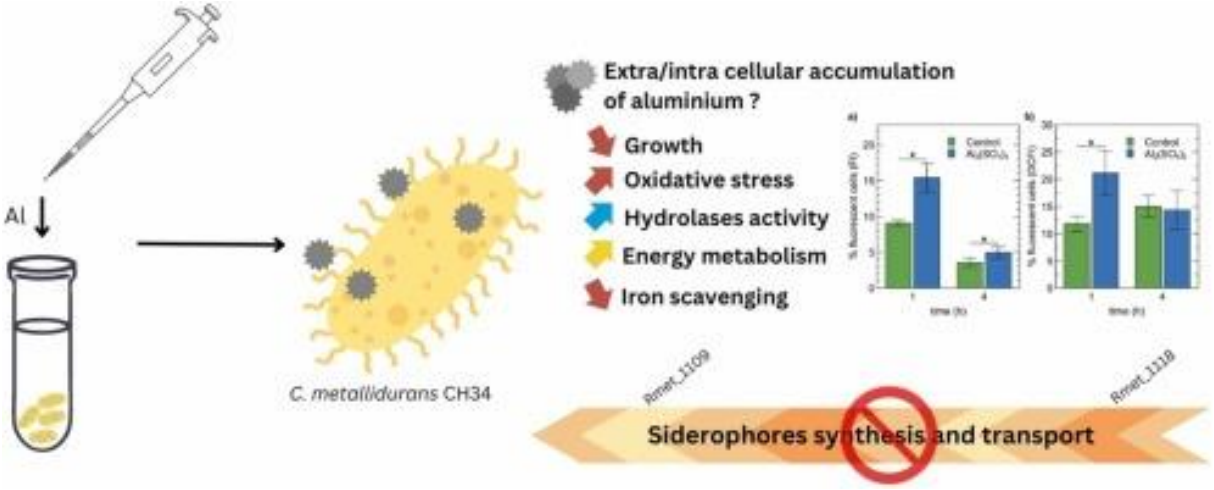
- Aluminium can inhibit the growth of metal-resistant *C.metallidurans* CH34.
- Aluminium increases metabolic activity and oxidative stress levels.
- Aluminium exposure induces differential proteomic response in *C.metallidurans* CH34.
- Biogenesis and transport of siderophores are inhibited by aluminium.
- Aluminium disrupts the architecture of mature biofilms.

Abstract

Aluminium (Al) is one of the most popular materials for industrial and domestic use. Nevertheless, research has proven that this metal can be toxic to most organisms. This light metal has no known biological function and to date very few aluminium-specific biological pathways have been identified. In addition, information about the impact of this metal on microbial life is scarce. Here, we aimed to study the effect of aluminium on the metal-resistant soil bacterium *Cupriavidus metallidurans* CH34 in different growth modes, i.e. planktonic cells, adhered cells and mature biofilms. Our results indicated that despite a significant tolerance to aluminium (minimal inhibitory concentration of 6.25 mM $Al_2(SO_4)_3.18H_2O$), the exposure of *C. metallidurans* to a sub-inhibitory dose (0.78 mM) caused early oxidative stress and an increase in hydrolytic activity. Changes in the outer membrane surface of planktonic cells were observed, in addition to a rapid disruption of mature biofilms. On protein level, aluminium exposure increased the expression of proteins involved in metabolic activity such as pyruvate kinase, formate dehydrogenase and poly(3-hydroxybutyrate) polymerase, whereas proteins involved in chemotaxis, and the production and transport of iron scavenging siderophores were significantly downregulated.

Graphical Abstract



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Introduction

Aluminium is the most abundant metallic element in Earth's crust. From 2018 to 2022, an average of 65.9 million metric tons of aluminium were produced worldwide [26]. It is a largely employed material and can be encountered everywhere in our daily activities, from construction and mechanical industries to cookware, cosmetic and pharmaceutical products. This intense use and continuous exposure, urges action to monitor aluminium pollution and its impact on the environment and organisms.

Chemically, aluminium is considered a light metal with a density of 2.7 $g.cm^{-3}$. It has strong positive charge and electron acceptor properties, in addition to high reactivity at room temperature. Aluminium is a strong Lewis acid and rapidly reacts with oxygen. It can form stable complexes with phosphates, sulfates, fluorides and organic matter. Ionic aluminium can be toxic for most organisms. Because it has no known biological function, very few aluminium-specific pathways have been identified in organisms [15]. In bacteria, one protein conferring tolerance to aluminium was reported in *Arthrobacter viscosus* [28]. In plants, Al-induced pathways to reduce toxicity are better documented, including

secretion of organic acids, the release of inorganic phosphorus, remodeling of the cell wall, pH modulation and biosynthesis of phytochelatins and metal-binding proteins [70].
Aluminium's strong affinity to phosphates [16], [34] drives its preferential binding to cellular phospholipidic membranes, which interferes with their activity [63], alters the bilayer structure and initiates oxidative stress. It also binds to nucleic acids, proteins and ATP [52]. The availability of ligands increases the solubility of aluminium and when bound to organic molecules it is still bioavailable [30]. This metal is reported to form complexes with several carboxylic acids of biological relevance such as citrate, oxalate, succinate, gluconate, lactate and aspartate [23]. Aluminium is able to impair metal-dependent enzymes, such as aconitase, succinate dehydrogenase and fumarase [8], as well as to promote the Fenton reaction [47]. It can inhibit growth of *E.coli* and various *Pseudomonas* species, and disrupt biofilm formation [6]. Its toxicity depends on the speciation, on the exposed microorganism and on the environmental conditions (pH, organic molecules) [21]. Due to its very complex chemistry, the speciation of aluminium in liquids is still not fully explored making the understanding of its biological interactions a difficult task. For instance, in aqueous solutions of aluminium chloride the dominant species is the hydrated Al^{3+} [18], in aqueous solutions of aluminium sulfate, the dominant forms are $AlSO_4^+$, $Al(SO_4)_2^-$ and hydrated Al^{3+}, and in sea water the main ionic species is $Al(OH)_4^-$ [60].
Still, numerous aluminium-tolerant bacteria have been isolated, such as *Brochothrix thermosphacta* and *Vibrio alginolyticus*, which could withstand 1.87 mM of $AlCl_3$ on aluminium-loaded nutrient agar [59]. *Pseudomonas pseudoalcaligenes* KF707 and *Pseudomonas fluorescens* ATCC13525 tolerated 6.25 mM and 12.5 mM of $Al_2(SO_4)_3$ in LB, respectively [6]. Some of the mechanisms for aluminium-detoxification were identified in *P. fluorescens* ATCC13525 and in *Pseudomonas putida* ATCC12633, including complexation to phosphatidylcholine and phosphatidylethanolamine [22], [44], [5]. However, to date the available information about the interaction of aluminium with microorganisms is scarce and insufficient for a satisfying understanding of its toxicity or removal mechanisms.
Nevertheless, the bacterial potential for environmental bioremediation of aluminium is being studied. For example, a community of dissimilatory sulfate-reducing bacteria isolated from

a municipal sludge in Portugal has been suggested as a bioremediation agent, it survived 1.3 mM of Al^{3+} and could reach 78% of metabolism-dependent aluminium precipitation [37]. Another example is the removal of aluminium from contaminated wastewater by *P. aeruginosa* with an efficiency up to 46% [53]. *Cupriavidus metallidurans* is a model bacterium to investigate metal resistance and processing [27], [39], [45]. Although type strain CH34, which was initially isolated from a zinc decantation tank [40], [42], has been studied for its adaptation to, resistance to and biomineralization of a variety of metal ions (e.g., Cu^{+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, CrO_4^{2-}, Pb^{2+}, Ag^{+}, Au^{+}, Au^{3+}, $HAsO_4^{2-}$, AsO_2^{2-}, Hg^{2+}, Cs^{+}, Bi^{3+}, Tl^{+}, SeO_3^{2-}, SeO_4^{2-}, Sr^{2+} and UO_2^{2+}) [12], [27], [45], [54], [55], its response to aluminium remained unstudied. Therefore, we explored the response of *Cupriavidus metallidurans* CH34 to aluminium stress.

Section snippets

Bacterial strains and growth conditions

C. metallidurans CH34 was routinely grown in Tris–buffered mineral medium (MM284), which contained 6.06 g.L-1 Tris/HCl, 4.68 g.L-1 NaCl, 1.49 g.L-1 KCl, 1.07 g.L-1 NH_4Cl, 0.43 g.L-1 Na_2SO_4, 0.2 g.L-1 $MgCl_2.6H_2O$, 0.03 g.L-1 $CaCl_2.2H_2O$, 0.04 g.L-1 $Na_2HPO_4.2H_2O$, 4.8 mg.L-1 Fe(III)(NH_4)citrate, 144 µg.L-1 $ZnSO_4.7H_2O$, 100 µg.L-1 $MnCl_2.4H_2O$, 62 µg.L-1 H_3BO_3, 190 µg.L-1 $CoCl_2.6H_2O$, 17 µg.L-1 $CuCl_2.2H_2O$, 24 µg.L-1 $NiCl_2.6H_2O$ and 36 µg.L-1 $Na_2MoO_4.2H_2O$). MM284 was supplemented with 2 g.L-1 sodium

Effect of Al on *Cupriavidus metallidurans* CH34

Since no detailed information on the toxicity of aluminium on *Cupriavidus metallidurans* exists, we first scored the minimal inhibitory concentration (MIC). After 24 h in MM284, no growth was observed from 6.25 mM $Al_2(SO_4)_3.18H_2O$ onwards (Fig. 1a), and this remained for at least an additional 48 h (data not shown). Increasing concentrations of Al were accompanied by a decreasing pH. Lowering of the pH improves the solubility of aluminium and promotes the formation of aggressive Al^{3+}. It is worth

Conclusions

In this work, we investigated the effect of aluminium on the bacterium *Cupriavidus metallidurans* in particular, as the interaction of this metal with environmental species is overlooked. We studied its effect on growth, morphology, biofilms, oxidative stress status and the proteomic response. Results showed that aluminium had a strong impact, even at a micromolar level. Mature biofilms were affected by rapid disruption. Proteome analysis in *C. metallidurans* CH34 strongly suggests a shutdown of

Environmental implication

Aluminium a highly reactive element and heavily utilized in industry with over 60 million tons produced annually worldwide. However, a growing body of research indicates its hazardous character towards living organisms. As such, it is considered a contaminant of potential concern. Because little is known about its interactions with environmental bacteria, we contribute to a better understanding of its effects, especially on the soil bacterium *Cupriavidus metallidurans*, at neutral pH. We

CRediT authorship contribution statement

Gillan David: Conceptualization, Methodology, Supervision, Writing – review & editing. **Ben Miloud Yahia Najla:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Landoulsi Ahmed:** Conceptualization, Project administration, Writing – review & editing. **Chatti Abdelwaheb:** Conceptualization, Supervision, Writing – review & editing. **Wattiez Ruddy:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Abdeljelil Nissem:** Conceptualization,

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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