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Isolation of aluminum-tolerant bacteria capable of nitrogen removal in activated sludge

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ABSTRACT

Four strains of bacteria capable of withstanding 20 mM concentration of aluminum were isolated from activated sludge in a bioreactor. 16S rRNA identification and morphological characteristics indicated that these strains were *Chryseobacterium* sp. B1, *Brevundimonas diminuta* B3, *Hydrogenophaga* sp. B4, and *Bacillus cereus* B5. Phylogenetic analysis revealed the position and interrelationships of these bacteria. *B. diminuta* B3 and *Hydrogenophaga* sp. B4 could achieve nitrate nitrogen removal of 94.0% and 76.8% within 36 h of its initial concentration of 148.8 and 151.7 mg/L, respectively. Meanwhile, B3 and B4 could degrade ammonia with little nitrite accumulation. Results of this study provide more information about aluminum-resistant bacteria and laid the foundation for aluminum salt when it is simultaneously used for chemical precipitation.

1. Introduction

Aluminum, the most abundant metal in the earth's crust, surprisingly finds little use in biological systems. Aluminum can diminish the absorption of calcium, magnesium, iron, and other elements necessary for the function of organisms, thereby interfering with their normal metabolic processes. It is a well-established neurotoxin for animals, and is suspected to be associated with various neurodegenerative diseases (Bondy, 2014). Solubilized aluminum in the soil water absorbed by plant roots severely inhibits root elongation (Matsumoto, 2000). Studies conducted on Al exposure to microorganisms have further elaborated the mechanisms underlying toxic effects of this metal (Auger et al., 2013). Al with concentration < 3 mM inhibited the growth of *Escherichia coli* (Auger et al., 2013), and >1 mM exerted a toxic effect on *Desulfovibrio desulfuricans* (Amonette et al., 2003).

The toxicity of aluminum relies on the fact that it can impede enzymes that are dependent on Fe for their effective function, because of their similar trivalent states, thus creating an oxidative environment. Programmed cell death was invoked by aluminum in both plants (Tamas et al., 2005) and yeast (Zheng et al., 2007). Despite its toxicity, some species can be resistant to aluminum by changing their metabolic pathways. Organic acids such as oxaloacetate and oxalate permit microbes to expel Al (Auger et al., 2013). *Pseudomonas fluorescens* exhibits a fine metabolic balancing act to adapt aluminum (Lemire et al., 2010).

To date, most studies on aluminum tolerance have been performed on plants and fungi, and very few bacteria have been reported to exhibit aluminum tolerance. In bacteria, the mechanism of aluminum resistance was well presented for *P. fluorescens* (Auger et al., 2013). Strains of *Burkholderia* sp. isolated from acidic swamps and forest soils were tolerant to aluminum (Aizawa et al., 2010; Kunito et al., 2012). *Acidocella aluminiidurans* was another newly isolated aluminum-tolerant bacterium (Kimoto et al., 2010). Moreover, aluminum salt was usually added to wastewater treatment systems to enhance phosphorus removal (Ji et al., 2015). Recognition of aluminum-resistant bacteria in the activated sludge is very important.

In this study, four strains of bacteria were isolated and identified from activated sludge, which could grow well at 20 mM concentration of aluminum. In addition, their ability to remove nitrogen was tested. The results provide more information about aluminum-resistant bacteria and laid the foundation for aluminum salt when it is simultaneously used for chemical precipitation (Ji et al., 2015) in wastewater treatment.

2. Materials and methods

2.1. Enrichment and isolation of Al-resistant bacteria

Sludge samples were collected from a modified sequencing batch biofilm reactor (Yang et al., 2014) for domestic wastewater treatment in Wuhan, China. Aluminum-resistant bacteria were enriched in 100 mL of beef extract peptone medium in a 250-mL Erlenmeyer flask, to which 20 mM of Al as $Al_2(SO_4)_3\ 18H_2O$ was added and pH was adjusted to 7.2 by adding 1 M NaOH and HCl. The medium was sterilized by autoclaving at 121 °C for 20 min. The sludge samples were used to inoculate (1%, *w*/*v*) the sterile medium amended with Al and incubated for 24 h, with shaking (150 rpm, 30 °C). Subsequently, 1 mL of enrichment

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Table 1
Characteristics of activated sludge used.

Parameters	Density ($g\ m^{-3}$)	MLSS ($g\ m^{-3}$)	VSS ($mL\ g^{-1}$)	DO ($mg\ L^{-1}$)	pH (−)
Value	1.002–1.004	2500–3600	80–120	0.2–4.0	6.8–7.6

Table 2
Morphology and cultural characteristics of the isolates.

Strain	Descriptions of morphology and cultural characteristics
B1	Gram-negative, none-motile, rod-shaped, yellow-pigmented, convex, complete border
B3	Gram-negative, motile, short rods, smooth, opaque, slight convex with entire edge
B4	Gram-negative, motile, straight to slight curved rods, smooth, raised
B5	Gram-positive, motile, whitish to cream in color, vary in shape from circular to regular

culture was used to inoculate 99 mL of the aforementioned sterile medium. This procedure was performed thrice. Al-resistant bacteria were then purified by repeated streaking on nutrient agar plates containing 20 mM aluminum.

2.2. Identification of isolates and phylogenetic analysis

Total bacterial DNA was extracted using a genomic DNA extraction kit (TianGen, China) following the manufacturer's instructions. The collected DNA was mixed with a polymerase chain reaction (PCR) mixture (20 μL): 2 μL of 10 × PCR buffer, 1.7 μL of deoxynucleotide triphosphates (dNTPs) (each 2.5 mM), 0.5 μL of F-primer (CAGAGTTTGA TCCTGGCT, 100 μM), 0.5 μL of R-primer (AGGAGGTGATCCAGCCGCA, 100 μM), 2 μL of DNA template, 0.35 μL of Taq polymerase ($5\ U\ \mu L^{-1}$), and 12.95 μL of double-distilled water. PCR was conducted as follows: treatment at 94 °C for 5 min, 94 °C for 30 s, annealing at 55 °C for 1 min, 72 °C for 2 min, 30 cycles; 72 °C for 10 min; cooling and holding at 4 °C. The PCR products were purified and sequenced by a DNA Sequencer (3730, ABI, USA). DNA Sequence Similarity GenBank Basic Local Alignment Search Tool (BLAST) was used for homology searches. The phylogenetic tree was constructed with MEGA 5.0 using the neighbor-joining algorithm (1000 replications).

2.3. Characteristics of nitrogen removal

The modified denitrifying medium (DM) comprised $Na_2HPO_4 \cdot 7H_2O$ ($7.9\ g\ L^{-1}$); KH_2PO_4 (1.5); NH_4Cl (0.3); $MgSO_4 \cdot 7H_2O$ (0.1); NaAc (4.7); $NaNO_3$ ($0.85\ g\ L^{-1}$); and trace element solution ($2\ ml\ L^{-1}$). The trace element solution contains EDTA ($50.0\ g\ L^{-1}$); $ZnSO_4$ (2.2); $CaCl_2$ (5.5); $MnCl_2 \cdot 4H_2O$ (5.06); $FeSO_4 \cdot 7H_2O$ (5.0); $(NH_4)_6Mo_7O_2 \cdot 4H_2O$ (1.1); $CuSO_4 \cdot 5H_2O$ (1.57); and $CoCl_2 \cdot 6\ H_2O$ ($1.61\ g\ L^{-1}$); pH = 7.0–7.5. The precultured isolated bacteria with beef extract peptone medium (7.9×10^7, 0.5% *v*/*v*) were inoculated into the Erlenmeyer flask with DM (200 mL). The Erlenmeyer flask was sealed with tampons and the cultures were incubated in a shaking incubator maintained at 30 °C and 150 rpm for 36 h.

2.4. Analysis methods

Chemical oxygen demand (COD), ammonia nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), nitrite (NO_2^-–N), total nitrogen (TN), volatile suspended solids (VSS), and mixed liquor suspended solids (MLSS) of the samples were determined according to the standard methods (Eaton et al., 2005). Water pH was measured by an 828 Orion pH meter. Dissolved oxygen (DO) and water temperature were measured by a 52 YSI DO meter. Bacteria were scanned by high-resolution field emission scanning electron microscopy (ZEISS, ΣIGMA HD/VP). Bacterial samples were pretreated by fixing with 2.5% pentanediol in a 0.1 M phosphate buffer. Then, the samples were washed and dehydrated in a graded series of ethanol solutions (50%, 70%, 80%, 90%, and 100%), dried, and coated with gold.

3. Results and discussion

3.1. Isolation of Al-resistant bacteria and phylogenetic analysis

Properties of activated sludge are shown in Table 1. It is evident that the activated sludge from the bioreactor was typical for wastewater treatment. On the basis of the differences in colony form, four bacterial strains, which could tolerate 20 mM concentration of aluminum, were isolated. Descriptions of morphology and cultural characteristics of the four strains are presented in Table 2. Their nucleotide sequences of 16S rRNA were determined and submitted to the GenBank database

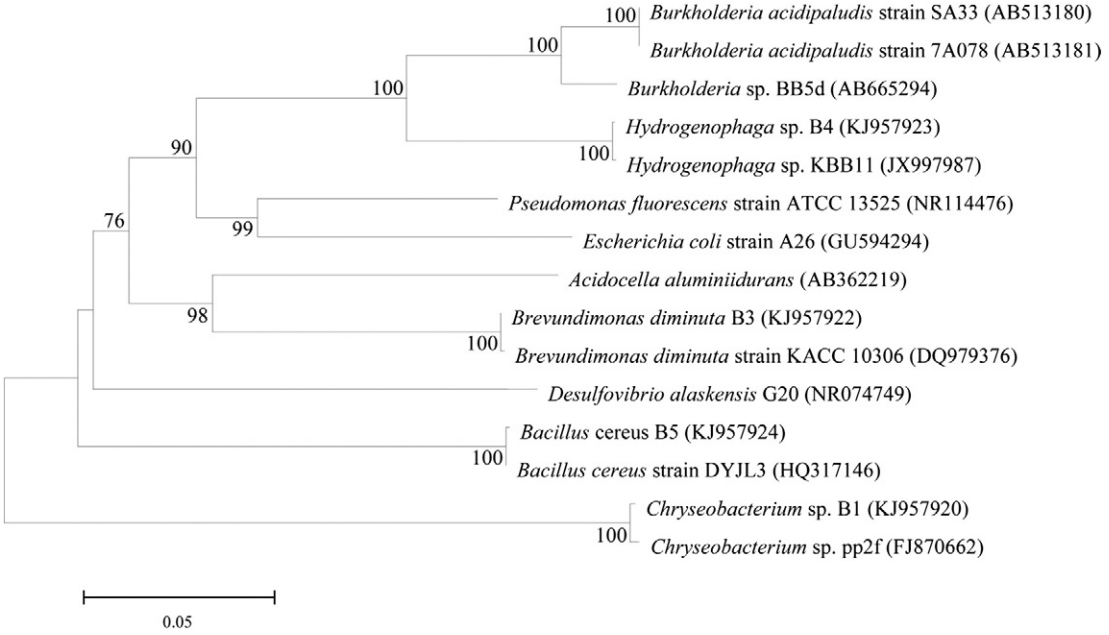

Fig. 1. Phylogenetic tree based on partial 16S rRNA sequences (1463 bp). Bootstrap values are shown at each node.