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Erratum to "Proteomic analysis of differential protein expression in *Acidithiobacillus ferrooxidans* cultivated in high potassium concentration" [Microbiol. Res. 168 (7) (2013) 455–460]

Jianping Ouyang[1], Wenbin Guo[1], Bo Li[1], Li Gu, Huijun Zhang, Xinhua Chen*

Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, Fujian, China

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ABSTRACT

Acidithiobacillus ferrooxidans is a chemolithoautotrophic acidophile that oxidizes ferrous iron or sulfur compounds to obtain energy in the presence of various ions. To investigate the potassium ion response of *A. ferrooxidans*, we conducted a proteomics analysis. We identified eight proteins that were differentially expressed in the presence of high potassium concentration, including four up-regulated and four down-regulated proteins. Transcription levels of the genes encoding differential expressed proteins were subsequently analyzed by Northern blot in the presence of high potassium concentration. Among the up-regulated proteins, GDP-mannose 4,6-dehydratase, ribose 5-phosphate isomerase A and ribose-phosphate pyrophosphokinase were known to be implicated in the synthesis of glycocalyx, suggesting that the formation of glycocalyx might be involved in the *A. ferrooxidans* response to high potassium concentration. Thickening of the glycocalyx layer was also observed in cells cultivated under high potassium concentration via transmission electronic microscopy (TEM) analysis. Among the down-regulated proteins, ATP synthase F1 delta subunit and ATP synthase F1 beta subunit were two important components of ATP synthase. ATP synthase (P-ATPase) is directly linked to the transport of potassium into the cell, thus *Acidithiobacillus ferrooxidans* might just reduce the quantity of ATP synthase to offset the high potassium level in the culture medium. Therefore, the results obtained here provide some new clues to improve our understanding of the response of *A. ferrooxidans* to high potassium concentration.

1. Introduction

Potassium is a major monovalent cellular cation that plays a key role in maintaining the cell turgor pressure (Brown 1976), affects cytoplasmic pH (Epstein 2003), and even regulates the activity and expression of enzymes (Lee et al. 2007). The intracellular concentration of potassium (150–500 mM) (Dinnbier et al. 1988; Epstein and Schultz 1966) is generally much higher than that in the extracellular environment. Thus, controlling intracellular potassium levels with channels and active transporters is vital for cell growth and survivability in the host and the natural environment. Bacteria often have multiple potassium uptake systems including P-type ATPases (Magalhães et al. 2005), kdp (Gassel et al. 1998) and Trk system (Johnson et al. 2009). The P-type ATPase family is a large, physiologically important family of membrane proteins that can be divided into two major groups based on cation specificity. Members of the P1 group transport heavy metals, such as Cu^{2+}, Cd^{2+}, and Hg^{2+}, while the members of the P2 group transport a wide array of monovalent and divalent cations, including H^+, Na^+, K^+, Mg^{2+} and Ca^{2+} (Magalhães et al. 2005). Kdp, the inducible high affinity ATP-driven K^+-transport system of *Escherichia coli* belongs to the class of P-ATPases (Gassel et al. 1998). Maintenance of the proper gradients for essential ions across cellular membranes makes P-type ATPases crucial for cell survival.

Acidithiobacillus ferrooxidans (formerly called *Thiobacillus ferrooxidans*) is a chemolithoautotrophic gram-negative γ-proteobacterium that lives in acidic environments like mining wastes and acid mine drainages. *A. ferrooxidans* can oxidize ferrous ion, elemental sulfur and sulfide minerals to obtain energy (Rawlings 2005; Rohwerder and Sand 2003), and this characteristic has been applied successfully in biomining operations. The adaptability of *A. ferrooxidans* to extreme environments, high ion

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* Corresponding author at: Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Daxue Road 184 #, Xiamen 361005, Fujian, China. Tel.: +86 592 2195297; fax: +86 592 2085376.
E-mail address: Chenxinh@tom.com (X. Chen).
[1] These authors contributed equally to this work.

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concentrations (especially heavy metal ions) and low pH may increase its use in industrial mineral processing. The heavy metal resistance of *A. ferrooxidans* has been observed in many strains for heavy metals such as arsenic (Butcher et al. 2000), mercury (Sasaki et al. 2005; Valdes et al. 2008) and copper (Alvarez and Jerez 2004; Felicio et al. 2003). Arsenic is usually regarded as a hazardous heavy metal even though it is actually a semi-metal. In fact, four arsenic resistance genes, *arsB*, *arsC*, *arsH*, and a putative *arsR* gene, were identified in *A. ferrooxidans* (Butcher et al. 2000). Genome analysis of *A. ferrooxidans* ATCC 23270 identified three genes that may potentially encode the well described Mer components, i.e., the repressor accessory protein (MerD, AFE2483), the mercury reductase (MerA, AFE2481), and the mercuric ion transporter (MerC, AFE2480) (Valdes et al. 2008). Finally, Alvarez and Jerez proved that Cu^{2+} was discharged from inner accompanying the degradation of polyphosphoric acid (Alvarez and Jerez 2004). Moreover, studies also found that cells may reduce the quantity of Cu^{2+} transferring in internal environment by lowering the expression of some acidic channels and carrier proteins which were adhering to outer membranes (Ana et al. 2003).

Besides heavy metals, other ions such as potassium, sodium and magnesium have been detected in acid mine drainage (Valente and Gomes 2009) and in ore leach solutions (Yang et al. 2011). However, little is known about the response of *A. ferrooxidans* to these ions. Therefore, in this study, we investigated the *A. ferrooxidans* response to a high potassium concentration using two-dimensional electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ionization-time of flight tandem mass spectrometry (MALDI-TOF–TOF).

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterium *A. ferrooxidans* AF3 was isolated from the Yufu mine of Hunan province in China (Chen et al. 2009). *A. ferrooxidans* was grown in 9K medium containing ferrous iron that was modified as described previously (Kai et al. 1989). Cultivation of *A. ferrooxidans* in high potassium concentration was carried out by adding KCl to 9K medium to a final concentration of 0.1 M. The pH of both media was adjusted to 2.0.

2.2. Protein extraction

Cells in the late exponential phase of growth were harvested by centrifugation at $6000 \times g$ and then washed three times with diluted H_2SO_4 at pH 2.0. The cell pellet was resuspended in the lysis buffer containing 500 mM Tris–HCl (pH 7.8), 50 mM EDTA, 2% β-mercaptoethanol and 2 mM PMSF and then sonicated on ice. The cell lysate was extracted with an equal volume of water-saturated phenol. The sample was centrifugated at $13{,}000 \times g$ for 15 min and the organic phase was collected. Then a four-fold volume of prechilled methanol was added. After allowing the solution to stand for 3 h at −20 °C, the protein was harvested by centrifugation at $13{,}000 \times g$ for 10 min. The precipitated protein was washed with ice-cold methanol twice, lyophilized and dissolved in a rehydration solution consisting of 7.5 M urea, 2 M thiourea, 50 mM DTT, 2% CHAPS, 2% carrier ampholyte, and 5 mM PMSF for 2-DE analysis. Protein concentration was measured using a Coomassie protein assay kit with BSA as the standard protein (Pierce Biotechnology, USA).

2.3. 2-DE and image analysis

2-DE was performed as previously described (Peng et al. 2004). One milligram of the protein was analyzed for each sample. Isoelectric focusing (IEF) was carried out with an IPGphor unit (Amersham Biosciences, Piscataway, NJ, USA) using precast 18-cm pH 4-7 nonlinear IPG gel strips (Amersham Biosciences). Proteins were separated according to mass in the second dimension by SDS-PAGE with 12% gels. Protein molecule weight marker D530A (Takara, Japan) was applied to monitor the molecule weight of each protein. Coomassie Brilliant Blue G250-stained gels were scanned with a SHARP JX-330 laser densitometer. The spots were each quantitatively analyzed with ImageMaster v5.00 software (Amersham Biosciences). 2-DE of each sample was done in three replicates. The protein spots with expression variation of more than 1.5-fold were considered significant.

2.4. Protein digestions and identification by mass spectrometry (MS)

In-gel tryptic digestion and MS analysis were performed as reported previously (Wu et al. 2004). Protein spots showing distinctly different expression patterns, i.e. more than 1.5-fold expression variation, were excised from the preparative gels, destained, and in-gel digested with sequencing-grade trypsin (Promega, Madison, WI, USA). The peptides were extracted with 0.1% trifluoroacetic acid (TFA) in 60% acetonitrile, lyophilized and dissolved in 0.5% TFA for mass spectrometric analysis. The prepared peptide was mixed with an equal volume of the matrix solution containing α-cyano-4-hydroxy-cinnamic acid and acetonitrile, then analyzed on an Autoflex MALDI-TOF–TOF instrument (Bruker Daltonics, Billerica, MA, USA). All spectra were obtained with a positive-ion reflector. The resulting peptide mass fingerprinting (PMF), together with pI and M_W values estimated from 2-DE gels, were queried against UniProtKB/SwissProt database using the software GPS Explorer version 3.6 and MASCOT version 1.9 software tools (www.expasy.ch). The database was based on the whole genome sequence of *A. ferrooxidans* ATCC 23270 with NCBI accession number of NC_011761.

2.5. Northern blot analysis

Probes corresponding to each gene were synthesized with specific primers (Table 1) using DIG DNA Labeling Mix (Roche, Basel, Switzerland). Total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) from *A. ferrooxidans* cells grown under the same conditions and harvested at the same growth phase as that used for protein preparation. A total of 20 μg of total RNA per sample was used for Northern blotting analysis. Hybridization was carried out according to protocol provided with the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). The quantity of total RNA loaded was controlled at the same level based on the quantity of 16S rRNA in two compared samples.

2.6. Transmission electronic microscopy (TEM) analysis

Cells in the late exponential phase of growth were harvested by centrifugation at $6000 \times g$ and then washed with sterile H_2O at pH 2.0 until jarosite was completely removed. Preparation of specimens for TEM assay was performed as described previously (Liao et al. 2010). After fixation, the cells were infiltrated with the solution of acetone and epon-araldite over a period of 24 h, polymerized at 60 °C for 48 h. Fifty nanometer sections were sectioned, then the sections were placed on copper sieves and contrasted with uranyl acetate and lead citrate for 30 min each. The sections were viewed using a JEM-1230 TEM (JEOL Japan Electronics Co., Ltd., Japan).