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Chromium resistance characteristics of Cr(VI) resistance genes ChrA and ChrB in *Serratia sp*. S2

Yuan He[a,1], Lanlan Dong[a,1], Simin Zhou[a], Yan Jia[a,c], Ruijia Gu[a], Qunhua Bai[a,c], Jieying Gao[a,b], Yingli Li[a,b], Hong Xiao[a,b,*]

[a] Department of Health Laboratory Technology, School of Public Health and Management, Chongqing Medical University, Medical 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China
[b] Research Center for Medicine and Social Development, Chongqing Medical University, Chongqing 400016, China
[c] Innovation Center for Social Risk Governance in Health, Chongqing Medical University, Chongqing 400016, China

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ABSTRACT

objective: To find an efficient chromium (VI) resistance system, with a highly efficient, economical, safe, and environmentally friendly chromium-removing strain, ChrA, ChrB, and ChrAB fragments of the chromium (VI) resistance gene in *Serratia* sp. S2 were cloned, and their prokaryotic expression vectors were constructed and transformed into *E. coli* BL21. The anti-chromium (VI) capacity and characteristics of engineered bacteria, role of ChrA and ChrB genes in the anti-chromium (VI) processes, and the mechanism of chromium metabolism, were explored.

Methods: The PCR technique was used to amplify ChrA, ChrB, and ChrAB genes from the *Serratia* sp. S2 genome. ChrA, ChrB, and ChrAB genes were connected to the prokaryotic expression vector pET-28a and transferred into *E. coli* BL21 for prokaryotic expression. Cr-absorption and Cr-efflux ability of the engineered strains were determined. The effects of respiratory inhibitors and oxygenated anions on Cr-efflux of ChrA and ChrB engineered strains were explored.

Results: ChrA, ChrB, and ChrAB engineered strains were constructed successfully; there was no significant difference between the control strain and the ChrB engineered strain for Cr-metabolism ($P > 0.05$). Cr-absorption and Cr-efflux of ChrA and ChrAB engineered strains were significantly stronger than the control strain ($P < 0.05$). Oxyanions (sulfate and molybdate) and inhibitors (valinomycin and CN^-) could significantly inhibit the Cr-efflux capacities of ChrA and ChrAB engineered strains ($P < 0.05$), while NADPH could significantly promote such capacities ($P < 0.05$).

Conclusion: The Cr-transporter, encoded by ChrA gene, confer the ability to pump out intracellular Cr on ChrA and ChrAB engineered strains. The ChrB gene plays a positive regulatory role in ChrA gene regulation. The Cr-metabolism ability of the ChrAB engineered strain is stronger than the ChrA engineered strain. ChrA and ChrAB genes in the Cr-resistance system may involve a variety of mechanisms, such as sulfate ion channel and respiratory chain electron transfer.

1. Introduction

In recent years, wide applications of Cr in the industrial development of China have led to serious environmental contamination of Cr (VI). Biodegradability by using microorganisms is generally the preferred method of Cr(VI) pollution treatment, because of the advantages of microorganism methods, such as low cost, affordability, efficiency, environmental friendliness, etc. (Karthik et al., 2017). The bioremediation of microbial chromate has attracted the interest of numerous researchers, but there has not been much characterization of the mechanisms of Cr(VI) reduction (Das et al., 2016). A variety of wild bacteria, separate from a Cr(VI) contaminated environment, have been reported with Cr-resistance or Cr(VI)-reducing potential, including *Serratia* sp. S2 (Zhou et al., 2017), *Bacillus cereus SJ* (He et al., 2010), *Lysinibacillus fusiformis ZCl* (He et al., 2011), *Arthrobacter* sp. *strain FB24* (Henne et al., 2009), *Ochrobactrum tritici 5bvl1* (Branco and Morais, 2013), *Pseudomonas aeruginosa* (Kang et al., 2017), *Escherichia coli* (Chihomvu et al., 2015), *Leucobacter salsicius* (Yun et al., 2014), and

* Corresponding author at: Department of Health Laboratory Technology, School of Public Health and Management, Chongqing Medical University, Chongqing 400016, China.
E-mail address: fengzsm2018@sina.com (H. Xiao).
[1] Contributed equally.

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some other species. It has been reported that all these wild strains are resistant to, or can reduce chromate, but are pathogenic for human beings, so these strains are not suitable for the environment, to deal with Cr(VI) pollution. The metabolism of chromate in *Serratia* sp. S2, with a high potential for chromate resistance and the removal of chromate (HE Yuan et al., 2017), is probably accomplished by a complex system with several related genes (Dong et al., 2017). In this study, the ChrA and ChrB genes were chosen as the research objects. The ChrA protein is a transmembrane protein that belongs to the CHR superfamily, which has thousands of ChrA homologues in its protein family. Previous studies have shown that the ChrA protein can use H^+ to generate a transmembrane electrochemical proton gradient, to excrete intracellular Cr out of cells, thus decreasing the toxicity of Cr(VI) to cells (Aguilar-Barajas et al., 2008; Sarkar et al., 2015). Most of these wild strains with the chromium resistance gene ChrA are highly pathogenic, and they can easily become biological pollution. The ChrB protein has two main functions: a protein relating with Cr-resistance, and a Cr-sensitive regulator on the Chr operon, which can be activated by Cr(VI) (Branco and Morais, 2013; Chihomvu et al., 2015).

To find an efficient chromium (VI) resistance system, an efficient, economical, safe, and environmentally friendly chromium-removing strain was constructed, to lay the foundation for the microbiological treatment of chromium pollution in the future. In the actual environment, the viability and metabolic activity of many microorganisms are limited. Therefore, this study was carried out at the molecular level of microorganisms, to explore the metabolic processes of microorganisms and the influences of various factors on the microbial Cr(VI) resistance. This research group has studied a high Cr(VI) resistance strain with the genetic properties of *Serratia*. ChrA, ChrB, and ChrAB were constructed by gene cloning and recombination techniques. Recombinant expression vectors determine the anti-chromium (VI) capacity and characteristics of the engineered bacteria. The roles of the ChrA and ChrB genes in the anti-Cr(VI) process and the mechanism of chromium (VI) metabolism, were explored.

2. Materials and methods

2.1. ChrA, ChrB, and ChrAB genes

Serratia sp. S2 was preserved in at 80 °C (HE Yuan et al., 2017), and its genomic DNA was acquired according to the boiled template method (Tan et al., 2013). Plasmids used in the experiment were purified using the TIANprep Mini Plasmid Kit (Tiangen Biotech Co., Ltd., Beijing, China). ChrA, ChrB, and ChrAB gene sequences were searched for the whole-genome sequence of *Serratia* sp. S2. Special primers were designed by Primer Premier 5.0 and synthesized by Sagan corporation. All primer sequences are shown in Table 1. The whole-genome sequence of *Serratia* sp. *S2* was used as the PCR-amplified template of ChrA, ChrB, and ChrAB genes. The PCR amplification reaction mixture (25 μl) contained 2 × pfu PCR Master Mix (Tiangen Biotech Co., Ltd., Beijing, China). The PCR amplification program of ChrA was as follows: One cycle at 94 °C for 3 min; 30 cycles at 94 °C for 30 s, 67 °C for 30 s, and 72 °C for 90 s; and one cycle at 72 °C for 5 min. The PCR amplification program of ChrB was as follows: One cycle at 94 °C for 3 min; 30 cycles at 94 °C for 30 s, 69 °C for 30 s, and 72 °C for 1 min; and one cycle at 72 °C for 5 min. The PCR amplification program of ChrAB was as follows: One cycle at 94 °C for 3 min; 30 cycles at 94 °C for 30 s, 69 °C for 30 s, and 72 °C for 150 s; and one cycle at 72 °C for 5 min. All amplified products were identified by 1.5% agarose gel (Sangon Biotech Co., Ltd.) electrophoresis and subsequently purified by the TIANgel Midi Purification Kit (Tiangen Biotech Co., Ltd., Beijing, China).

Table 1
Primer pairs sequences.

Primer name	Primer sequences(5′→3′)	Restriction enzyme site
ChrA-F	CCG**GAATCC**ATGAGCAAAACGGTCGTTCT	EcoR I
ChrA-R	CCG**CTCGAG**TTCTGCGCCGGACAGT	Xho I
ChrB-F	CCG**GAATTC**ATGCGTGTCTGGCGAACCCTGA	EcoR I
ChrB-R	CCC**AAGCTT**TCACTCTGCGGAAGAACGA	*Hin*dIII
ChrAB-F	CCG**GAATTC**ATGCGTGTCTGGCGAACCCT	EcoR I
ChrAB-R	CCG**CTCGAG**TCATTCTGCGCCGGACAGTCC	Xho I

①The line portion is the restriction site.
②The wave line portion is protected base.

2.2. Construction of engineered strains

The purified PCR products and expression vector pET-28a(+) (preserved at −80 °C) deal with double enzyme cutting by a restriction enzyme (listed in Table 1). After purification, recovery, and connection, the target DNA fragments were ligated to pET-28a(+), to generate the recombinant vectors pET-28a(+)-ChrA, pET-28a(+)-ChrB and pET-28a(+)-ChrAB. Then the recombinant vectors were transferred into *E. coli* BL21 (DE3) (Tiangen Biotech Co., Ltd., Beijing, China) cells to construct engineered bacteria. The empty vector pET-28a(+) was used to construct the control strain. Positive clones, which contained pET-28a(+), were selected and subsequently verified by DNA sequencing confirmation (Sangon Biotech Co., Ltd.).

2.2.1. Expression of recombinant protein

The correctly identified colonies of *E. coli* BL21(DE3) carrying pET-28a(+)-ChrA, pET-28a(+)-ChrB, and pET-28a(+)-ChrAB, dubbed as ChrA, ChrB, and ChrAB, were grown at 37 °C in the LB medium with kanamycin (50 μg/ml) to an A600 of 0.6. Then, 0.5 mM of isopropyl-β-D-thiogalactoside (IPTG) was added, and incubation was continued for 6 h at 100 r/min, 30 °C. The control strain was induced at the same conditions as the blank. The ChrA protein was extracted by the Triton x114 detergent method (Aizawa et al., 2008), while the ChrB protein was extracted by the sonication method (Cerny et al., 1995). The protein concentration was detected by the BCA Protein Assay Kit (Sangon Biotech Co., Ltd.). Then, the proteins were diluted to the same concentration, and the expression levels of proteins were determined by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.2.2. Western blot of ChrA in engineered bacteria, ChrA and ChrAB

Induced detergent phase ChrA proteins of ChrA and ChrAB engineered bacteria were transferred to a PVDF membrane after SDS-PAGE. The blotted PVDF (Immobilon-P) was washed twice with Tris/0.05% Tween(R) (TBST), and the nonspecific binding sites were blocked with 5% nonfat dry milk at room temperature for 1 h. The PVDF membrane was probed with the 1:400 dilution primary antibody (Rabbit anti-His-tag) at 4 °C overnight. After being washed 3 times for 10 min, the antibody-antigen complexes were tagged with sheep-rabbit peroxidase on the secondary anti-IgG antibody, and shaken at room temperature for 1 h. Then the complexes were washed 3 times for 5 min. The PVDF film was placed on the plate of a Bio-Rad fluorescence imager and photographed using ECL as luminescent substrates (Bahouth, 2000; Tang et al., 2011).

2.3. Cr-resistance characteristics of engineered bacteria

2.3.1. Resistance to Cr(VI)

The overnight-shaken cultures of ChrA, ChrB, ChrAB, *Serratia* sp. S2, and the control strain were inoculated into fresh LB medium with $Cr_2O_7^{2-}$ (25, 50, 75, and 100 mg/L) for 24 h at 37 °C, and the A600 was measured by a UV–vis spectrophotometer. The growth curve was drawn to determine the Cr(VI) resistance of the strains.