Characterization and genomic analysis of chromate resistant and reducing *Bacillus cereus* strain SJ1

Minyan He¹,², Xiangyang Li¹, Liang Guo¹, Susan J Miller³, Christopher Rensing²*, Gejiao Wang¹*

**Abstract**

**Background:** Chromium is a toxic heavy metal, which primarily exists in two inorganic forms, Cr(VI) and Cr(III). Chromate [Cr(VI)] is carcinogenic, mutational, and teratogenic due to its strong oxidizing nature. Biotransformation of Cr(VI) to less-toxic Cr(III) by chromate-resistant and reducing bacteria has offered an ecological and economical option for chromate detoxification and bioremediation. However, knowledge of the genetic determinants for chromate resistance and reduction has been limited so far. Our main aim was to investigate chromate resistance and reduction by *Bacillus cereus* SJ1, and to further study the underlying mechanisms at the molecular level using the obtained genome sequence.

**Results:** *Bacillus cereus* SJ1 isolated from chromium-contaminated wastewater of a metal electroplating factory displayed high Cr(VI) resistance with a minimal inhibitory concentration (MIC) of 30 mM when induced with Cr(VI). A complete bacterial reduction of 1 mM Cr(VI) was achieved within 57 h. By genome sequence analysis, a putative chromate transport operon, chrIA1, and two additional chrA genes encoding putative chromate transporters that likely confer chromate resistance were identified. Furthermore, we also found an azoreductase gene azoR and four nitroreductase genes nitR possibly involved in chromate reduction. Using reverse transcription PCR (RT-PCR) technology, it was shown that expression of adjacent genes chrA1 and chrI was induced in response to Cr(VI) but expression of the other two chromate transporter genes chrA2 and chrA3 was constitutive. In contrast, chromate reduction was constitutive in both phenotypic and gene expression analyses. The presence of a resolvase gene upstream of chrIA1, an arsenic resistance operon and a gene encoding Tn7-like transposition proteins ABBCCCD downstream of chrIA1 in *B. cereus* SJ1 implied the possibility of recent horizontal gene transfer.

**Conclusion:** Our results indicate that expression of the chromate transporter gene chrA1 was inducible by Cr(VI) and most likely regulated by the putative transcriptional regulator ChrI. The bacterial Cr(VI)-resistant level was also inducible. The presence of an adjacent arsenic resistance gene cluster nearby the chrI1A suggested that strong selective pressure by chromium and arsenic could cause bacterial horizontal gene transfer. Such events may favor the survival and increase the resistance level of *B. cereus* SJ1.

**Background**

The wide use of chromium (Cr) in textile, leather tanning and electroplating industries with subsequent sewage disposal causes severe contamination of global soil-water systems [1,2]. Highly soluble, hexavalent chromium [chromate, CrO₄²⁻] is very toxic. As an analogue of sulfate, chromate can enter bacterial and mammalian cells readily via sulfate transport systems [3]. The subsequent reduction of Cr(VI) by glutathione, thiols and other metabolites, and coproduction of reactive oxygen species (ROS) that damage DNA and other cellular components are the cause of the carcinogenic, mutational, and teratogenic potential of chromate [4-6]. On the other hand, the trivalent chromium (Cr(III)) is less bioavailable, thermodynamically stable and less toxic [7]. Accordingly, the reduction of toxic Cr(VI) to
stable Cr(III) is an efficient way to remove chromate from soil and water systems.

Bioremediation of chromate-contaminated sites, especially when stimulating indigenous microbial communities, is getting more and more attention because of its economical and environmental friendly aspects compared to chemical and physical methods [8-10]. An increasing number of Cr(VI)-reducing bacteria have been detected and studied including a pseudomonad strain CRB5 [4], Brucella sp. [11], Bacillus sp. strain QC1-2 [12], Burkholderia cepacia MCMB-821 [13] and Thermus scotoductus strain SA-01 [14].

Bacteria have developed different strategies of chromate resistance including chromate efflux and chromate reduction. Efflux of chromate, which is mediated by the chromate transporter protein ChrA, has been confirmed in Pseudomonas aeruginosa [15,16], Ochrobactrum tritici 5bv11 [17] and Shewanella sp. ANA-3 [18]. Prior studies have not identified a chromate-responsive regulatory protein. Most chromate reduction studies have focused on soluble enzymes encoded by genes located on chromosomes [19]. However, very few of the proteins responsible for chromate reduction have been purified and characterized because of technical difficulties. When examining induction of chromate resistance and reduction genes, several strains including Shewanella oneidensis MR-1 [20], Ochrobactrum tritici 5bv11 [17] and Ralstonia metallidurans strain CH34 [21] have been shown to contain genes induced by chromate.

In this study, a chromate-resistant and reducing strain Bacillus cereus S1J was successfully isolated from chromium contaminated wastewater of a metal electroplating factory. Three chromate transporter related genes chrA, a chromate responsive regulator chrI, four nitR genes encoding nitroreductase and one azoR gene possibly involved in chromate reduction were identified by the draft genome sequence. Using RT-PCR technology, we found that all of the five genes encoding putative chromate reductases appeared to be expressed constitutively. In contrast, the gene chrA1 encoding a transporter with high homology to other transporters linked to chromate resistance was up-regulated by the addition of Cr(VI) together with the adjacent putative transcriptional regulator chrI. Since chrA1 is probably regulated by chrI, this suggests identification of the first known chromate-responsive regulator.

**Results**

**Identification of Cr(VI)-reducing B. cereus S1J that is highly chromate resistant**

Strain S1J showing both high Cr(VI) resistance and reduction abilities was isolated from industrial wastewater of a metal plating factory. S1J was a Gram positive, rod shaped bacterium. The 16S rDNA sequence was used for bacterial identification. S1J showed the highest identity (100%) with B. cereus 03BB102 [GenBank: CP001407] and was hereafter referred to as B. cereus S1J.

B. cereus S1J showed rapid reduction of Cr(VI) aerobically. Cell growth and Cr(VI) reduction by B. cereus S1J were monitored spectrophotometrically (Figure 1). The growth rate of S1J was rapid. It reached log-phase in 4-6 h in LB medium and the growth rate was decreased by addition of 1 mM chromate. In the first 12 h, the chromate reduction rate was shown to be fastest under optimum pH (7.0) and temperature (37°C) conditions (data not shown). After 57 h of incubation, up to 97% soluble Cr(VI) was reduced and white precipitate was visible at the bottom of the flasks [22]. Abiotic Cr(VI) reduction was not observed in cell-free LB medium (Figure 1).

![Figure 1 Chromate reduction and growth curves of B. cereus S1J](image-url)
After cultivation of *B. cereus* SJ1 for 24 h with 1 mM K₂CrO₄, the cell became shorter with roughness on the cell surfaces possible caused by the environmental stress of chromate (Figure 2)

**General features of *B. cereus* SJ1 draft genome and genes involved in chromate metabolism**

Draft genome sequence analysis of *B. cereus* SJ1 showed a genome size of about 5.2 Mb distributed in 268 contigs with an average GC content of 35.4%, containing 5,708 putative coding sequences (CDSs). There are 100 tRNA genes representing all 20 amino acids and 6 scattered ribosomal RNA genes identified on the draft genome. The likely origin of replication of the chromosome of *B. cereus* SJ1 was located in a 9.0 kb region that included co-localization of six genes (*rpmH, gyrA, gyrB, recF, dnaN* and *dnaA*). It was localized by comparing its draft genome to complete genomes of several strains of the *B. cereus* group though MUMmer3.20.

Three putative chromate transporter genes, *chrA1, chrA2* and *chrA3* were identified in the genome of *B. cereus* SJ1 (Additional file 1). The *chrA1* encoding ChrA protein showed the highest amino acid identity (97%) with a homologous protein annotated as chromate transporter in *Bacillus thuringiensis* serovar konkukian str. 97-27 [GenBank: YP036530]. Interestingly, *chrA1* gene (locus_tag: BCSJ1_04594, 1,194 bp) located downstream of a potential transcriptional regulator gene *chrI* (locus_tag: BCSJ1_04599, 309 bp). The region of *chrA1* and *chrI* also contained several CDSs encoding homologs of Tn7-like transposition proteins and a resolvase that could potentially have been involved in horizontal gene transfer events (Figure 3a). This region covered 26 kb sequence and showed lower GC content (32.8%) compared with the average GC content of *B. cereus* SJ1’s whole genome (35.4%). A similar region was also observed in *B. thuringiensis* serovar konkukian str. 97-27 (Figure 3b), but was absent in other *B. cereus* genomes. Remarkably, differing from *B. thuringiensis* serovar konkukian str. 97-27, this region of *B. cereus* SJ1 contained several genes related to arsenic resistance including genes encoding an arsenic resistance operon repressor ArsR, arsenic resistance protein ArsB, arsenate reductase ArsC, arsenic chaperon ArsD and arsenic pump ATPase ArsA (Figure 3a). This may indicate a very recent horizontal gene transfer (HGT) event since genes located upstream of *chrIA1* and downstream of arsenic resistance genes were resolvase and Tn7-like transposition protein ABBCCCD in both strains. Furthermore, four *nitR* genes encoding nitroreductases and a gene *azoR*, encoding an azoreductase that had previously been reported to catalyze chromate reduction [19,23] were found in the draft genome (Additional file 1, 99-100% amino acid identities).

**Heavy metal tolerance of *B. cereus* SJ1 and putative genes responsible for heavy metal resistance**

Since *B. cereus* SJ1 was isolated from industrial wastewater containing various toxic elements in addition to chromium, the MICs of *B. cereus* SJ1 for these heavy metals were determined. For *B. cereus* SJ1, the highest resistance was found for As(V), while Hg(II) was the most toxic compared to the other metal ions. When *B. cereus* SJ1 was incubated with increasing As concentration, no viable cells were recovered at concentrations above 50 mM As(V) and 4 mM As(III). The MICs of

![Figure 2 SEM micrographs of *B. cereus* SJ1 cells. (a), *B. cereus* SJ1 cells grown in LB medium for 24 h without K₂CrO₄, (b), *B. cereus* SJ1 cells grown in LB medium amended with 1 mM K₂CrO₄ for 24 h. Scale bars: 1 μm.](http://www.biomedcentral.com/1471-2180/10/221)
B. cereus SJ1 for Cu(II), Co(II), Ni(II), Cd(II), Ag(I) and Hg(II) were 0.9 mM, 0.8 mM, 0.7 mM, 0.2 mM, 0.02 mM and 0.007 mM, respectively. In order to survive in such unfavorable habitat, B. cereus SJ1 must have various determinants to tolerate such harsh conditions. For example, the copper concentration of the wastewater was as high as 0.65 mM and the MIC of B. cereus SJ1 to copper was 0.9 mM in R2A medium. When we analyzed the genome sequence of B. cereus SJ1, several genes related to copper resistance including copper-exporting P-type ATPase CopA, copper export protein CopC, copper resistance protein CopD, copper homeostasis protein CutC and two multicopper oxidases were identified. Furthermore, many other putative heavy metal resistance genes including those for As, Zn, Mn, Co, Cd, Te and Hg were also identified in the B. cereus SJ1 draft genome (Additional file 2).

Chromate reduction is constitutive
The difference in chromate reducing ability of B. cereus SJ1 with and without Cr(VI) induction was not significant (Figure 4A). Although less rapid chromate reduction was observed in B. cereus SJ1 cells induced before inoculation during the first 32 h, both cultures emerged at approximately 85% chromate reduced within 55 h. No abiotic Cr(VI) reduction was observed in LB medium without bacterial inoculation. Induction of genes possibly responsible for chromate reduction was also evaluated by RT-PCR. As shown in Figure 5, all the four nitR genes and the azoR gene were expressed constitutively. The results of RT-PCR were in agreement with the bacterial chromate induction experiment (Figure 4A), strongly indicating no significant regulatory influence of chromate on chromate reduction.

Expression of chrA1 is inducible by chromate
Using the procedure described in Methods, we found that the uninduced and induced cells grew to similar cell densities in medium containing 5 mM Cr(VI) as determined spectrophotometrically at OD$_{600}$. However, the induced cells grew to higher cell densities than the uninduced cells at higher Cr(VI) concentrations in the growth medium. The MIC of induced B. cereus SJ1 to K$_2$CrO$_4$ was 30 mM whereas that of the uninduced strain was 20 mM (Figure 4B).
Figure 4 Chromate resistance and reduction of *B. cereus* SJ1. Chromate reduction (A) and resistance (B) analysis of *B. cereus* SJ1 uninduced (◊) and induced with (■) 1 mM K₂CrO₄ for 8 h before bacterial inoculation in LB medium (pH 7.0). *B. cereus* SJ1 was incubated for 48 h before growth was measured for Cr resistance determination. (▲), amended with 1 mM K₂CrO₄ without bacterial inoculation as a control. Error bars represent standard deviation of triplicate samples.

Figure 5 RT-PCR analysis of putative chromate reduction genes *nitR* and *azoR*. M, 1 kb DNA ladder. r, negative control for RT, obtained using total RNA (after DNase I treatment) as the template for PCR amplification, to verify that no genomic contamination was present in the RNA extract; c, RT-PCR product using the first strand cDNA as the template; g, PCR positive control obtained using genomic DNA from *B. cereus* SJ1 as the template. 0, 1 and 3 after r and c represent samples uninduced and induced by 0.3 mM K₂CrO₄ for 1 h and 3 h, respectively. Lanes 1-7, *nitR1* (locus_tag: BCSJ1_00500, 592 bp); Lanes 8-14, *azoR* (locus_tag: BCSJ1_06081, 413 bp); Lanes 15-21, *nitR2* (locus_tag: BCSJ1_14230, 480 bp); Lanes 22-28, *nitR3* (locus_tag: BCSJ1_17540, 546 bp); Lanes 29-35, *nitR4* (locus_tag: BCSJ1_02410, 477 bp); Lanes 36-38, RT-PCR of 16S rRNA genes. The arrow indicates a non-specific band.
Induction of the different \textit{chrA} genes was also evaluated by RT-PCR using RNA isolated from cultures grown in the presence and absence of 0.3 mM Cr(VI) from 0 h to 3 h (Figure 6A). A \textit{chrA}1-specific fragment was clearly visible when Cr(VI) was added that was absent when no Cr(VI) was added (Lane 4 vs 5 and 6), indicating expression of \textit{chrA}1 was induced by the addition of Cr(VI). In contrast, RT-PCR of the other two \textit{chrA} genes, \textit{chrA}2 and \textit{chrA}3, showed that both were expressed constitutively. No products were found using total RNA as the template for PCR amplification, thus indicating the absence of DNA contamination in the total RNA preparations.

\textit{chrI}, encoding a transcriptional regulator, is regulated by chromate

The \textit{chrI} gene located upstream of \textit{chrA}1 encodes a protein with 98% amino acid sequence identity to the PadR-family transcriptional regulator from \textit{B. thuringiensis} serovar konkukian str. 97-27 [GenBank: YP036529]. As \textit{chrI} was a potential transcriptional regulator, it should be responsive to the inducer (Cr), so we analyzed the transcription of \textit{chrI} at 5 and 15 min after addition of K$_2$CrO$_4$. A very weak PCR product was detected with cDNA from uninduced cells as shown in Figure 6B. The level of the \textit{chrI} gene transcript was 16-fold higher (analyzed using BandScan 5.0 program) in cells induced for 15 min compared to the uninduced culture (lane 4 vs 6), confirming substrate-mediated regulation of \textit{chrI}. To confirm the hypothesis that \textit{chrI-chrA}1 was transcribed as a single transcription unit, RT-PCR was carried out with mRNA prepared from \textit{B. cereus} SJ1 grown with and without K$_2$CrO$_4$ (0.3 mM) as described above. PCR products of the expected size (1,130 bp) were obtained with cDNA from both induced and uninduced cultures as the templates (Figure 6B), which indicated \textit{chrI} and \textit{chrA}1 were arranged as an operon. No PCR products...
were amplified using total RNA as the template that was designed to detect DNA contamination.

The arrangement of chrI genes in an operon together with chrA encoding a chromate transporter can be detected in both Gram positive and Gram negative bacteria (Additional file 3). An alignment of ChrI homologs was constructed using ChrI of *B. cereus* SJ1 and other related proteins encoded in operons having a chrI gene adjacent to a chrA gene (Additional file 4). The more-conserved domains were located in the N- and C-terminal regions. Within the conserved domains, two amino acids, lysine and arginine, were identified that might be involved in chromate binding and recognition.

**Discussion**

Chromate-reducing bacteria have been discovered in both contaminated and non-polluted environments [1,13,24,25]. In this study, a chromate-resistant strain *B. cereus* SJ1 was isolated from chromium contaminated wastewater of a metal plating factory in China. *B. cereus* SJ1 showed a rapid growth rate in chromate containing medium and efficient chromate-reducing ability under aerobic conditions. Since the isolation site for *B. cereus* SJ1 was contaminated with as much as 1.89 mg Cr per liter (36.28 μM), we reasoned that genes conferring chromate resistance could be present in this strain. By genome sequencing analysis, we were able to identify a putative chromate transport operon chrIA1 and two additional chrA genes encoding putative chromate transporters that confer chromate resistance. Furthermore, we also found an azoreductase gene azoR and four nitR genes that encode nitroreductases which may catalyze reduction of chromate [19,23].

The membrane transporter protein ChrA has been shown to be responsible for extrusion of chromate ions across the cytoplasmic membrane in *P. aeruginosa* [15,16], *Ochrobactrum tritici* 5bvl1 [17] and *Shewanella* sp. ANA3 [18]. It was demonstrated that the chromate transporter ChrA functions as a chemiosmotic pump that extrudes chromate using proton-motive force [15]. ChrA protein belongs to the CHR superfamily which includes dozens of putative homologs from all three domains of life [26]. Cr(VI) induction of *B. cereus* SJ1 in this study conferred the ability to survive at a higher chromate concentration. Exposure to chromate resulted in the up-regulation of chrA1 and higher chromate resistance. Possibly increased level of ChrA1 is responsible for higher chromate resistance.

The chrI gene product located upstream of chrA1 showed a high homology to PadR-family transcriptional regulators. The *padA* gene encoding phenolic acid decarboxylase, is a member of the PadR family that has been identified as a transcriptional repressor in *Pediococcus pentosaceus* [27] and *Lactobacillus plantarum* [28]. Although genes encoding PadR homologs located either upstream or downstream of putative chromate transporter gene *chrA* have been identified in many genera, such as *B. thuringiensis* serovar konkukian str. 97-27 [GenBank: YP036529], *Oceanobacillus iheyensis* HTE831 [GenBank: NP694199], *B. licheniformis* ATCC 14580 [GenBank: YP093604] and *Alkaliphilus orenlandii* OHL-LAs [GenBank: YP001512811], the real function of a PadR homolog associated with chromate resistance has never been reported. In this study, this gene encoding a PadR homolog was renamed as chrI since it was induced by chromate. By an alignment of most PadR-like regulators which form an operon with the chromate transporter gene *chrA*, highly conserved basic amino acids (lysine and arginine) were identified in ChrI and the homologs that might be involved in chromate binding and recognition because they would carry a positive charge under physiological conditions. Possibly the negatively charged oxanion CrO$_4^{2-}$ would preferentially bind the basic, positively charged amino acids conserved in the putative transcriptional regulator ChrI.

A strong selective pressure for transformation of metal- and metalloid-related resistance genes is present in heavy metal contaminated environments [29,30]. Horizontal gene transfer (HGT) events driven by mobile genetic elements, such as phages, plasmids, insertion sequences, integrons and transposons, have been shown to provide microbes with a wide variety of adaptive traits for microbial survival under hostile environmental conditions. In this study, *B. cereus* SJ1 was isolated from wastewater contaminated with multiple heavy metals. The presence of a resolvase gene upstream of chrI1A1 and an arsenic resistance gene cluster, and the Tn7-like transposition proteins ABCCCD gene downstream of the arsenic resistance operon in *B. cereus* SJ1 but absent in other strains of *B. cereus* implied the possibility of a recent HGT event. Interestingly, other strains of *B. cereus* harbor a gene encoding CHRD-domain-containing protein adjacent to the *chrA* gene. Whether these proteins have a regulatory role is currently unknown [31]. In addition, ChrA1 from *B. cereus* SJ1 is only distantly related to ChrA proteins from other strains of *B. cereus* indicating potential horizontal gene transfer from other Gram-positive bacteria as an adaptation to survive in a highly chromate contaminated environment.

Chromate can be reduced nonenzymatically as well as by various bacterial enzymes. Dihydrolipoamide dehydrogenase from *Thermus scotoductus* SA-01 [32], azoreductase in *Shewanella oneidensis* [19] and flavoproteins from *P. putida* and *E. coli* [3] were previously reported to be associated with Cr(VI) reduction. Compared to the one electron transfer chromate reductase gene *chrR* from *P. putida*, *yieF* from *E. coli* was proposed to be a more appropriate gene for bioremediation applications.
because of the three-electron transfer ability of its gene product and consequently, the generation of fewer reactive oxygen species (ROS) [33]. In our study, one azoreductase gene azoR and four nitR genes encoding nitroreductase obtained from B. cereus SJ1 showed high identities with other Cr(VI) reductases and were expressed constitutively. Since Cr(VI) reduction of strain SJ1 was not inducible by chromate, other potential chromate reductases in B. cereus SJ1 must also be constitutively expressed and the enzyme activity is probably adventitious.

Conclusion
This study describes insights into the chromate resistance and reduction capabilities of B. cereus SJ1 using both physiological and molecular techniques. The expression of the chromate transporter gene chrA1 was inducible by Cr(VI) and most likely regulated by chrI. Even though the physiological function of ChrI has not been verified due to the absence of a genetic system for this Gram positive strain, ChrI is most likely the first identified chromate responsive regulator. In addition, genome analysis identified a number of putative genes encoding gene products with possible functions in chromate resistance and reduction which may be the basis for the observed high chromate resistance and reduction ability of this strain. Furthermore, possible horizontal gene transfer events indicated in this study may have enabled B. cereus SJ1 to survive in metal (loid) contaminated environments.

Methods
Isolation of Cr(VI)-resistant and reducing bacteria
Industrial wastewater samples were obtained from a metal electroplating factory in Guangdong, China. The total concentrations of Cr, Cu, Zn, Mn, Pb, Co, As and Cd in this sample determined by atomic absorption spectrometry were 36.28 μM, 0.65 mM, 24.88 μM, 7.83 μM, 0.49 μM, 0.41 μM, 0.32 μM, and 0.007 μM, respectively. Isolation of chromate-resistant and reducing bacteria was performed as described [34]. The abilities of the chromate-resistant bacteria to reduce Cr(VI) (K₂CrO₄) were determined using a spectrophotometric method using the reagent 1, 5-diphenylcarbazide (DPC) [34]. Several chromate-resistant bacteria were isolated and strain SJ1 was chosen for this study. The 16 S rDNA of strain SJ1 was obtained from the genome sequence (see below) and analyzed by BlastN searching tools http://www.ncbi.nlm.nih.gov/blast. Cell morphologies were examined under a scanning electron microscope (SEM; JSM-6390, JEOL, Japan) with 20,000 V accelerating voltage and 15,000 times enlargement.

Determination of the minimal inhibitory concentrations (MICs) of heavy and transition metals and metalloids
The MIC, defined as the lowest concentration of heavy metals that inhibited growth in R2A broth (Becton Dickinson, MD, USA), was performed with strain SJ1. A 1% inoculum of an overnight culture was introduced into R2A medium amended with different concentrations of CuCl₂, NiCl₂, Co(NO₃)₂, Na₂HAsO₄, NaAsO₂, HgCl₂, CdCl₂ and AgNO₃, incubated at 37°C on a rotary shaker at 200 rpm for 3 days. MIC values were determined spectrophotometrically at OD₆₀₀.

Chromate resistance and reduction assays
The exponential phase cultures of uninduced, and induced with 1 mM K₂Cr₂O₆ for 8 h, were adjusted to the same OD₆₀₀. One hundred microliters of each culture was added to 10 ml fresh LB medium with increasing amounts of K₂CrO₄ and incubated at 37°C with 200 rpm shaking for 3 days. The OD₆₀₀ values were then determined spectrophotometrically. For chromate reduction, the uninduced and induced cultures were prepared as above and inoculated into 100 ml LB medium amended with 1 mM K₂CrO₄ and incubated at 37°C on a rotary shaker at 200 rpm for about 60 h. The residual Cr(VI) concentration was monitored as described above. LB medium with 1 mM K₂CrO₄ without bacterial cells was incubated as a negative control to monitor abiotic chromate reduction.

Sequencing of the B. cereus SJ1 genome
High-molecular-mass genomic DNA isolated from B. cereus SJ1 using Blood & Cell Culture DNA Mini Kit (Qiagen, MD, USA) was used to construct a 4 kb to 40 kb random genomic library. Whole genome shotgun sequencing was performed by the University of Arizona Genetics Core facility, using a Roche 454 Genome Sequencer FLX instrument. The B. cereus SJ1 DNA sample was loaded onto one region of a standard four-region plate. A local Linux computing cluster was used for signal processing on the images produced by the FLX instrument. The Roche gsassembler software version 2.0.01 was used for de novo assembly of the 271,408 reads. Using the default assembly parameters, 141 contigs of length greater than 500 bp were built, along with 127 shorter contigs. These 268 contigs were submitted to the RAST annotation server [35] for subsystem classification and functional annotation. Genome comparison was performed through SEED server http://rast.nmpdr.org/seedviewer.cgi and the subsequent results were modified manually. GC content was analyzed using CLC Main Workbench 5 program http://www.clcbio.com. The NCBI Prokaryotic Genomes Automatic Annotation Pipeline was used for gene annotation.
in preparation for data submission to GenBank. http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html.

Gene expression and co-transcription analyses

RT-PCR was used to assess induced expression and co-transcription of the chromate resistance and reduction related genes of strain SJ1. Total RNA was obtained from mid-exponential phase strain SJ1 cells grown from 0 h to 3 h in the presence or absence of 0.3 mM K$_2$CrO$_4$ in LB medium. Total RNA was isolated by the RNeasy Mini Kit (Qiagen) and then digested with DNase I (Fermentas, MD, USA) to remove any DNA. The OD$_{260}$ values were then determined spectrophotometrically for the total RNA concentration. Equal amounts of total RNA were used to perform cDNA synthesis using iScript™ Select cDNA Synthesis Kit (Biorad, CA, USA). Standard PCR programs were used to generate amplicons from 3 μl of the reverse transcription reaction mixture using the specific primer pairs listed in Additional file 5. PCR amplification using RNA as template was served as the control to investigate the potential presence of DNA contamination. The relative levels of the cDNAs of RT-PCR were determined by densitometric analyses using BandScan 5.0 software (GLyko Inc., Novato, CA, USA) using 16 S rRNA genes as references.

Deposition of strain and nucleotide sequences

*B. cereus* SJ1 was deposited in The Agricultural Research Service Culture Collection, USA (NRRL http://nrrl.ncuar.usda.gov) under the accession number of NRRL B-59452. The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank http://www.ncbi.nlm.nih.gov/sites/genome under the accession number of ADFM00000000. The version described in this paper is the first version, ADFM01000000.

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Author details

1State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China. 2Department of Soil, Water and Environmental Science, The University of Arizona, Tucson, AZ 85721, USA. 3Biotecnomology Computing Facility, Arizona Research Laboratories, The University of Arizona, Tucson, AZ 85721, USA.

Authors’ contributions

All authors participated in the design of the study and data analyses. MH carried out bacterial isolation, resistant and reduction assay, molecular genetic studies and manuscript preparation. XL carried out the genome shotgun submission. LG performed the electron microscope analysis. CR participated in the design of the experiments and helped to draft the manuscript. GW is the principal investigator of the funded project. She coordinated the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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References

1. Pattanapipitsapa P, Brown NL, Macaskie LE: Chromate reduction and 16 S rRNA identification of bacteria isolated from a Cr (VI)-contaminated site. Appl Microbiol Biotechnol 2001, 57:257-261.
2. Morales-Barrera L, Cristan-Urbina E: Hexavalent chromium removal by a *Thiobedema inhamatum* fungal strain isolated from tannery effluent. Water Air Soil Pollut 2008, 187:327-336.
3. Ackerley DF, Gonzalez CF, Park CH, Blake R, Keyhan M, Matin A: Chromate-reducing properties of soluble flavoproteins from *Pseudomonas putida* and *Escherichia colii*. Appl Environ Microbiol 2004, 70:873-882.
4. McLean J, Beveridge TJ: Chromate reduction by a pseudomonad isolated from a site contaminated with chromated copper arsenate. Appl Environ Microbiol 2001, 67:1076-1084.
5. Chardin B, Dolla A, Chaspoul F, Fardeau ML, Gallice P, Bruschi M: Bioremediation of chromate: thermodynamic analysis of effects of Cr(VI) on sulfate reducing bacteria. Appl Microbiol Biotechnol 2002, 60:352-360.
6. Klonowska A, Clark ME, Thieinan SB, Bles BI, Wall JD, Fields MW: Hexavalent chromium reduction in *Desulfovibrio vulgaris* Hildenborough causes transitory inhibition of sulfate reduction and cell growth. Appl Microbiol Biotechnol 2008, 78:1007-1016.
7. Thacker U, Parikh R, Shouche Y, Madamwar D: Hexavalent chromium reduction by *Providencia sp*. Proc Biochem 2006, 41:1332-1337.
8. Smith WL, Gadd GM: Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms. J Appl Microbiol 2000, 88:983-991.
9. Veta M, Curutchet G, Donati E: A combined bacterial process for the reduction and immobilization of chromium. Int Biodeterior Biodegrad 2003, 52:31-34.
10. Poopal AC, Laxman RS: Hexavalent chromium reduction by immobilized *Streptomyces griseus*. Biotech Lett 2008, 30:1003-1010.
11. Thacker U, Parikh R, Shouche Y, Madamwar D: Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr(VI) contaminated sites. Biotechnol Biofuels 2009, 2:1541-1547.
12. Campos J, Martinez-Pacheco M, Cervantes C: Hexavalent-chromium reduction by a chromate-resistant *Bacillus* sp. strain. Antonie van Leeuwenhoek 1995, 68:203-208.
13. Wani R, Kodam KM, Gawai KR, Dhakephalkar PK: Chromate reduction by *Burkholderia cepacia* MCMB-821, isolated from the pristine habitat of alkaline crater lake. Appl Microbiol Biotechnol 2007, 75:627-632.
14. Opperman DJ, Heerden EV: Aerobic Cr (VI) reduction by a chromate-resistant *Bacillus* sp. strain. FEMS Microbiol Lett 1999, 181:7398-7400.
15. Pimentel BE, Moreno-Sanchez R, Cervantes C: Efflux of chromate by *Pseudomonas aeruginosa* cells expressing the ChrA protein. FEMS Microbiol Lett 2002, 212:249-254.
16. Branco R, Chung AP, Johnston T, Gurel V, Morais P, Zhitkovich A: The chromate-inducible chrA/B/C/D/F operon from the transposable element *TnO/Chr* confers resistance to chromium(VI) and superoxide. J Bacteriol 2008, 190:6969-7003.
17. Aguilar-Barajas E, Palouco E, Cervantes C, Rensing C: Expression of chromate resistance genes from *Shewanella* sp. strain ANA-3 in *Escherichia coli*. FEMS Microbiol Lett 2008, 285:97-100.
18. Mugerfield I, Law BA, Wickham GS, Thompson DK: A putative azoreductase gene is involved in the *Shewanella oneidensis* response to heavy metal stress. Appl Microbiol Biotechnol 2009, 82:1131-1141.
19. Thompson MR, Verberkmoes NC, Chourey K, Shah M, Thompson DK, Hettich RL: Dosage-dependent proteome response of *Shewanella oneidensis* MR-1 to acute chromate challenge. J Proteome Res 2007, 6:1745-1757.
20. Juhrik S, Petzich N, Hubener N, Grobe C, Nies DH: New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 2002, 179:15-25.
22. Puzon GJ, Roberts AG, Kramer DM, Xun L. Formation of soluble organo-chromium(III) complexes after chromate reduction in the presence of cellular organics. Environ Sci Technol 2005, 39:2811-2817.

23. Kwak YH, Lee DS, Kim HB. Vibrio harveyi nitroreductase is also a chromate reductase. Appl Environ Microbiol 2005, 69:4390-4395.

24. Pal A, Dutta S, Paul AK. Reduction of Hexavalent Chromium by Cell-Free Extract of Bacillus sphaericus AND 303 Isolated from Serpentine Soil. Curr Microbiol 2005, 51:327-330.

25. Yewalkar SN, Dhumal KN, Sainis JK. Chromium (VI)-reducing Chlorella spp. isolated from disposal sites of paper-pulp and electroplating industry. J Appl Phycol 2007, 19:459-465.

26. Diaz-Perez C, Cervantes C, Campos-Garcia J, Julian-Sanchez A, Riveros-Rosas H. Phylogenetic analysis of the chromate ion transporter (CHR) superfamily. FEBS J 2007, 274:6215-6227.

27. Barthelmebs L, Lecomte B, Divies C, Cavin J. Inducible metabolism of phenolic acids in Pediococcus pentosaceus is encoded by an autoregulated operon which involves a new class of negative transcriptional regulator. J Bacteriol 2000, 182:6724-6731.

28. Gury J, Barthelmebs L, Tran NP, Divies C, Cavin J. Cloning, deletion, and characterization of PadR, the transcriptional repressor of the phenolic acid decarboxylase-encoding padA gene of Lactobacillus plantarum. Appl Environ Microbiol 2004, 70:2146-2153.

29. Ryan RP, Ryan DJ, Dowling DN. Multiple metal resistant transferable phenotypes in bacteria as indicators of soil contamination with heavy metals. J Soil Sed 2005, 5(2):95-100.

30. Cai L, Liu GH, Rensing C, Wang GJ. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiol 2009, 9:

31. Hyvonen M. CHRD, a novel domain in the BMP inhibitor chordin, is also found in microbial proteins. Trends Biochem Sci 2003, 28:470-473.

32. Opperman DJ, Heerden EV. Amembrane-associated protein with Cr (VI)-reducing activity from Thermus scotoductus SA-01. FEMS Microbiol Lett 2007, 280:210-218.

33. Ackerley DF, Gonzalez CF, Keyhan M, Blake R, Marin A. Mechanism of chromate reduction by the Escherichia coli protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. Environ Microbiol 2004, 6:851-860.

34. Yang J, He M, Wang G. Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of Intrasporangium sp. Q5-1. World J Microbiol Biotechnol 2009, 25:1579-1587.

35. Aziz RK, Bartels D, Best AA, DeLongh M, Diaz T, Edwards RA, Formsmo K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vasivea O, Vonstein V, Wilke A, Zagnitko O. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008, 9:75.