High quality draft genome sequence of the heavy metal resistant bacterium *Halomonas zincidurans* type strain B6\(^T\)

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**Abstract**

*Halomonas zincidurans* strain B6\(^T\) was isolated from a deep-sea heavy metal rich sediment from the South Atlantic Mid-Ocean Ridge. The strain showed significant resistance to heavy metals, especially to zinc. Here we describe the genome sequence and annotation, as well as the features, of the organism. The genome contains 3,325 protein-coding genes (2,848 with predicted functions), 61 tRNA genes and 6 rRNA genes. *H. zincidurans* strain B6\(^T\) encodes 31 genes related to heavy metal resistance. And HGT may play an important role in its adaption to the heavy metal rich environment. *H. zincidurans* strain B6\(^T\) may have potential applications in the bioremediation of heavy metal-contaminated environments.

**Keywords:** *Halomonas*, Heavy metal resistant, The South Atlantic Ocean, Genome

**Introduction**

Heavy metals, either essential (e.g. Mn, Zn, Cu, Co, Ni and Mo) or toxic (e.g. Hg, Ag and Cd), are generally harmful to microbial cells even at low concentrations, as to other living organisms [1,2]. However, some microorganisms are able to resist to certain kinds and concentrations of heavy metals through several mechanisms, such as incorporating or precipitating heavy metals into complexes, oxidizing or reducing metals to less toxic valence states, and direct transporting metals out of the cell [3,4]. These heavy metal resistant microorganisms have been attracting great interests because of their potential biotechnological applications in bio-mining of expensive heavy metals and bioremediation of heavy metal-contaminated environment [2].

*Halomonas*, the largest genus of the family *Halomonadaceae*, can be found in most saline environments, including marine environments, salterns, saline lakes and soils, as well as salty foods, etc. [5,6]. *Halomonas zincidurans* strain B6\(^T\), a moderately halophilic bacterium, was isolated from a deep-sea sediment from the South Atlantic Mid-Ocean Ridge [5]. The strain was able to grow in medium containing high concentrations of heavy metals, especially Zn\(^{2+}\) ion, which is not detected in the reference strains and other moderately halophiles [5,7]. Therefore, the novel isolate was named as *H. zincidurans* due to its particular resistance to zinc ion [5]. Here, we present a summary classification and a set of features of *H. zincidurans* strain B6\(^T\), together with the description of the genomic sequencing and annotation.

**Organism information**

A deep-sea sediment sample, TVG10, was collected from the South Atlantic Mid-Ocean Ridge (Table 1). There were many small hard orange red-colored lumps mixed in the sediment sample, which might be the particles containing ferric oxide and diffusing with hydrothermal plumes [8]. Not surprisingly, the concentrations of heavy metals in sample TVG10 were much higher than those in the samples collected from deep-sea seamount sediment [9], offshore sediment [10] and continental crust [11] (Additional file 1: Table S1), including Fe (98.99 mg/g), Mn (42.48 mg/g), Cu (0.839 mg/g), Ni (0.338 mg/g), Zn (0.285 mg/g), Cr (0.195 mg/g) and Co (0.064 mg/g). With consideration of the heavy metal rich environment, marine broth
medium (MB, BD) containing 20 mM Mn$^{2+}$ was used to isolate heavy metal resistant strains. Subsequently, strain B6T was obtained [5].

*H. zincidurans* strain B6T is a Gram-stained negative, rod-shaped (Figure 1), moderately halophilic bacterium growing at 0.5-15% (w/v) NaCl (Table 1). Strain B6T exhibited the highest 16S rRNA gene sequence similarity with *H. xinjiangensis* (96.1%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain B6T and *H. xinjiangensis* clustered together in a distinct branch within the genus *Halomonas* with a high bootstrap value (Figure 2). Strain B6T was able to resist high concentrations of heavy metals in liquid HM medium, including Mn$^{2+}$ (200 mM), Co$^{2+}$ (1.0 mM), Cu$^{2+}$ (2.5 mM) and Zn$^{2+}$ (14 mM). Its resistance to Zn$^{2+}$ could be much higher (30 mM) when incubated on marine agar 2216 medium (MA, BD) [5], comparing to only 1 mM Zn$^{2+}$ resisted by *H. xinjiangensis* TRM0175T. And the maximum zinc resistance concentration for 250 moderately halophilic bacteria, reported by Nieto et al., was only 2.5 mM [7]. Therefore, *H. zincidurans* strain B6T is of significant interest due to its prominent resistance to zinc.

### Genome sequencing information

#### Genome project history

The next-generation shotgun-sequencing and quality assurance was performed at the Beijing Genome Institute.

### Table 1 Classification and general features of *H. zincidurans* B6$^T$ according to the MIGS recommendations [12]

| MIGS ID | Property | Term | Evidence code$^a$ |
|---------|----------|------|-------------------|
| MIGS-6  | Habitat  | Deep-sea sediment | TAS [5] |
| MIGS-6.3| Salinity  | Moderately halophilic, 0.5-15% NaCl | TAS [5] |
| MIGS-22 | Oxygen   | Strictly aerobic | TAS [5] |
| MIGS-15 | Biotic relationship | Free-living | NAS |
| MIGS-14 | Pathogenicity | Not reported | |
| MIGS-4  | Geographic location | South Atlantic Ocean | TAS [5] |
| MIGS-5  | Sample collection time | Feb 20, 2012 | NAS |
| MIGS-4.1| Latitude  | 13.60° S | TAS [5] |
| MIGS-4.2| Longitude | 14.52° W | TAS [5] |
| MIGS-4.3| Depth    | 2950 m | TAS [5] |
| MIGS-4.4| Altitude | -2950 m | TAS [5] |

Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [25].
The gap closure and annotation processes were performed by the authors. The Whole Genome Shotgun project of *H. zincidurans* strain B6 has been deposited at DDBJ/EMBL/GenBank under the accession JNCK00000000. The version described in this paper is version JNCK01000000. Table 2 presents the project information and its association with MIGS version 2.0 compliance [12].

**Growth conditions and DNA isolation**

*H. zincidurans* strain B6 was aerobically cultivated in MB medium at 30°C. Total genomic DNA was extracted using the method described by Marmur [32]. The quality and quantity of the genomic DNA was determined by 0.6% agarose gel electrophoresis with λ-Hind III digest DNA marker (TaKaRa, Dalian, China) and by a Qubit® fluorometer (Invitrogen, CA, USA) with Qubit dsDNA BR Assay kit (Invitrogen, CA, USA). About 350 μg DNA with a concentration of 450 ng/μl was obtained.

**Genome sequencing and assembly**

Whole-genome shotgun DNA sequencing of *H. zincidurans* strain B6 was performed using Solexa paired-end sequencing technology (HiSeq2000 system, Illumina, USA) [33]. Two libraries with insert size 494 bp and 2,586 bp were constructed and a total of 519 Mb and 416 Mb raw data were produced before filtering. After removing the adapter, duplicated reads and short inserts from the data of large library, there remained 433 Mb (~120-folds genome coverage) and 328 Mb (~90-folds genome coverage) clean data from the small and large libraries for assembling, respectively. Then these sequences were assembled into 15 contigs using the SOAPdenovo v.1.05 [30], the contig N50 length of which was 1,864,365 bp. PCR primers for gap closure were designed with Primer Premier v.5. PCR reactions were performed with PrimeSTAR HS Polymerase (TaKaRa, Dalian, China) and the amplicons were sequenced using Sanger and primer walking technologies. The sequenced fragments were subsequently assembled with the contigs using SeqMan of the Lasergene package (DNASTar, Madison, WI) into 2 contigs.

**Genome properties**

The genome was assembled into 2 contigs, one with a size of 3,546,937 bp and the other with 7,823 bp (Table 3). The G+C content determined based on the total 3,554,760 bp sequences was 66.41%. A total of 3,392 genes were predicted, including 3,325 protein-coding genes, 61 tRNA genes and two copies of 16S-23S-5S rRNA gene operons (Table 4 and Figure 2). Among the protein coding genes, 2,848 were assigned to putative functions, and the remaining was annotated as hypothetical proteins. In total, 1,938 and 442 protein coding genes were assigned to KEGG and subsystems, respectively. The detailed properties and the statistics of the genome as well as the distribution of genes into COG functional categories are summarized in Tables 3, 4 and 5, Figure 3 and Additional file 2: Table S2.

**Insights into the genome**

The genome of *H. zincidurans* strain B6 contains 31 genes related to heavy metal resistance, especially to zinc resistance (Table 6). Zinc is an essential but also toxic metal for living being [2,43]. The concentration of zinc inside bacterial cells is maintained by importing limitation, efflux, accumulation and sequestration [44,45]. *H. zincidurans* strain B6 possesses four heavy metal translocating P-type ATPases (HALZIN_733, HALZIN_1240, HALZIN_2196 and HALZIN_2262), which may participate in the transport of Zn2+, Mn2+, Cu2+, Cd2+, Pb2+, Ag+ and Hg2+.
against the concentration gradient to the periplasm [2,44].

Especially the two ZntA P-type ATPases (HALZIN_733 and HALZIN_2196) may mediate resistance to Zn\(^{2+}\), Cd\(^{2+}\) and Pb\(^{2+}\) [46,47]. Zn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Cd\(^{2+}\) and Ni\(^{2+}\) are able to be transported by RND family efflux transporter protein (HALZIN_54, HALZIN_1411, HALZIN_2047, HALZIN_2208 and HALZIN_2209) from both the cytoplasm and the periplasm to outside [2,44]. Usually the P-type ATPases are regulated by MerR family regulators responding to the intracellular heavy metal concentration [44,48,49]. Six analogues of MerR family regulators (HALZIN_399, HALZIN_922, HALZIN_2261, HALZIN_2264, HALZIN_2469 and HALZIN_2675) were found in the genome of H. zincidurans strain B6\(^{T}\). Additionally, a zinc uptake regulation protein ZUR (HALZIN_1413), which is a repressor regulator during zinc uptake, is also detected [44,50]. The presence of these genes is in accordance with zinc resistance phenotype of H. zincidurans strain B6T.

Among the 31 ORFs related to heavy metal resistance, it is noteworthy of two mer-operons. One mer-operon encodes a mercuric transport protein (MerE, HALZIN_916) for organic mercury uptake [51], a transcriptional regulator (MerD, HALZIN_917), three alkylmercury lyases (MerB, HALZIN_918-920) catalyzing organomercurials yielding Hg\(^{2+}\) [52] and a transcriptional regulator (MerR,
HALZIN_922). The other one encodes a transcriptional regulator (MerR, HALZIN_2469), two mercuric transport proteins (MerT and MerP, HALZIN_2470-2471) for inorganic mercury uptake [51] and a mercuric reductase (MerA, HALZIN_2472) catalyzing Hg²⁺ to Hg⁰ [53]. According to the genomic data, *H. zincidurans* strain B6T is able to survive in both inorganic and organic mercury environments. Interestingly, the four ORFs of the inorganic mer-operon showed the highest sequence identities to those of *Halomonas lutea*. Nevertheless, all the six ORFs of the organic mer-operon did not show the highest sequence identities to those of the genus *Halomonas*, but to the genera *Burkholderia*, *Pseudomonas*, *Gladiecola* and *Stenotrophomonas*, which indicates that the organic mer-operon might be acquired by HGT. Of special interest are the three alkylmercury lyases (MerB, HALZIN_918-920), which had obvious differences between the G+C content (56.6%; 57.1, 56.6 and 56.0% for these three gene sequences, respectively) as well as the G+C content at the third-codon positions (60.3%; 60.4, 61.0 and 59.4% for these three gene sequences, respectively) and those of the total protein-coding genes (65.4 and 82.8%, respectively). Besides, the RSCUs of nearly half of the 59 codons used by these three genes (23, 27 and 26 codons for HALZIN_918-920, respectively) change more than 2 folds, compared with those used by total protein-coding genes. 13 of the 31

| Table 2 Project information |
|-----------------------------|
| **MIGS ID** | **Property** | **Term** |
| MIGS-31 | Finishing quality | High-quality draft |
| MIGS-28 | Libraries used | One pair-end 494 bp library and one pair-end 2,586 bp library |
| MIGS-29 | Sequencing platforms | Illumina HiSeq 2000 |
| MIGS-31.2 | Fold coverage | 120 x (494 bp library) and 90 x (2,586 bp library) |
| MIGS-30 | Assemblers | SOAPdenovo [30] |
| MIGS-32 | Gene calling method | Glimmer v3.02 [31] |
| Locus Tag | HALZIN |
| Genbank ID | JNCK00000000 |
| Genbank Date of Release | July 21, 2014 |
| GOLD ID | GI0069861 |
| BIOPROJECT | PRJNA234075 |
| Project relevance | Type strain, environmental, heavy metal resistance |
| MIGS-13 | Source Material Identifier | CGMCC 1.12450, JCM 18472 |

| Table 4 Nucleotide content and gene count levels of the genome |
|--------------------------|
| **Attribute** | **Genome (total)** |
| **Value** | **% of total** |
| Genome size (bp) | 3,554,760 |
| DNA coding (bp) | 3,153,982 |
| DNA G+C (bp) | 2,289,453 |
| DNA scaffolds | 2 |
| Total genes | 3,392 |
| Protein coding genes | 3,325 |
| RNA genes | 67 |
| Genes with function prediction | 2,916 |
| Genes assigned to COGs | 2,764 |
| 1 or more conserved domains | 2,764 |
| 2 or more conserved domains | 329 |
| 3 or more conserved domains | 74 |
| 4 or more conserved domains | 23 |
| Genes with Pfam domains | 2,188 |
| Genes with signal peptides | 180 |
| Genes with transmembrane helices | 697 |
| CRISPR repeats | 1 |

| Table 5 Number of genes associated with the 25 general COG functional categories |
|--------------------------|
| **Code** | **Value** | **% of total** | **Description** |
| J | 164 | 5.14 | Translation |
| A | 1 | 0.03 | RNA processing and modification |
| K | 230 | 7.21 | Transcription |
| L | 188 | 5.89 | Replication, recombination and repair |
| B | 4 | 0.13 | Chromatin structure and dynamics |
| D | 32 | 1.00 | Cell cycle control, mitosis and meiosis |
| Y | - | - | Nuclear structure |
| V | 33 | 1.03 | Defense mechanisms |
| T | 127 | 3.98 | Signal transduction mechanisms |
| M | 182 | 5.71 | Cell wall/membrane biogenesis |
| N | 64 | 2.01 | Cell motility |
| Z | - | - | Cytoskeleton |
| W | - | - | Extracellular structures |
| U | 62 | 1.94 | Intracellular trafficking and secretion |
| O | 109 | 3.42 | Posttranslational modification, protein turnover, chaperones |
| C | 215 | 6.74 | Energy production and conversion |
| G | 216 | 6.77 | Carbohydrate transport and metabolism |
| E | 325 | 10.19 | Amino acid transport and metabolism |
| F | 76 | 2.38 | Nucleotide transport and metabolism |
| H | 145 | 4.55 | Coenzyme transport and metabolism |
| I | 118 | 3.70 | Lipid transport and metabolism |
| P | 171 | 5.36 | Inorganic ion transport and metabolism |
| Q | 108 | 3.39 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 391 | 12.26 | General function prediction only |
| S | 229 | 7.18 | Function unknown |
| T | 628 | 18.51 | Not in COGs |
ORFs (41.9%) were not related to *Halomonadaceae* genes according to the gene sequence similarity analysis, 9 of the 13 ORFs had RSCU change larger than 2 folds in more than 25% codons. These results indicated the existence of HGT events among the heavy metal resistance-related genes. Thus, HGT events might be an important way for *H. zincidurans* strain B6$^T$ to acquire heavy metal resistant ability and to adapt to the heavy metal rich environment.

**Conclusion**

The draft genome sequence of the heavy metal resistant bacteria *H. zincidurans* strain B6$^T$ isolated from the South Atlantic Mid-Ocean Ridge provide an insight into the genomic basis of its heavy metal resistance ability. And HGT may play an important role in its adaptation to the heavy metal rich environment. On the basis of analysis and characterization of genome, *H. zincidurans* strain B6$^T$ might be resistant more kinds of heavy metal than we tested, such as $\text{Hg}^{2+}$, $\text{Cd}^{2+}$, $\text{Pb}^{2+}$, $\text{Ni}^{2+}$ and $\text{Ag}^+$, etc. And it may have the potential for the bio-remediation of multi-metal-contaminated environments. In addition, further analysis will be performed to confirm its resistant ability to other heavy metals and determine the mechanism of heavy metal resistance that we don’t know yet.
| Protein id   | Position | Size/aa | Strand | Predicted function                                      | Organism               | Class          | Identity | Accession no. |
|--------------|----------|---------|--------|--------------------------------------------------------|------------------------|----------------|----------|---------------|
| HALZIN_54    | 48442-49500 | 352     | +      | RND family efflux transporter, MFP subunit             | Idiomarina sediminum   | Gammaproteobacteria | 44%      | WP_025860724  |
| HALZIN_399   | 433553-434005 | 150     | +      | MerR family Cd(II)/Pb(II)-responsive transcriptional regulator | Halomonas lutea        | Gammaproteobacteria | 75%      | WP_019019418  |
| HALZIN_733   | 77827-780812 | 846     | +      | Heavy metal translocating P-type ATPase ZntA           | Gracilimonas tropica   | Sphingobacteria  | 59%      | WP_020403952  |
| HALZIN_916   | 977118-976882 | 78      | -      | Mercutic transport protein MerE                        | Burkholderia cepacia   | Betaproteobacteria | 99%      | WP_006965885  |
| HALZIN_917   | 977480-977115 | 121     | -      | Transcriptional regulator MerD                         | Pseudomonas putida     | Gammaproteobacteria | 98%      | WP_012806008  |
| HALZIN_918   | 978239-977592 | 215     | -      | Alkylmercury lyase MerB                                | Paraglaciecola polaris | Gammaproteobacteria | 94%      | WP_007106069  |
| HALZIN_919   | 979028-978390 | 212     | -      | Alkylmercury lyase MerB                                | Paraglaciecola polaris | Gammaproteobacteria | 94%      | WP_007106069  |
| HALZIN_920   | 979808-979179 | 209     | -      | Alkylmercury lyase MerB                                | Paraglaciecola polaris | Gammaproteobacteria | 90%      | WP_007106069  |
| HALZIN_922   | 980118-980540 | 140     | +      | Transcriptional regulator MerR                         | Stenotrophomonas maltophilia | Gammaproteobacteria | 99%      | WP_005413398  |
| HALZIN_934   | 994405-993521 | 294     | -      | Magnesium and cobalt efflux protein CorC               | Chromohalobacter saleigens | Gammaproteobacteria | 81%      | WP_011507633  |
| HALZIN_1240  | 1334217-1331998 | 739     | -      | Heavy metal translocating P-type ATPase                | Halomonas sp.          | Gammaproteobacteria | 97%      | WP_023004666  |
| HALZIN_1392  | 1499237-1498659 | 192     | -      | Superoxide dismutase                                   | Halomonas smyrnensis   | Gammaproteobacteria | 85%      | WP_016854001  |
| HALZIN_1411  | 1521826-1522995 | 389     | +      | RND family efflux transporter, MFP subunit             | Halomonas lutea        | Gammaproteobacteria | 76%      | WP_019017686  |
| HALZIN_1413  | 1526330-1526785 | 151     | +      | Zinc uptake regulation protein ZUR                     | Halomonas lutea        | Gammaproteobacteria | 82%      | WP_019017691  |
| HALZIN_2047  | 2179598-2182789 | 1063    | +      | RND family efflux transporter protein                   | Pseudoxanthomonas suwonensis | Gammaproteobacteria | 85%      | WP_013533339  |
| HALZIN_2196  | 2338252-2335574 | 892     | -      | Heavy metal translocating P-type ATPase ZntA           | Halomonas lutea        | Gammaproteobacteria | 65%      | WP_019020337  |
| HALZIN_2208  | 2355137-2351976 | 1053    | -      | RND family efflux transporter protein                   | Pseudomonas alcaligenes | Gammaproteobacteria | 58%      | WP_021217164  |
| HALZIN_2209  | 2356423-2351976 | 428     | -      | RND family efflux transporter, MFP subunit             | Halomonas lutea        | Gammaproteobacteria | 53%      | WP_019020155  |
| HALZIN_2226  | 2411989-2410787 | 400     | -      | Multicopper oxidase                                    | Sphingopyxis boekryngensis | Alphaproteobacteria | 55%      | WP_022673021  |
| HALZIN_2261  | 2412630-2413034 | 134     | +      | Transcriptional regulator MerR                         | Halomonas lutea        | Gammaproteobacteria | 90%      | WP_019017365  |
| HALZIN_2262  | 2413107-2415596 | 829     | +      | Heavy metal translocating P-type ATPase                | Halomonas lutea        | Gammaproteobacteria | 92%      | WP_019017357  |
| HALZIN_2264  | 2416527-2416976 | 149     | +      | Transcriptional regulator MerR                         | Halomonas lutea        | Gammaproteobacteria | 89%      | WP_026300314  |
| HALZIN_2266  | 2423176-2423622 | 148     | +      | CopG family transcriptional regulator                  | Halomonas lutea        | Gammaproteobacteria | 80%      | WP_019017364  |
| HALZIN_2271  | 2424931-2425086 | 51      | +      | Copper resistance protein CopC                        | Hyphomonas neptunium    | Alphaproteobacteria | 51%      | WP_011646711  |
| HALZIN_2272  | 2425115-2425978 | 287     | +      | Copper resistance protein CopD                        | Thialkalivibrio sp.     | Gammaproteobacteria | 43%      | WP_018881395  |
| HALZIN_2469  | 2658088-2657690 | 132     | -      | Transcriptional regulator MerR                         | Halomonas lutea        | Gammaproteobacteria | 90%      | WP_019020805  |
| HALZIN_2470  | 2658244-2658588 | 114     | +      | Mercuric transport protein MerT                        | Halomonas lutea        | Gammaproteobacteria | 78%      | WP_019020806  |
| HALZIN_2471  | 2658620-2658925 | 101     | +      | Periplasmic mercury(+2) binding protein MerP           | Halomonas lutea        | Gammaproteobacteria | 82%      | WP_019020807  |
| HALZIN_2472  | 2658988-2660622 | 544     | +      | Mercuric reductase, MerA family                        | Halomonas lutea        | Gammaproteobacteria | 93%      | WP_019020808  |
| HALZIN_2675  | 2872087-2872584 | 165     | +      | Transcriptional regulator MerR                         | Halomonas sp.           | Gammaproteobacteria | 66%      | WP_023005510  |
| HALZIN_3265  | 3489632-3489021 | 203     | -      | Superoxide dismutase                                   | Halomonas lutea        | Gammaproteobacteria | 74%      | WP_019019731  |
Additional files

Additional file 1: Table S1. Concentrations of heavy metals in deep-sea sediment collected from the South Atlantic Mid-Ocean Ridge (1) and the sediments from the Central Pacific seamount (2), offshore sediment (3) and continental crust (4).

Additional file 2: Table S2. Associated MIGS record.

Abbreviations
HGT: Horizontal gene transfer; RSUCl: Relative synonymous codon usage.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
YH designed and performed experiments, analyzed data and wrote the paper; ZL performed experiments; HC analyzed genome data; CW analyzed data; XX designed the experiments and wrote the paper. All authors read and approved the final manuscript.

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