High-quality-draft genome sequence of the multiple heavy metal resistant bacterium Pseudaminobacter manganicus JH-7T

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Abstract

Pseudaminobacter manganicus JH-7T (= KCTC 52258T = CCTCC AB 2016107T) is a Gram-staining-negative, aerobic and non-motile strain that was isolated from a manganese mine. The strain JH-7T shows multiple heavy metal resistance and can effectively remove Mn²⁺ and Cd²⁺. In addition, it is able to produce exopolysaccharides (EPS), which may contribute to metal remove/adsorption. Thus, strain JH-7T shows a great potential in bioremediation of heavy metal-contaminated environment. In this study, we report the draft genomic sequence of P. manganicus JH-7T and compare it to related genomes. Strain JH-7T has a 4,842,937 bp genome size with a G + C content of 61.2%, containing 4504 protein-coding genes and 71 RNA genes. A large number of putative genes associated with heavy metal resistance and EPS synthesis are found in the genome.

Keywords: Cadmium, Exopolysaccharides, Heavy metal resistance and adsorption, Manganese, Pseudaminobacter

Introduction

Genus Pseudaminobacter was established by Kämpfer et al. in 1999 and contains three species represented by Pseudaminobacter salicylatoxidans BN12T (type species) [1], Pseudaminobacter defluvii THI 051T [1] and Pseudaminobacter manganicus JH-7T [2]. The common characteristics of Pseudaminobacter strains are Gram-staining-negative, rod-shaped and aerobic [1, 2]. P. salicylatoxidans BN12T contains a peculiar ring-fission dioxygenase with the ability to cleave salicylate in 1, 2-position to 2-oxohepta-3, 5-dienedioic acid [3].

P. manganicus JH-7T was isolated from a sludge sample of a wastewater ditch in Dalong manganese mine in 2015 [2]. It shows multiple heavy metal resistance and can effectively remove Mn²⁺ and Cd²⁺. In addition, the strain produces EPS, which may facilitate heavy metal resistance and adsorption [4–6]. These features show great interests because of its potential applications in bioremediation of heavy metal contaminated environments. So far, only the genome of an atypical Pseudaminobacter strain Pseudaminobacter salicylatoxidans KCT001 has been sequenced [7]. Strain KCT001 can utilize tetrathionate as the substrate for sulfur-oxidizing chemolithotrophic growth [8]. For better understanding the mechanism of bacterial resistance and removal of heavy metals, here we analyze the genome of P. manganicus JH-7T.

Organism information

Classification and features

The phylogenetic relationship of P. manganicus JH-7T to the related members is shown in a 16S rRNA gene based neighbor-joining tree. Strain JH-7T is closely related to P. salicylatoxidans BN12T, P. defluvii THI 051T and P. salicylatoxidans KCT001 (Fig. 1). Strain JH-7T is Gram-staining-negative, aerobic, non-motile and rod-shaped (0.3–0.8 × 1–2 μm) (Fig. 2). The colonies are white, circular, entire, slightly raised and smooth on LB agar plates. It is positive for oxidase and catalase activities and hydrolysis of casein [2]. The major fatty acids are C₁₈:₁ω₇c, C₁₉:₀ cyclo ω8c and C₁₆:₀ and the G + C content is 61.2 mol% [2]. The major polyamine is sym-homospermidine and the respiratory quinone is
ubiquinone-10. The polar lipids are phosphatidylmonomethylethanolamine, diphosphatidylglycerol, phosphatidylcholine, two aminolipids and two lipids [2]. Table 1 shows the general features of *P. manganicus* JH-7T.

The resistant levels of *P. manganicus* JH-7T to multiple metal(loid)s were tested with the MIC on LB agar plates incubated at 28 °C for 7 days. The MICs for MnCl₂, CdCl₂, PbCl₂, CuCl₂, ZnSO₄ and NiSO₄ are 100, 2, 10, 5, 5 and 5 mmol/L respectively. The MICs for K₂CrO₄ and Na₃AsO₃ are both 0.1 mmol/L that are lower than the above six metals. Specifically, strain JH-7T could remove nearly 60% of 5 mmol/L Mn²⁺ and nearly 80% of 0.1 mmol/L Cd²⁺ (Fig. 3), respectively. In addition, strain JH-7T could produce EPS based on the aniline blue reaction incubated on LB agar in 3–7 days [9] (data not shown). This phenomenon is consistent with the cell image observed by TEM (Fig. 2). A layer of shadow around the strain was similar to the EPS observed in strain *Bifidobacterium longum* 35,624 [10].

**Genome sequencing information**

***Genome project history***

This organism was selected for sequencing particularly due to its multiple heavy metals resistance and heavy metal removal ability. Genome sequencing was performed by Wuhan Bio-Broad Co., Ltd., Wuhan, China in 2016. The draft genome sequence of strain *P. manganicus* JH-7T has been deposited at DDBJ/EMBL/GenBank under accession number MDET00000000. The project information is summarized in Table 2.

**Growth conditions and genomic DNA preparation**

*P. manganicus* JH-7T was grown under aerobic conditions in LB medium at 28 °C for 40 h. DNA extraction
Fig. 3 Mn$^{2+}$ and Cd$^{2+}$ removed by *P. manganicus* JH-7$^T$. Control stands for null LB medium. Strain JH-7$^T$ was incubated until OD$_{600}$ reach 1.0, and then amended with 5000 μmol/L MnCl$_2$ (a) and 100 μmol/L CdCl$_2$ (b), respectively. The cultures were removed at 24 h intervals. After centrifuging at 12,000 rpm for 10 min, the supernatant was used to determine the residual concentration of Mn$^{2+}$ and Cd$^{2+}$ by the atomic absorption spectrometry AAS (AAS; 986A, Beijing Puxi General Instrument 197 Co., Beijing, China). Bars represent the mean ± SD of three biological replicates.
was performed using the QIAamp kit (Qiagen, Germany) as the manufacturer’s instructions. A NanoDrop Spectrophotometer 2000 was used to determine the quality and quantity of the DNA. Seven microgram of DNA was sent to Bio-broad Technology Co., Ltd., Wuhan, China for sequencing.

**Genome sequencing and assembly**
The genome of strain JH-7$^T$ was sequenced on Illumina Hiseq2000 [11] and assembled by Bio-broad Technogoly Co., Ltd., Wuhan using SOAPdenovo v2.04 [12]. An Illumina standard shotgun library was constructed and sequenced, which generated 19,404,755 reads totaling 2,885,684,230 bp and average of 625 times genome coverage. The total size of the genome is 4,842,937 bp and a total of 60 scaffolds were obtained after arranging 68 contigs together. The part gaps of assembly were filled and the error bases were revised using GapCloser v1.12 [13].

**Genome annotation**
The draft genome was annotated through the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and

### Table 2 Project information

| MIGS ID | Property                        | Term                        |
|---------|---------------------------------|-----------------------------|
| MIGS-31 | Finishing quality               | High-quality draft          |
| MIGS-28 | Libraries used                  | Illumina Paired-End library (300 bp insert size) |
| MIGS-29 | Sequencing platforms            | Illumina Miseq 2000         |
| MIGS-31.2 | Fold coverage               | 624.94x                     |
| MIGS-30 | Assemblers                     | SOAPdenovo v2.04            |
| MIGS-32 | Gene calling method             | GeneMarkS$^+$               |
|         | Locus TAG                      | BFN67                       |
|         | Genbank ID                     | MDET00000000                |
|         | Genbank Date of Release        | 31, March, 2017             |
|         | GOLD ID                        | Gp0291525                   |
|         | Bioproject                     | PRJNA38732                  |
| MIGS-13 | Source material identifier      | CCTCC AB 2016107$^T$        |
|         | Project relevance              | Bioremediation              |

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|         | Project relevance              | Bioremediation              |

### Table 3 Genome statistics

| Attribute                  | Value         | % of total$^b$ |
|----------------------------|---------------|----------------|
| Genomic size (bp)          | 4,842,937     | 100            |
| DNA coding (bp)            | 4,238,496     | 87.5           |
| DNA G+C (bp)               | 2,963,726     | 61.2           |
| DNA scaffolds              | 60            | 100            |
| Total genes$^b$            | 4685          | 100            |
| Protein-coding genes       | 4504          | 96.2           |
| RNA genes                  | 71            | 1.7            |
| Pseudo genes               | 110           | 2.3            |
| Genes in internal clusters | 1725          | 38.3           |
| Genes with function prediction | 3228       | 68.9           |
| Genes assigned to COGs     | 3729          | 79.6           |
| Genes with Pfam domains    | 3926          | 83.8           |
| Genes with signal peptides | 392           | 8.4            |
| Genes with transmembrane helices | 1119       | 23.9           |
| CRISPR repeats             | 5             |                |

$^a$The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome

$^b$Also includes 110 pseudogenes, 54 tRNA genes, 12 rRNAs and 5 ncRNA

### Table 4 Number of genes associated with the 25 general COG functional categories

| Code | Value | % of total$^a$ | Description                                                                 |
|------|-------|----------------|------------------------------------------------------------------------------|
| J    | 181   | 4.02           | Translation                                                                  |
| A    | 0     | 0.00           | RNA processing and modification                                              |
| K    | 299   | 6.64           | Transcription                                                                |
| L    | 233   | 5.17           | Replication, recombination and repair                                        |
| B    | 3     | 0.07           | Chromatin structure and dynamics                                             |
| D    | 39    | 0.87           | Cell cycle control, mitosis and meiosis                                      |
| Y    | 0     | 0.00           | Nuclear structure                                                            |
| V    | 46    | 1.02           | Defense mechanisms                                                           |
| T    | 134   | 2.98           | Signal transduction mechanisms                                               |
| M    | 217   | 4.82           | Cell wall/membrane biogenesis                                                |
| N    | 35    | 0.78           | Cell motility                                                                |
| Z    | 0     | 0.00           | Cytoskeleton                                                                 |
| W    | 0     | 0.00           | Extracellular structures                                                     |
| U    | 106   | 2.35           | Intracellular trafficking and secretion                                      |
| O    | 156   | 3.46           | Posttranslational modification, protein turnover, chaperones                 |
| C    | 240   | 5.33           | Energy production and conversion                                             |
| G    | 312   | 6.93           | Carbohydrate transport and metabolism                                        |
| E    | 482   | 10.70          | Amino acid transport and metabolism                                          |
| F    | 87    | 1.93           | Nucleotide transport and metabolism                                          |
| H    | 158   | 3.51           | Coenzyme transport and metabolism                                           |
| I    | 153   | 3.40           | Lipid transport and metabolism                                               |
| P    | 209   | 4.64           | Inorganic ion transport and metabolism                                       |
| Q    | 91    | 2.02           | Secondary metabolites biosynthesis, transport and catabolism                |
| R    | 453   | 10.06          | General function prediction only                                             |
| S    | 444   | 9.86           | Function unknown                                                             |
| –     | 775  | 17.21          | Not in COGs                                                                  |

$^a$The total is based on the total number of protein coding genes in the annotated genome
genes were identified using the gene caller GeneMarkS+ with the similarity-based gene detection approach [14]. The predicted CDSs were translated and were submitted to the Pfam protein family database [15] and KEGG database [16]. The genes in internal clusters were performed by OrthoMCL [17, 18]. The protein function classification, transmembrane helices and signal peptides were predicted by WebMGA [19], TMHMM v. 2.0 [20] and SignalP 4.1 [21], respectively. In addition, the CRISPRfinder program [22] was used to predict CRISPRs in the genome.

**Genome properties**

The draft genome size of strain JH-7T is 4,842,937 bp with 61.2 mol% G + C content and contains 60 scaffolds. The genome properties and statistics are shown in Table 3. From a total of 4685 genes, 4504 (96.2%) are protein coding genes, 110 (2.3%) are pseudo genes and the rest are 71 predicted RNA genes, including 54 tRNA, 12 rRNAs and 5 ncRNA. In addition, 3729 (82.8%) protein coding genes are distributed into COG functional categories (Table 4).

**Insights from the genome sequence**

Strain JH-7T could tolerate multiple heavy metals (Mn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}) and remove Mn^{2+} and Cd^{2+}, suggesting that it has developed a number of evolutionary strategies to adapt to the mine environment. According to the genome annotation results, strain JH-7T harbors various putative proteins related to heavy metal(loid)s resistance including transporters, resistance proteins and metal reductases (Additional file 1: Table S1). MntH [23] and metal ABC transport system [24] are involved in cation uptake. Heavy metal-transporting ATPase is responsible for the efflux of Pb^{2+}, Zn^{2+}, Cd^{2+} and Ni^{2+} [25–28]. The genome contains Cu^{2+} efflux system CopABC [29], mercuric reductase MerA and regulator MerR [30]. Although the MICs for Cr^{6+} and As^{3+} are not high, the Cr^{6+} efflux protein ChrA [27, 31] and As^{3+} resistant proteins (ArsRHC and ACR3) [32–34] are present.

EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives [35]. Stain JH-7T could produce EPS and all essential proteins for EPS production are found in the genome.

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**Fig. 4** Putative nucleotide sugars biosynthesis pathway and EPS synthesis genes in *P. manganicus* JH-7T. **a** The predicted nucleotide sugars biosynthesis pathway. The numbers refer to the enzymes involved: 1, Glucokinase; 2, α-D-glucose phosphate-specific phosphoglucomutase; 3, UTP-glucose-1-phosphate uridylyltransferase; 4, UDP-glucose 4-epimerase GaIE; 5, Glucose-6-phosphate isomerase; 6, Fructokinase; 7, Glutamine-fructose-6-phosphate aminotransferase; 8, Phosphoglucosamine mutase; 9, UDP-N-acetylglucosamine; 10, Glucose-6-phosphate isomerase; 11, Mannose-6-phosphate isomerase; 12, PTS-Man-EIIA, ManX; 13, Phosphoglucomutase; 14, Mannose-1-phosphate guanylyltransferase. **b** The EPS synthesis gene cluster in strain JH-7T.
Four complete nucleotide sugar synthesis (EPS precursor) pathways are identified based on KEGG analysis (Additional file 1: Table S2) including the syntheses of UDP-glucose, UDP-galactose, UDP-GlcNAc and GDP-D-mannose (Fig. 4a). EPS assembly gene clusters were also found in the genome of strain JH-7T [36] (Additional file 1: Table S3, Fig. 4b). Based on gene analysis, it is suggested that the EPS assembly in strain JH-7T might belong to Wzx/Wzy-dependent pathway [37], e.g., repeat units are assembled by glycosyltransferases (EpsI) and translocated across the cytoplasmic membrane to periplasm by flippase (Wzx) [37] and WbaP [38]. Next, Wzy (RfaL), polysaccharide co-polymerase (GumC) and the outer membrane polysaccharide exporter (GumB) transports the polymerized repeat units to cell surface [37, 39]. EPS has been reported to contribute to heavy metal removal/adsorption in bacteria [3–6]. Hence, the ability of EPS may contribute to Mn^{2+} and Cd^{2+} removal.

To gain more insight, the genomic features of strain JH-7T is compared with the available genome P. salicylatoxidans KCT001 [7]. Strain JH-7T has similar genome size (4.84 Mbp) and G + C content (61.2 mol%) compared to strain KCT001 (4.61 Mbp; 62.8 mol%). A total of 2408 core proteins are shared between the two strains. Strain JH-7T has 1724 strain-specific CDSs. Figure 5 shows the genome comparison results of strain JH-7T with strain KCT001 using CGview comparison tool [40]. Comparing to P. salicylatoxidans KCT001, strain JH-7T was unable to utilize tetrathionate for chemolithoautotrophy (data not shown). However, it harbors high quantitative and diverse heavy metal resistance genes.

**Conclusions**

To the best of our knowledge, this study provides the first typical strain genomic information of the genus *Pseudaminobacter* and revealed a consistency of important characters between genotypes and phenotypes. Strain JH-7T is resistant to multiple heavy metals and capable of removal Mn^{2+}/Cd^{2+}. Genome analysis reveal various genes responsible for multiple heavy metal resistance, which provides the genomic basis for this strain to adapt the harmful environment.
Additional file

**Additional file 1**: Table S1. Putative heavy metal(loid) resistance proteins. Table S2. Putative nucleotide sugars biosynthesis proteins for EPS production. Table S3. Putative proteins for EPS production. (XLSX 11 kb)

**Abbreviations**

EPS: Exopolysaccharides; MIC: Minimal inhibition concentration

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**Authors’ contributions**

XX and JL performed the sequence annotation and genomic analysis and prepared the draft manuscript. ZZ, DW and JH performed the heavy metals resistance and removal tests. GW designed the study and revised the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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