High-quality-draft genome sequence of the heavy metal resistant and exopolysaccharides producing bacterium *Mucilaginibacter pedocola* TBZ30T

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**Abstract**

*Mucilaginibacter pedocola* TBZ30T (= CCTCC AB 2015301T = KCTC 42833T) is a Gram-negative, rod-shaped, non-motile and non-spore-forming bacterium isolated from a heavy metal contaminated paddy field. It shows resistance to multiple heavy metals and can adsorb/remove Zn$^{2+}$ and Cd$^{2+}$ during cultivation. In addition, strain TBZ30T produces exopolysaccharides (EPS). These features make it a great potential to bioremediate heavy metal contamination and biotechnical application. Here we describe the genome sequence and annotation of strain TBZ30T. The genome size is 7,035,113 bp, contains 3132 protein-coding genes (2736 with predicted functions), 50 tRNA encoding genes and 14 rRNA encoding genes. Putative heavy metal resistant genes and EPS associated genes are found in the genome.

**Keywords:** *Mucilaginibacter pedocola*, Genome sequence, Heavy metal resistance, Exopolysaccharides

**Introduction**

The genus *Mucilaginibacter* was first established by Pankratov et al. in 2007 and the type species is *Mucilaginibacter paludis* [1]. The common characteristics of this genus are Gram-negative, non-spore-forming, non-motile, rod-shaped and producing exopolysaccharides (EPS) [1, 2]. EPS are long-chain polysaccharides and consist of branched, repeating units of sugars or sugar derivatives [3]. EPS producing bacteria play an important role in environmental bioremediation such as water treatment, sludge dewatering and metal removal [4]. So far, genomic features of *Mucilaginibacter* strains are less studied.

*Mucilaginibacter pedocola* TBZ30T (= CCTCC AB 2015301T = KCTC 42833T) was isolated from a heavy metal contaminated paddy field in Hunan Province, P. R. China [5]. Here we show that strain TBZ30T is resistant to multiple heavy metals and remove Zn$^{2+}$ and Cd$^{2+}$. In addition, strain TBZ30T is able to produce EPS. The genomic information of strain TBZ30T are provided.

**Organism information**

**Classification and features**

Similarity analysis was performed using neighbor-joining method based on the 16S rRNA gene sequences and a phylogenetic tree was constructed using MEGA version 6.0 software (Fig. 1). Bootstrap analysis with 1000 replications was conducted to obtain confidence levels of the branches. Strain TBZ30T showed the highest 16S rRNA gene sequence similarity with *Mucilaginibacter gynuensis* YC7003T (95.8%), *Mucilaginibacter mallensis* MP1X4T (95.4%) and *Mucilaginibacter litoreus* BR-18T (95.4%) [6–8] and grouped together with *M. gynuensis* YC7003T (95.8%), *M. mallensis* MP1X4T (95.4%) and *M. litoreus* BR-18T (95.4%) (Fig. 1).

Strain TBZ30T is Gram-negative, non-motile, and non-spore-forming. Cells are rod-shaped (0.3–0.4 × 1.1–1.3 μm) (Fig. 2). Colonies are circular, pink, convex and smooth on R2A agar. Growth occurs aerobically at 4–28 °C (optimum, 25 °C), pH 5.0–8.5 (optimum, pH 7.0), and in the presence of 0–1.0 (w/v) NaCl (optimum, without NaCl) (Table 1) [5]. Oxidase- and catalase-positive [5]. It can use...
glucose, mannose, L-arabinose, maltose, melibiose, rhamnose and glycogen as the sole carbon sources [5]. Strain TBZ30\textsuperscript{T} can produce EPS testing by aniline blue staining method [9] (Fig. 3). The colonies of strain TBZ30\textsuperscript{T} and the known EPS producing strain *M. litoreus* BR-18\textsuperscript{T} are pink on LB plates (Fig. 3a and b), while the colonies are blue on LB-aniline blue plate (Fig. 3d and e). However, the colonies are always white for the negative control *Nocardiodales albus* KCTC 9186\textsuperscript{T} on either LB or LB-aniline blue plates (Fig. 3c and f). All of the above strains were incubated at 28 °C for 7 days. In addition, strain TBZ30\textsuperscript{T} is resistant to multiple heavy metals. The minimal inhibition concentration (MIC) tests for different heavy metals were performed on R2A agar plates at 28 °C for 7 days. The MICs for ZnSO\textsubscript{4}, CdCl\textsubscript{2}, PbSO\textsubscript{4}, CuSO\textsubscript{4} and NaAsO\textsubscript{2} are 3.5 mM, 1.5 mM, 0.4 mM, 1.2 mM and 0.35 mM, respectively. Furthermore, strain TBZ30\textsuperscript{T} could adsorb/remove nearly 60% of Zn\textsuperscript{2+} and 55% of Cd\textsuperscript{2+} in the R2A liquid medium (added with 0.3 mM ZnSO\textsubscript{4} and 0.25 mM CdCl\textsubscript{2}, respectively) (Fig. 4). The amount of the heavy metals were detected by an atomic absorption spectrometer.

**Genome information**

**Genome project history**

*M. pedocola* TBZ30\textsuperscript{T} was sequenced on the basis of its abilities of heavy metals resistance and removal, which has a great potential for bioremediation. The draft genome was sequenced by Wuhan Bio-Broad Co., Ltd., Wuhan, China. The high-quality-draft genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession number MBTF0000000.1. The project information is shown in Table 2.

**Growth condition and DNA isolation**

*M. pedocola* TBZ30\textsuperscript{T} was grown in R2A medium at 28 °C for 36 h with continuous shaking at 120 rpm. Bacterial cells were harvested through centrifugation (13,400×g for 5 min at 4 °C) and the total genomic DNA was extracted using the QiAamp kit (Qiagen, Germany). The quality and quantity of the DNA were determined using a spectrophotometer (NanoDrop 2000, Thermo).

**Genome sequencing and assembly**

Whole-genome DNA sequencing was performed in Bio-broad Co., Ltd., Wuhan, China using Illumina standard shotgun library and Hiseq2000 pair-end sequencing strategy [12]. For accuracy of assembly, low quality of the original sequence data reads were removed. The
Table 1 Classification and general features of *Mucilaginibacter pedocola* TBZ30\(^T\) [39]

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
|         | Classification | Domain **Bacteria** | TAS [40] |
|         | Phylum | Actinobacteria | TAS [41, 42] |
|         | Class | Sphingobacteria | TAS [43, 44] |
|         | Order | Sphingobacteriales | TAS [45, 46] |
|         | Family | Sphingobacteriaceae | TAS [47] |
|         | Genus | *Mucilaginibacter* | TAS [1] |
|         | Species | *pedocola* | TAS [5] |
|         | Strain | TBZ30\(^T\) (= CCTCC AB 2015301\(^T\) = KCTC 42833\(^T\)) | |
|         | Gram stain | negative | TAS [5] |
|         | Cell shape | rod | TAS [5] |
|         | Motility | non | TAS [5] |
|         | Sporulation | non-sporulating | NAS |
|         | Temperature range | 4–28 °C | TAS [5] |
|         | Optimum temperature | 25 °C | TAS [5] |
|         | pH range; Optimum | 5.0–8.5, 7.0 | TAS [5] |
|         | Carbon source | glucose, mannose, L-arabinose, maltose, melibiose, rhamnose, rhamnose and glycogen | TAS [5] |
|         | MIGS-6 | Habitat | paddy field with heavy metal | TAS [5] |
|         | MIGS-6.3 | Salinity | 0–1% NaCl (w/v), optimal at 0% | TAS [5] |
|         | MIGS-22 | Oxygen requirement | aerobic | TAS [5] |
|         | MIGS-15 | Biotic relationship | free-living | TAS [5] |
|         | MIGS-14 | Pathogenicity | non-pathogen | NAS |
|         | MIGS-4 | Geographic location | Linxiang city, Hunan province, China | TAS [5] |
|         | MIGS-5 | Sample collection | 2014 | TAS [5] |
|         | MIGS-4.1 | Latitude | N30°17′54″ | TAS [5] |
|         | MIGS-4.2 | Longitude | E109°28′16″ | TAS [5] |
|         | MIGS-4.4 | Altitude | not reported | |

*Evidence code-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) [48]*

Fig. 3 EPS detection using the aniline blue staining method [9]. **a**, **b** and **c** strain TBZ30\(^T\), positive control *Mucilaginibacter litoreus* BR-18\(^T\) and negative control *Nocardiales albus* KCTC 9186\(^T\) cultivated in LB plates, respectively; **d**, **e** and **f** the above three strains cultivated in LB-aniline blue plates, respectively.
assembly of TBZ30\textsuperscript{T} genome is based on 16,967,512 quality reads totaling 2,523,391,653 bases with a 377.50\times average genome coverage. The final reads were assembled into 39 contigs (> 200 bp) using SOAPdenovo v2.04 [13]. The part gaps of assembly were filled and the error bases were revised using GapCloser v1.12 [14].

**Genome annotation**
The genome of strain TBZ30\textsuperscript{T} was annotated through the NCBI PGAP, which combined the gene caller GeneMarkS\textsuperscript{+} with the similarity-based gene detection approach [15]. Pseudo genes were predicted using the NCBI PGAP. Internal gene clustering was performed by the OrthoMCL program using Match cutoff of 50\% and E-value Exponent cutoff of 1-e5 [16, 17]. The COGs functional categories were assigned by the WebMGA server with E-value cutoff of 1-e10 [18]. The translations of the predicted CDSs were used to search against the Pfam protein family database and the KEGG database [19, 20]. The transmembrane helices and signal peptides were predicted by TMHMM v. 2.0 and SignalP 4.1, respectively [21, 22].

![Fig. 4](image)

**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 | 377.50x |
| MIGS-30 | Assemblers | SOAPdenovo v2.04 |
| MIGS-32 | Gene calling method | GeneMarkS\textsuperscript{+} |
| Locus TAG | BC343 |
| Genbank ID | MBTF00000000.1 |
| Genbank Date of Release | 04, 25, 2017 |
| GOLD ID | Gs0134261 |
| Bioproject | PRJNA331061 |
| MIGS-13 | Source material identifier | Strain CCTCC AB 2015301 |
| Project relevance | Bioremediation |

**Table 3** Nucleotide content and gene count levels of the genome

| Attribute | Value | % of total |
|-----------|-------|------------|
| Genome size (bp) | 7,035,113 | 100 |
| DNA coding (bp) | 6,126,065 | 87.1 |
| DNA G + C (bp) | 46.1\% | 100 |
| DNA scaffolds | 38 | 100 |
| Total genes | 6072 | 100 |
| Protein-coding genes | 5935 | 97.7 |
| RNA genes | 67 | 1.1 |
| Pseudo genes | 70 | 1.2 |
| Genes in internal clusters | 587 | 9.7 |
| Genes with function prediction | 2736 | 45.1 |
| Genes assigned to COGs | 4046 | 66.6 |
| Genes with Pfam domains | 4434 | 73.0 |
| Genes with signal peptides | 1005 | 16.6 |
| Genes with transmembrane helices | 1407 | 23.2 |
| CRISPR repeats | 11 | 0.2 |

The total is based on the size of the genome in base pairs and the total number of protein coding genes in the annotated genome.
Table 4 Number of genes associated with the 21 general COG functional categories

| COG class | count | % of total | description                                      |
|-----------|-------|------------|--------------------------------------------------|
| J         | 160   | 2.70       | Translation, ribosomal structure and biogenesis   |
| A         | 1     | 0.02       | RNA processing and modification                   |
| K         | 406   | 6.84       | Transcription                                     |
| L         | 224   | 3.77       | Replication, recombination and repair             |
| B         | 1     | 0.02       | Chromatin structure and dynamics                  |
| D         | 35    | 0.59       | Cell cycle control, cell division, chromosome partitioning |
| V         | 88    | 1.48       | Defense mechanisms                                |
| T         | 459   | 7.73       | Signal transduction mechanisms                    |
| M         | 389   | 6.55       | Cell wall/membrane/envelope biogenesis            |
| N         | 23    | 0.39       | Cell motility                                     |
| U         | 87    | 1.47       | Intracellular trafficking, secretion, and vesicular transport |
| O         | 123   | 2.07       | Posttranslational modification, protein turnover, chaperones |
| C         | 185   | 3.12       | Energy production and conversion                  |
| G         | 337   | 5.68       | Carbohydrate transport and metabolism             |
| E         | 247   | 4.16       | Amino acid transport and metabolism               |
| F         | 73    | 1.23       | Nucleotide transport and metabolism               |
| H         | 156   | 2.63       | Coenzyme transport and metabolism                 |
| I         | 162   | 2.73       | Lipid transport and metabolism                    |
| P         | 200   | 3.37       | Inorganic ion transport and metabolism            |
| Q         | 106   | 1.79       | Secondary metabolites biosynthesis, transport and catabolism |
| R         | 593   | 9.99       | General function prediction only                  |
| S         | 431   | 7.26       | Function unknown                                  |
| –         | 1449  | 24.41      | Not in COGs                                      |

The total is based on the total number of protein coding genes in the genome

Fig. 5 A graphical circular map of Muclaginibacter pedocola TBZ30T. From outside to center, rings 1, 4 show protein-coding genes colored by COG categories on forward/reverse strand; rings 2, 3 denote genes on forward/reverse strand; rings 5 show G+C % content; ring 6 shows G+C % content plot and the innermost ring shows GC skew.
Genome properties
The genome size of strain TBZ30^T is 7,035,113 bp with an average G + C content of 46.1% (Table 3). It has 6072 genes including 5935 protein-coding genes, 70 pseudo genes and 14 rRNA, 50 tRNA, and 3 ncRNA genes. The information of the genome statistics is shown in Table 3 and the classification of genes into COGs functional categories is summarized in Table 4. The graphical genome map is provided in Fig. 5.

Insights from the genome sequence
Strain TBZ30^T could be resistant to multiple heavy metals (Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and As^{3+}) and adsorb/remove Zn^{2+} and Cd^{2+} during cultivation. Analyzing of its genome, various putative proteins related to multiple heavy metals resistance are found (Table 5). RND efflux systems (CzcABC), CDF efflux systems (CzcD and YieF) and P-type ATPases (HMA and ZntA) are responsible for the efflux of Zn^{2+}, Cd^{2+} and Pb^{2+} [23–27].

| Heavy metals or EPS production | Putative function | Locus_tag of the predicted protein |
|-------------------------------|------------------|-----------------------------------|
| Zinc-Cadmium-Led resistance   |                  |                                   |
| RND efflux systems            | CusA/CzcA heavy metal efflux RND transporter | BC343_14685, BC343_14785 |
|                              | Efflux RND transporter periplasmic adaptor subunit CzcB | BC343_14680, BC343_14795 |
|                              | Outer membrane protein CzcB | BC343_14800 |
| CDF efflux systems            | Cation transporter CzcD | BC343_11185 |
|                              | Cation transporter YieF | BC343_27350 |
| P-type ATPase                 | Heavy metal translocating P-type ATPase HMA | BC343_08790 |
|                              | Heavy metal translocating P-type ATPase ZosA | BC343_14675 |
|                              | Cadmium-translocating P-type ATPase ZntA | BC343_00930 |
| Zip super family              | Zip family metal transporter | BC343_14670 |
| Copper resistance             | Zip family metal transporter | BC343_14670 |
|                              | Heavy metal translocating P-type ATPase ZosA | BC343_14675 |
|                              | Copper homeostasis protein CutC | BC343_23340 |
| Arsenic resistance            | Arsenite efflux pump ACR3 | BC343_02735 |
|                              | Arsenate reductase ArsC | BC343_02740, BC343_24635 |
|                              | Arsenite S-adenosylmethyltransferase ArsM | BC343_24640 |
|                              | Arsenical resistance repressor ArsR | BC343_24645, BC343_02755 |
| Nucleotide sugars biosynthesis for EPS production | | |
| CDP-Glc                       | Sugar kinase | BC343_21040, BC343_04390 |
|                              | Phosphoglucomutases | BC343_18360 |
|                              | Glucose-1-phosphate cytidylyltransferase RfbF | BC343_04660 |
| ADP-Glc                       | Glucose-1-phosphate adenyllytransferase | BC343_23820 |
| GDP-D-man                     | Glucose-6-phosphate isomerase | BC343_14065 |
|                              | 6-phosphofructokinase | BC343_20710, BC343_25175 |
|                              | Mannose-6-phosphate isomerase ManA | BC343_15810, BC343_21400 |
|                              | Phosphoglucomamine mutase phosphomannomutase | BC343_21600 |
|                              | Mannose-1-phosphate guanylyltransferase | BC343_03170 |
| EPS biosynthesis              | 3-Deoxy-D-manno-octulosonic-acid transferase KdtA | BC343_09425 |
|                              | Priming glycosyltransferase CpsE | BC343_04560 |
|                              | Glycosyltransferase | BC343_04600, BC343_09445 |
|                              | ABC transporter KpsMT | BC343_09400, BC343_09585 |
|                              | Polysaccharide co-polymerase protein PCP | BC343_04670 |
|                              | Outer membrane polysaccharide protein OPX | BC343_04675 |
|                              | Flippase Wzx | BC343_08105 |
|                              | Capsular biosynthesis protein PHP | BC343_09405 |
Zip family metal transporter and P-type ATPase ZosA are associated with the efflux of Zn$^{2+}$, Cd$^{2+}$ or Cu$^{2+}$ [28–30], and CutC is involved in Cu$^{2+}$ homeostasis [30–32]. Moreover, As$^{3+}$-resistant proteins including arsenite efflux pump ACR3, arsenate reductase ArsC, arsenite S-adenosylmethyltransferase ArsM and arsenic resistance repressor ArsR are also found [33–35] (Table 5).

Strain TBZ30$^T$ produces EPS during cultivation. According to KEGG analysis, the complete biosynthesis pathway of repeating units of nucleotide sugars are identified in the genome, including the biosynthesis of CDP-Glc, ADP-Glc and GDP-D-man (Table 5). Genes related to long-chain polysaccharide assembly are also found (Table 5). The EPS production pathway in strain TBZ30$^T$ appears to belong to ABC transporter dependent pathway [36]. First, the 3-deoxy-D-manno-octulosonic-acid transferase (KdtA) is responsible for the synthesis of poly-Kdo linker using either diacil or monoacil phosphatidylglycerol as the substrate [36]; Then priming glycosyltransferase (CpsE) catalyzes the transformation of the first repeating unit to the poly-Kdo linker; Next, glycosyltransferases catalyze the synthesis of EPS repeat-unit; Finally, the polymerized repeat-units are exported through an envelope-spanning complex consisting of ABC transporter (KpsMT), polysaccharide co-polymerase protein (PCP) and outer membrane polysaccharide protein (OPX) [37, 38]. In addition, strain TBZ30$^T$ genome owns a flippase (Wzx) which catalyzes the translocation of repeat-units crossing the cytoplasmic membrane. EPS have been reported to play an important role in metal removal [3]. Therefore, it is possible that the EPS of strain TBZ30$^T$ participate in Zn$^{2+}$ and Cd$^{2+}$ removal by adsorption.

Conclusions
To the best of our knowledge, this study presents the first genomic information of a Muclaginibacter type strain. The data reveal good correlation between geno-

Abbreviations
EPS: Exopolysaccharides; MIC: Minimal inhibition concentration

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Authors' contributions
XF and JT performed the phenotypic characterization, the data analysis and wrote the manuscript. LN participated in phenotypic experiments. JH participated in data analysis. GW was responsible for research design 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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