The first complete genome sequence of species *Shewanella decolorationis*, from a bioremediation competent strain Ni1-3

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Abstract

*Shewanella decolorationis* are Gram-negative γ-Proteobacteria with environmental bioremediation potential because they can perform anaerobic respiration using various types of pollutants as terminal electron acceptors. So far, three isolated and cultured strains of *S. decolorationis* have been reported. However, no complete *S. decolorationis* genome has been published yet, which limited exploring their metabolism and feasibility in application. Here, *S. decolorationis* Ni1-3 isolated from an electroplating wastewater treatment plant showed strong reduction capabilities on azo dyes and oxidized metals. In order to construct the complete genome, high-quality whole-genome sequencing of strain Ni1-3 were performed by using both Nanopore MinION and Illumina NovaSeq platforms, from which the first complete genome of *S. decolorationis* was obtained by hybrid assembly. The genome of strain Ni1-3 contains a megaplasmid and a circular chromosome which encodes more proteins than that of the strains LDS1 and S12 belonging to the same species. In addition, more Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are identified in strain Ni1-3 genome. Importantly, 32 cytochrome-c and AzoR azoreductase coding genes are identified in the genome, which make strain Ni1-3 competent to degrade the azo dyes and versatile to bioremediate some other environmental pollution. The complete genome sequence of strain Ni1-3 can expand our knowledge toward its metabolic capabilities and potential, meanwhile, provide a reference to reassemble genomes of other *S. decolorationis* strains.

Keywords: *Shewanella decolorationis*; complete genome; Nanopore; cytochrome c; bioremediation

Introduction

The *Shewanella* of Gram-negative are facultative anaerobic γ-Proteobacteria which are widely distributed in aquatic environments, such as fresh water and marine sediments (Hau and Gralnick 2007). To date, about 70 different species of *Shewanella* have been discovered and reported (Hau and Gralnick 2007; Lemaire et al. 2020). Their hallmark features include unparalleled respiratory diversity and the capacity to thrive at harsh conditions, and most *Shewanella* strains can grow at low temperatures (<5°C) (Hau and Gralnick 2007). *Shewanella* strains generally have versatile strategies of extracellular electron transfer, which enable them to utilize a wide range of organic carbon sources and transfer electrons to insoluble electron acceptors, such as electrode and metallic precipitate (Lemaire et al. 2020). Meanwhile, *Shewanella* strains can reduce/degrade various inorganic or organic substances, such as Cr(VI) (Wielinga et al. 2001), nitrate (Samuelsson 1985), selenite (Klonowska et al. 2005), dimethyl sulfide (Myers and Nealon 1988), nitroaromatic compounds (Zhao et al. 2005; 2007), and so on. Their metabolic diversity and the nonpathogenicity of most strains make *Shewanella* have a great potential in bioremediation of metal and organic pollution. Moreover, they are useful in producing probiotics, proteins or compounds, as well as constructing microbial fuel cells (Nealson and Scott 2006; Hau and Gralnick 2007; Lemaire et al. 2020).

*Shewanella decolorationis* are unique bacteria in *Shewanella* genus, which can carry out anaerobic respiration using some azo dyes as terminal electron acceptors (Hong et al. 2007; Hong and Gu 2009). The first reported *S. decolorationis* strain S12 isolated from the activated sludge of a textile-printing wastewater treatment plant (Xu et al. 2005). *S. decolorationis* S12 can effectively decolorize Reactive Brilliant Blue K-GR, amaranth, methyl red, and other dyes under anaerobic conditions (Xu et al. 2006; Hong et al. 2007). *S. decolorationis* are very likely to have important application potential in the bioremediation of dyeing wastewater due to their remarkable capacity to decolorize dyes.

To the best of our knowledge, so far only three draft genomes of *S. decolorationis* strains have been reported, namely S12, Ni1-3, and LDS1 (Xu et al. 2013; Cai et al. 2019; Lemaire et al. 2019). Strain Ni1-3 was isolated in our laboratory from the sludge of an electroplating wastewater treatment plant (Cai et al. 2019). This study aims to complete the genome of strain Ni1-3 by assembling the
sequencing reads from Nanopore MinION and Illumina NovaSeq platforms, and to characterize the genome through annotation and comparative genomics.

**Materials and methods**

**Bacterial strain and genomic DNA extraction**

*S. decolorationis* Ni1-3 was from laboratory stock. The original strain was previously isolated from an electroplating wastewater treatment plant and the genomic DNA extraction of strain Ni1-3 was described in our previous work (Cai et al. 2019). The purity of the extracted genomic DNA was robustly checked using the Synergy HTX multi-mode reader (BioTek, USA) and its integrity was verified by the agarose gel electrophoresis.

**Genome sequencing**

The genomic DNA was sequenced using Nanopore and Illumina technologies. The 1.5 μg genomic DNA of strain Ni1-3 was used for the library construction with the SQK-LSK108 kit (Oxford Nanopore Technologies, UK) according to the manufacturer’s instruction, and Nanopore MinION sequencing was performed using MinKNOW v1.15.4 with the FLO-MIN106 flow cell (Oxford Nanopore Technologies, UK). Base calling was conducted using Guppy v3.1.5 which was configured in MinKNOW, and the reads of whose quality less than Q7 were filtered. Illumina sequencing conducted on the NovaSeq platform was described in our previous work (Cai et al. 2019).

**Genome assembly and annotation**

The quality control of Nanopore reads was conducted using NanoPlot v1.35.5 (De Coster et al. 2018). The quality of the Illumina raw reads and clean reads was analyzed using FastQC v0.11.8 (https://github.com/s-andrews/FastQC). Quality trimming, read filtering, and removing adapter were performed using fastp v0.20.1 (Chen et al. 2018). After the quality control of the two data sets, the genome was de novo assembled using Unicycler v0.4.9b (Wick et al. 2017) with normal hybrid assembly mode.

The genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatiana et al. 2016), and the resulting proteome was further annotated with eggNOG-Mapper. The cytochrome-c CDSs of strains Ni1-3, LDS1, and S12 were identified by amino-acid sequence alignment against the 41 cytochrome-c CDSs of Shewanella oneidensis MR-1 (Carnacho et al. 2009), and the alignment results were considered to be positive when meeting the criteria (query coverage > 90%, identity > 80% and e-value < 1 x 10^-5) described by Assis et al. (2017).

**Results and discussion**

**Genomic assembly and features**

To construct the complete genome of strain Ni1-3, both Nanopore sequencing and Illumina sequencing were conducted. Briefly, 74,257 reads were obtained from Nanopore sequencing (Supplementary Table S1). Mean read length is 13,776 bp with average quality of 9.8. For Illumina sequencing, 9,067,958 clean reads were obtained, and the Phred scores higher than Q20 accounts for 96.96% (Supplementary Table S2). A circular chromosome (5,001,960 bp) and a megaplasmid (286,397 bp) were obtained from Unicycler hybrid assembly (Supplementary Table S3), neither of them contains N.

The circular chromosome represents 94.58% of the whole genome of strain Ni1-3 containing 4,164 CDSs, 28 rRNAs, 106 tRNAs (Table 1). Compared with *S. oneidensis* MR-1, the chromosomes of *S. decolorationis* (i.e., strains Ni1-3, LDS1, and S12) showed higher GC contents. Notably, the chromosome of strain Ni1-3 encodes more proteins than that of strain MR-1 and the other two *S. decolorationis* strains (LDS1 and S12). The complete genome sequence of strain Ni1-3 suggests that *S. decolorationis* and *S. oneidensis* may carry similar counts of rRNA and tRNA. Meanwhile 312 CDSs have been predicted in the megaplasmid.

| Organism                  | Accessiona | Assembly level | Length (bp) | GC% | CDSb | rRNAc | tRNAd | Other RNAe |
|---------------------------|------------|----------------|-------------|-----|------|-------|-------|------------|
| *S. decolorationis* Ni1-3 | CP031775.3 | Complete genome | 5,001,960   | 47.2| 4,164| 28    | 106   | 4          |
| *S. decolorationis* LDS1  | CP037898.1 | Chromosome     | 4,719,362   | 47.1| 4,024| 12    | 96    | 5          |
| *S. decolorationis* S12   | GCA_000485795.1 | Scaffold | 4,843,966 | 47.1| 4,094| 9     | 77    | 3          |
| *S. oneidensis* MR-1      | AE014299.2 | Complete genome | 4,969,811   | 46.0| 4,068| 28    | 104   | 6          |

*a* GenBank assembly accession number.

b Number of protein-coding sequences.

c Number of rRNAs.

d Number of tRNAs.

*e* Number of other RNAs.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) in the genome of strain Ni1-3 and other Shewanella strains was carried out using CRISPR Recognition Tool (CRT) (Bland et al. 2007). The features of chromosome and plasmid were visualized using CGView (Stothard and Wishart 2005).

**Phylogenetic and comparative analysis**

To explore the phylogenetic relationship between *S. decolorationis* Ni1-3 and other Shewanella strains, a phylogenetic tree based on the whole genome sequence was constructed using CVTree3 (Zuo and Hao 2015) with all the CDSs of strain Ni1-3 and other 27 Shewanella strains downloaded from the NCBI and JGI databases. Collinearity analysis of the chromosome sequences of strains Ni1-3 and LDS1 was performed using Mauve v2.0150226 (Darling et al. 2004), and the value of locally collinear blocks (LCBs) weight was set as 145. Average nucleotide identity (ANI) calculation of the whole genome of strains Ni1-3, LDS1, and S12 was performed using JSpeciesWS (Richter et al. 2016). To compare the functional genes of strain Ni1-3 with those of other two *Shewanella* strains, the proteomes of strains MR-1 and LDS1 were also comprehensively annotated using eggNOG-Mapper. The cytochrome-c CDSs of strains Ni1-3, LDS1, and S12 were identified by amino-acid sequence alignment against the 41 cytochrome-c CDSs of *Shewanella oneidensis* MR-1 (Carnacho et al. 2009), and the alignment results were considered to be positive when meeting the criteria (query coverage > 90%, identity > 80% and e-value < 1 x 10^-5) described by Assis et al. (2017).
Interestingly, as shown in Supplementary Table S4, there are five CRISPRs in the chromosome of strain Ni1-3, and the numbers of repeats and spacers are 181 and 176, respectively. However, CRISPR is not found in the genome of strains S12 and MR-1, and only one CRISPR is found in the genome of strain LDS1. In the meantime, Cas1, Cas2, and Cas3 have been all found in the chromosome of strain Ni1-3. These indicate that strain Ni1-3 possesses the type I-F CRISPR-Cas system and may have experienced many invasions of exogenous DNA in the past, and now it possesses an excellent immune system. Furthermore, 12 genomic GIs have been identified in the chromosome using the prediction method of IslandPath-DIMOB (Figure 1A). There are also 3 GIs predicted in the plasmid (Figure 1B).

**Phylogenetic characterization**

As shown in the phylogenetic tree of 28 Shewanella strains based on the whole genome (Figure 2), strain Ni1-3 is phylogenetically closest to strains LDS1 and S12, which are clustered in one
terminal tree branch (marked by green shadow in Figure 2), and divergent from S. oneidensis and other Shewanella species (Figure 2). Besides, strain Ni1-3 has high ANI values (>97%) with strains LDS1 and S12 indicating that it belongs to S. decolorationis. The chromosome collinearity analysis of strain Ni1-3 and strain LDS1 resulted in 28 LCBs as shown in Supplementary Figure S1. There are lots of homologous sequences between strains Ni1-3 and LDS1, but there are many inversions, translocations, deletions, and insertions as well, which caused the rearrangement of chromosomal sequences of strain Ni1-3.

**Functional annotations**

A total of 3,714 genes in the chromosome of strain Ni1-3 are annotated based on COG database, and they account for 89% of all the CDSs. Among them, 790 genes accounting for 19% are annotated to unknown functions, and they may be a potential gene set deserved further study. There are 1,475 genes classified by KEGG pathway database annotation, and the most abundant groups of genes are involved in biosynthesis of secondary metabolites, biosynthesis of cofactors and carbon metabolism. As for the plasmid, 178 genes are annotated as hypothetical proteins by RAST, accounting for 57% of all the annotated CDSs. Interestingly, mercuric ion reductase (MerA), transporter (MerT), resistance operon regulatory protein (MerR), and binding protein (MerP) are all found in the plasmid. Besides, four efflux RND transporters related to copper or silver, two copper resistance proteins, and five tellurium resistance proteins are also found in the plasmid. These results suggest that the plasmid provides strain Ni1-3 great power for environmental adaptation and evolution. Moreover, the functions of these hypothetical proteins with high proportion deserve further investigation.

As shown in Figure 3, annotation of strain Ni1-3 chromosome on COG database resulted in 323 genes which are classified as C (Energy production and conversion), and the count of annotated genes is greater than that of strains LDS1 and MR-1. Eighty-seven genes of strain Ni1-3 are involved in G (Carbohydrate transport and metabolism), while 83 and 77 genes carried by strains LDS1 and MR-1 are responsible for carbohydrate and metabolism, respectively. The count of annotated genes involved in P (Inorganic ion transport and metabolism) is more than that of strains LDS1 and MR-1, and both the resistance genes of nickel and copper are found in genome of strain Ni1-3. This result is consistent with that strain Ni1-3 was isolated from the wastewater containing a large amount of heavy metal ions. Moreover, strain Ni1-3 has been proved to possess strong resistance to nickel and copper (Cai et al. 2019). In the meantime, a total of 48 genes are annotated in CAZy database, which are also more than that of strains LDS1 and MR-1. These indicate that strain Ni1-3 may utilize more
organic carbon sources for respiration and possess a stronger metabolic system than strains LDS1 and MR-1.

**Cytochrome-c proteins**

The outstanding feature of *Shewanella* species is possessing a variety of cytochrome-c proteins, which are involved in electron transfer of bacterial respiration (Meyer *et al.* 2004; Breuer *et al.* 2015). To date, excluding one pseudogene, about 41 cytochrome-c CDSs have been identified in strain MR-1 (Meyer *et al.* 2004). Strain MR-1 is a model strain of both *Shewanella* and exoelectrogens (Logan 2009), most of its cytochrome-c proteins have been functionally annotated and well-studied. Thus, we used its 41 cytochrome-c CDSs as references to identify the homologous cytochrome-c proteins. There are 32 cytochrome-c proteins in strains Ni1-3 and S12 identified by BLAST, and strain LDS1 has only 31 cytochrome-c proteins (Figure 4). Consistently, except for periplasmic monoheme cytochrome c4, the cytochrome-c proteins of the three strains are the same. OmcA and MtrC are not found in all the three strains by BLAST, but three or four OmcA/MtrC family decaheme c-type cytochromes are found in their genome by NCBI annotation. The results indicate that their OmcA and MtrC are quite different from those of strain MR-1, and they also possess CymA-Mtr electron transfer chains which are mainly composed of cytochrome-c proteins (Beblawy *et al.* 2018). Consequently, they could obtain electrons from organic carbon sources via that electron transfer chains, and these electrons can be ultimately transferred to extracellular environments for the degradation of azo dyes (Cai *et al.* 2012; Cao *et al.* 2017). Meanwhile, strain Ni1-3 contains AzoR which has been proved to be azoreductase (Yang *et al.* 2013). In fact, strain Ni1-3 has been observed degrading/reducing several azo dyes (*e.g.*, amaranth, Sudan red I, and methyl red) or oxidized cathodic materials with high capacity in our further researches (data not shown), and it may be applicable for dyeing wastewater treatment relying on its respiratory diversity and adaptive capacity to harsh environments.

**Conclusions**

We report a high-quality complete genome of strain Ni1-3 which was isolated from a wastewater treatment plant containing plenty of heavy metal ions. Phylogenetic and ANI calculation analyses indicate that strain Ni1-3 belongs to the species *S. decolorationis* and is closely related to *S. decolorationis* LDS1 and *S. oneidensis* MR-1. This complete genome sequence could work as a reference for assembling genomes of other *S. decolorationis* strains and provide genomic insights for expanding the application potentials of *Shewanella* strains.

**Data availability**

The chromosome and plasmid sequences of *S. decolorationis* Ni1-3 can be accessed under the GenBank accession number CP031775.3 and CP076856.1 respectively. The data sets of Nanopore and Illumina reads are available in the NCBI SRA database via the BioProject accession number PRJNA723063. Supplemental material is available at figshare: https://doi.org/10.25387/g3.14974596.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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