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Complete genome of a metabolically-diverse marine bacterium *Shewanella japonica* KCTC 22435^T

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ABSTRACT

Shewanella japonica KCTC 22435^T is a facultatively anaerobic, Gram-negative, mesophilic, rod-shaped bacterium isolated from sea water at the Pacific Institute of Bio-organic Chemistry of the Marine Experimental Station, Troitza Bay, Gulf of Peter the Great, Russia. Here, we report the complete genome of *S. japonica* KCTC 22435^T, which consists of 4,975,677 bp (G + C content of 40.80%) with a single chromosome, 4036 protein-coding genes, 97 tRNAs and 8 rRNA operons. Genes detected in the genome reveal that the strain possesses a type II secretion system, cytochrome *c* family proteins with various numbers of heme-binding motifs, and metabolic pathways for utilizing diverse carbon sources, supporting the potential of KCTC 22435^T to generate electricity in salinity culture conditions.

1. Introduction

The bacterial genus *Shewanella* (family: *Shewanellaceae*, order: *Alteromonadales*, Class: *Gammaproteobacteria*) was first described by MacDonell and Colwell (1985) and currently consists of >60 species. *Shewanella* species can utilize a wide range of carbon sources and a number of organic and inorganic compounds as terminal electron acceptors under anaerobic conditions (Hau and Gralnick, 2007) allowing these species to survive in diverse habitats including seawater, freshwater, activated sludge, sediment and spoiled food (Liu et al., 2015). Thanks to their remarkable metabolic versatility, *Shewanella* species have been regarded as promising model organisms in the bioremediation of environmental pollutants (e.g., radionuclides, halogenated organics, petroleum, etc.) and microbial fuel cell (MFC) research (Hau and Gralnick, 2007).

MFC devices utilize microorganisms as biocatalysts to convert waste organic matter and biomass into electricity. The process involves production of electrons from carbon sources, transfer of electrons to the extracellular electron acceptors, and generation of electricity by reduction of acceptors (Rabaey and Verstraete, 2005). The choice of the biocatalyst depends upon the metabolic versatility of microorganisms, their ability to transfer electrons outside the cell, and the potential to operate in diverse environmental conditions (e.g. freshwater vs. marine, aerobic vs, anaerobic). *Geobacter*- and *Shewanella*-containing MFCs have recently become popular because their metabolic pathways are well-described in literature (Lovely, 2006; Fredrickson et al., 2008). *Geobacter* species however operate under strict anaerobic conditions and thus their utility is rather limited. In turn, *Shewanella* species can operate well in air-exposed conditions, can utilize a wide range of substrates, and have become attractive models for utilization in MFCs. For example, *Shewanella oneidensis* MR-1 is a fresh-water microbe that is widely used in MFCs. However, freshwater accounts for only ~2.5% of total Earth's water (https://water.usgs.gov/edu/earthwherewater.html), necessitating the need to discover novel microorganisms capable of generating electricity in high salinity environments. Recently *Shewanella marisflavi* EP1 was shown to produce electricity at high ionic strengths (up to 1488 mM or 8% NaCl) (Huang et al., 2010). Unfortunately, respiration of EP1 can apparently be conducted under limited carbon sources (e.g., lactate). Therefore, search for microorganisms capable of transforming wide range of organic substrates into electrical energy and under saline conditions remains an active area of research.

Shewanella japonica KCTC 22435^T is a facultatively anaerobic, Gram-negative, mesophilic, rod-shaped bacterium isolated from sea water at the Pacific Institute of Bio-organic Chemistry of the Marine Experimental Station, Troitza Bay, Gulf of Peter the Great, Russia (Table 1). This type strain was originally reported as agar-digesting bacteria (Ivanova et al., 2001). However, a recent study has revealed that *S. japonica* strain ATCC: BAA-316 (= KCTC 22435^T) can respire diverse carbon sources

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Table 1
General features of *Shewanella japonica* KCTC 22435^T and MIGS mandatory information.

Item	Description
General features	
Classification	Domain *Bacteria* Phylum *Proteobacteria* Class *Gammaproteobacteria* Order *Alteromonadales* Family *Shewanellaceae* Genus *Shewanella*
Type strain	KCTC 22435^T
Gram strain	Negative
Cell shape	Straight rod
Motility	Not reported
Sporulation	Non-spore-forming
Temperature range	10–37 °C, optimally at 20–25 °C
Salinity range	NaCl, 0–3%
pH range	6–9, and optimally at 7.5
Investigation	
Submitted to INSDC	Accession number CP020472
Investigation type	bacteria_archaea
Project name	Genome sequence of *Shewanella japonica* KCTC 22435
Environment	
Geographic location	Gulf of Peter the Great, Russia
Depth	1 m
Collection date	1994-01
Environment (biome)	Temperate marginal sea biome (ENVO:01000856)
Environment (feature)	Bay (ENVO:00000032)
Environment (material)	Water (ENVO:00002006)
Environment (package)	Sea water (ENVO:00002149)
Isolation and growth conditions	PMID: 11411670
Sequencing	
Sequencing platform	PacBio RS II with P6-C4 chemistry
Fold coverage	204.89×
Assembler	SMRT Analysis v2.3.0
Annotation source	Prodigal v2.6.3

ranging from monosaccharides to sucrose to agar and generate electricity in marine environments, consequently supplementing the weaknesses of *S. oneidensis* and *S. marisflavi* and thus becoming a promising model for utilization in MFC devices (Biffinger et al., 2011). Despite the industrial relevance of strain KCTC 22435^T, its genomic information was hitherto unavailable. Therefore, we report the complete genome of the strain KCTC 22435^T and also evaluate its potential to be utilized as biocatalyst in MFC devices.

2. Data description

S. japonica KCTC 22435^T was grown for three days at 25 °C on marine agar. Isolated colonies were picked using a sterile toothpick and genomic DNA was extracted using the i-genomic BYF mini kit (iNtRON Biotechnology, Seongnam, Republic of Korea) following manufacturer's protocols. Genome sequencing was performed using PacBio RS II SMRT sequencing technology (Pacific Biosciences, Menlo Park, CA, USA). A 20-kb insert SMRTbell library was constructed and sequenced yielding >204× average genome coverage. De novo assembly of 138,026 subreads with 8909 nucleotides on average (1,229,796,822 bp in total) was conducted using the hierarchical genome-assembly process (HGAP) pipeline of the SMRT Analysis v2.3.0 (Chin et al., 2013). The overlapping regions at both ends of a contig were manually identified and trimmed to generate a unique stretch on both ends. Then, a new version for the contig was generated by cutting the contig into two halves and switching the first half with the second. The newly generated contig served as reference to which raw PacBio reads were mapped using the resequencing module of the SMRT Analysis. This enabled us to correct possible sequencing errors at contig ends, where mapping coverage is relatively lower.

Next, we identified protein-coding genes using Prodigal v2.6.3 (Hyatt et al., 2010). The predicted CDSs were BLAST-searched against UniProt (Wu et al., 2006), Pfam (Punta et al., 2011) and COG (Tatusov et al., 2003) databases to gain insights about the molecular functions and family classifications of predicted genes. Signal peptides and transmembrane helices were predicted using SignalP v4.1 (Petersen et al., 2011) and TMHMM v2.0 (Krogh et al., 2001). rRNA, tRNA and other miscellaneous features were predicted using RNAmmer v1.2 (Lagesen et al., 2007), tRNAscan-SE v1.21 (Lowe and Eddy, 1997) and Rfam v12.0 (Griffiths-Jones et al., 2005). The graphic circular map of genome was constructed and visualized using Circos v0.67 (Krzywinski et al., 2009). Automatic detection of clustered regularly interspaced palindromic repeats (CRISPRs) was performed using MinCED v.0.2.0 (Bland et al., 2007).

The complete genome of *S. japonica* KCTC 22435^T is composed of a circular chromosome of 4,975,677 bp with G + C content of 40.8% (Fig. 1; Table 2). The protein coding regions cover 84.19% of the genome (4,189,134 bp) and encode 4036 proteins (Table 2). The genome also encodes eight rRNAs (seven operons of 5S, 16S and 23S, in that order, and one operon of two copied 5S, 16S and 23S in that order), 97 tRNAs and three other RNAs (one tmRNA and two ncRNAs). Signal peptides and transmembrane helices were found in 606 and 1086 protein coding genes, respectively (Table 2). Plasmids and CRISPR repeats were not detected.

Molecular mechanisms of electron transfer in *Shewanella* species have not been fully explored. However, both a type II secretory pathway and outer membrane cytochromes are likely to play a critical role in transferring electrons to the extracellular electron acceptors (Hau and Gralnick, 2007). The genome of *S. japonica* KCTC 22435^T encodes a complete gene cluster of the type II secretion system (locus tags SJ2017_4026 to SJ2017_4037), in addition to genes encoding Type VI secretion system apparatus and secretion proteins (SJ2017_2446 to SJ2017_2448, SJ2017_2456 to SJ2017_2463, respectively). The type VI secretion system confers bacteria the abilities of cell adherence and biofilm formation (Linares et al., 2016). Consequently, the presence of type VI secretion system suggests the possibility that the strain KCTC 22435^T can be attached to an electrode surface and form biofilms on the surface. The genome of *S. japonica* KCTC 22435^T also encodes diverse *c*-type cytochrome family proteins that play an important role as electron carriers. The numbers of CXXCH heme-binding motifs of the cytochrome *c* family proteins ranged from one to eleven, but hexaheme cytochrome *c* was not detected in the genome. As the most abundant type, 14 out of the 34 family proteins (e.g., SJ2017_3429) possessed tetraheme-binding motifs. The existence of cytochrome *c* family proteins with diverse numbers of heme-binding motifs support that the strain KCTC 22435^T is able to efficiently attach electrons to the family proteins (Kranz et al., 2009).

Besides, the genome of *S. japonica* KCTC 22435^T indicates that the strain can utilize a diverse range of carbohydrates as carbon source. The genome encodes four beta-agarases (e.g., SJ2017_1905 for degrading agarose), three beta-xylanases (e.g., SJ2017_2021 for xylane), a glycogen debranching protein GlgX (SJ2017_2159 for starch), and an endoglucanase (SJ2017_3472 for cellulose). In addition, the strain may degrade hexoses (e.g., glucose or fructose) using both the pentose phosphate pathway (e.g., SJ2017_3279, SJ2017_3945) and the Entner-Doudoroff pathway (SJ2017_1967 to SJ2017_1970), instead of using the Embden-Meyerhof pathway. The presence of a UDP-galactose-4-epimerase (SJ2017_2010) and galactose-1-phosphate uridylyltransferase (SJ2017_2306) supports that the strain may directly use galactose as a carbon source. The genome also encodes all enzymes (SJ2017_1933, SJ2017_1940, and SJ2017_1941) involved in De Ley-Doudoroff pathway, indicating that galactonate can be degraded by the strain. Finally, the strain may also use carbon sources gluconolactone and gluconate since the genome encodes a 2-dehydro-3-deoxygluconokinase (SJ2017_1903), 2-dehydro-3-deoxyphosphogluconate aldolase (SJ2017_1904), and gluconolactonase (SJ2017_1906).