Desulfosporosinus youngiae sp. nov., a spore-forming, sulfate-reducing bacterium isolated from a constructed wetland treating acid mine drainage

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Strain JW/YJL-B18^T, a spore-forming, sulfate-reducing bacterium, was isolated from constructed wetland sediment. Cells were curved rods, 0.7–1.2 µm in diameter and 3–7 µm long. Despite being phylogenetically a member of the Gram-type-positive phylum *Firmicutes*, cells stained Gram-negative at all growth phases. Strain JW/YJL-B18^T grew at 8–39 °C, with an optimum at 32–35 °C and no growth at 4 °C or below or at 42 °C or above. The pH^{25 °C} range for growth was 5.7–8.2, with an optimum at pH^{25 °C} 7.0–7.3, and no growth was detected at or below pH 5.2 or at or above pH 8.4. The salinity range for growth was 0–3% (NaCl/KCl 9:1). Strain JW/YJL-B18^T utilized as carbon and energy sources beef extract, yeast extract, formate, succinate, lactate, pyruvate, ethanol and toluene. Fumarate, sulfate, sulfite and thiosulfate were reduced in the presence of lactate. Arsenate (V) was not used as an electron acceptor. Strain JW/YJL-B18^T showed no indication of growth under autotrophic conditions. The predominant cellular fatty acids were C_{16:1} and C_{16:0}. The genomic DNA G+C content was 36.6 mol% (HPLC). 16S rRNA gene sequence analysis indicated that strain JW/YJL-B18^T fell into the genus *Desulfosporosinus*, with *Desulfosporosinus auripigmenti* OREX-4^T as its closest neighbour with a validly published name (97.9% similarity). Based on molecular genetic evidence and physiological and biochemical characters including differences in the DNA G+C content, we propose to place strain JW/YJL-B18^T (=DSM 17734^T =ATCC BAA-1261^T) as the type strain of a novel species, *Desulfosporosinus youngiae* sp. nov.

Sulfate-reducing bacteria (SRB) are responsible for a variety of biogeochemical processes. For example, SRB reduce sulfate to dissolved sulfide (S²⁻), which may react with metals to form metal sulfides; thus, they are considered to play a major role in metal removal and alkalinity generation in treating acid mine drainage. The SRB are an ecologically and metabolically diverse group of anaerobes which include members of the phylum *Firmicutes* (formerly the phylogenetically Gram-type-positive bacteria; Wiegel, 1981) residing within the family *Peptococcaceae* in the order *Clostridiales*. Among them, the genus *Desulfosporosinus* currently consists of five species of obligately anaerobic, spore-forming bacteria (<http://www.bacterio.cict.fr/d/desulfosporosinus.html>). Stackebrandt *et al.* (1997) distinguished this group from the genus *Desulfotomaculum* on the basis of phylogenetic evidence and their ability to grow under autotrophic conditions.

However, the property of autotrophic growth (i.e. 50% of the biomass is derived from inorganic carbon) has not been demonstrated unequivocally for all species. Vatsurina *et al.* (2008) recently reported *Desulfosporosinus orientis* 343 as the type strain of a novel species in the same genus, *Desulfosporosinus hippei*, which does not oxidize H₂ with CO₂ or acetate. Here, we report another novel *Desulfosporosinus* species that is not capable of growing lithotrophically, isolated from a constructed treatment wetland receiving acid mine drainage.

A sediment sample was taken from a constructed treatment wetland receiving acid sulfate water (Lee *et al.*, 2006) and inoculated into the medium for enrichment prepared as described by Widdel & Bak (1992). The medium contained lactate (15 mM) as the main carbon and energy source and was supplemented with 0.05% yeast extract. The pH^{25 °C} of the medium was 6.8. The enrichment culture was incubated at 37 °C for 4 weeks. Two isolates, designated strains JW/YJL-B18^T and JW/YJL-B19, were obtained and purified by three subsequent rounds of single colony isolation using the agar (1.5% w/v)-shake-roll-tube

Abbreviation: SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JW/YJL-B18^T is DQ117470.

technique (Ljungdahl & Wiegel, 1986). Colonies of both isolates appeared after 1–2 days in an agar-roll tube and were white, irregular and sometimes filamentous, 0.1–0.8 mm in diameter. The cells of both isolates were curved rods that typically measured 0.7–1.2 µm in diameter and 3.0–7.0 µm in length. Endospores detected in the late exponential growth phase were oval and central to subterminal. Cells stained Gram-negative at all growth phases.

DNA was extracted from the isolates as described previously (Lee *et al.*, 2005) and the 16S rRNA gene was amplified with the primers 27 forward and 1492 reverse (Lane, 1991). PCR amplification and subsequent sequencing of 16S rRNA genes were performed as described previously (Lee *et al.*, 2007). The similarities of partial sequences were determined using Sequencher version 4.1.4 (Gene Codes). Retrieved 16S rRNA gene sequences were analysed using BLASTN, aligned with closely related sequences using CLUSTAL_X version 1.81 (Thompson *et al.*, 1997) and edited using GeneDoc version 2.6.02 (<http://www.nrbcs.org/gfx/genedoc/>). Phylogenetic trees were constructed using algorithms provided in PHYLIP version 3.6a2.1 (Felsenstein, 2001).

The nearly complete 16S rRNA gene sequences of strains JW/YJL-B18^T and JW/YJL-B19 were identical; thus, only strain JW/YJL-B18^T was chosen for further analyses because they were both isolated from the same enrichment culture. The 16S rRNA gene sequence consisting of 1443 bp (positions 52–1470 according to the *Escherichia coli* numbering scheme; GenBank accession no. X80725) placed strain JW/YJL-B18^T within the radius of the genus *Desulfosporosinus* (Stackebrandt *et al.*, 1997). The closest relative identified was the unpublished *Peptococcaceae* strain Y5 (99.8% similarity in the 16S rRNA gene sequence) (Liu *et al.*, 2004). The next closest neighbour was *Desulfosporosinus auripigmenti* OREX-4^T, with 97.9% similarity (Fig. 1). DNA–DNA hybridization of strain JW/YJL-B18^T against *Desulfosporosinus auripigmenti* DSM 13351^T showed 3.1% (5.1% in duplicate) relatedness, indicating that strain JW/YJL-B18^T does not belong to the species *Desulfosporosinus auripigmenti* based on the recommendations of Wayne *et al.* (1987).

Strain JW/YJL-B18^T grew at 8–39 °C at pH 7.0, with an optimum at 32–35 °C. No growth was detected at 4 °C or below or at 42 °C or above. The pH range for growth was pH 5.7–8.2 at 35 °C, with an optimum at pH 7.0–7.3. No growth was detected at or below pH 5.2 or at or above pH 8.4. The salinity range for growth was 0–3% (w/v) NaCl at a NaCl/KCl ratio of 9:1. However, strain JW/YJL-B18^T grew with up to 6% NaCl when iron citrate (0.1 mM) was added to the medium.

Utilization of various carbon sources was determined using the basal medium from Widdel & Bak (1992) supplemented with 0.02% yeast extract. Strain JW/YJL-B18^T utilized beef extract (0.2%), yeast extract (0.2%), formate (20 mM), succinate (20 mM), lactate (20 mM), pyruvate (20 mM), ethanol (20 mM) and toluene (0.1 mM) as carbon and energy sources. There was no evidence of growth on Casamino acids, peptone, tryptone, acetate, arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, sucrose, inositol, mannitol, sorbitol, xylitol, glutamate, propionate, benzene, phenol (1 mM) or benzoate (1 mM). Moreover, strain JW/YJL-B18^T did not show evidence of growth under chemolithoautotrophic or chemolithoheterotrophic conditions, i.e. with H₂/CO₂ or H₂/arsenate/acetate (Liu *et al.*, 2004). Utilization of electron acceptors was tested with 0.2% lactate as an electron donor, and the following electron acceptors at a concentration of 20 mM were utilized: fumarate, forming succinate, and sulfate, thiosulfate and sulfite (2 mM), forming sulfide. Arsenate, anthraquinone 2,6-disulfonate (AQDS), elemental sulfur, ferric citrate, ferric oxyhydroxide, manganese, nitrate, nitrite, nickel, molecular oxygen and selenium were not utilized as terminal electron acceptors. The inability of strain JW/YJL-B18^T to reduce arsenate was further suggested by the failure to obtain a PCR product for the arsenate reductase gene (*arrA*) (Malasarn *et al.*, 2004). Several other morphological and physiological characters separate the novel isolate from other *Desulfosporosinus* species (Table 1). Strains JW/YJL-B18^T and JW/YJL-B19 failed to yield a PCR product using primer sets for the α -subunit of the adenosine-5'-phosphosulfate reductase gene (*apsA*) and the dissimilatory sulfite reductase genes (*dsrAB*) (Friedrich,

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequences showing the position of strain JW/YJL-B18^T amongst members of the genus *Desulfosporosinus*. The tree was constructed using the neighbour-joining method with Jukes and Cantor distance corrections. Numbers at nodes represent bootstrap values (percentages of 1000 replicates); values above 80% were considered significant. Bar, 2 substitutions per 100 nucleotide positions.

Table 1. Biochemical and chemotaxonomic characteristics of strain JW/YJL-B18^T and its closest relatives

Strains: 1, JW/YJL-B18^T (this study); 2, *Desulfosporosinus auripigmenti* OREX-4^T (Newman *et al.*, 1997; Stackebrandt *et al.*, 2003); 3, *Desulfosporosinus meridiei* S10^T (Robertson *et al.*, 2001); 4, *Desulfosporosinus orientis* DSM 765^T (Campbell & Postgate, 1965; Stackebrandt *et al.*, 2003); 5, *Desulfosporosinus lacus* STP12^T (Ramamoorthy *et al.*, 2006); 6, *Desulfosporosinus hippei* 343^T (Vatsurina *et al.*, 2008). ND, No data available.

Character	1	2	3	4	5	6
Source	Constructed wetland sediment	Lake sediment	Anaerobic groundwater	Soil	Lake sediment	Permafrost deposit
Cell diameter (µm)	0.7–1.2	0.4	0.7–1.1	0.7–1.0	0.5–0.7	1.0–1.2
Temperature for growth (°C)						
Range	8–39	ND	10–37	<42	4–32	5–37
Optimum	32–35	25–30	28	30–37	30	28
pH for growth						
Range	5.7–8.2	ND	ND	ND	6.5–7.5	6.5–7.5
Optimum	7.0–7.3	6.4–7.0	7.0	7.0	ND	7
Salinity (% NaCl)	0–3	ND	0–3	0–4.5	ND	<2.0
DNA G + C content (mol%)	36.6 (HPLC)	41.6 (HPLC)	46.9 (<i>T_m</i>)	45.9 (<i>T_m</i>)	42.7 (HPLC)	42.1 (HPLC)
Gram stain	–	+	Variable	–	–	–
Spore shape and position*	O, C–ST	O, ST–T	O, ST	O, ST	O, ST	R, T
Oxidation of H ₂	–	+	+	+	+	–
Electron donors used						
Formate	+	+	+	+	+	–
Fumarate	–	–	–	+	–	–
Malate	–	+	–	–	–	–
Succinate	+	–	–	–	–	–
Glycerol	–	+	+	–	+	–
Electron acceptors used						
Arsenate	–	+	–	–	–	–
Fumarate	+	+	–	+	–	–
Sulfur	–	+	+	+	–	–
Iron (III)	–	–	–	–	+	–

*C, Central; O, oval; R, round; ST, subterminal; T, terminal.

2002; Wagner *et al.*, 1998). However, these results are probably due to deviations from consensus sequences in regions complementary to the two primer sets.

Bacterial cell-membrane phospholipid fatty acids were extracted, fractionated, saponified and methylated to obtain fatty acid methyl ester profiles of the phospholipids as described previously (Guckert *et al.*, 1985). Fatty acid methyl esters were analysed using GC-MS (Agilent 7972 MSD) as described previously (Lee *et al.*, 2006). The major fatty acids were the even-numbered carbon, straight-chain saturated and mono-unsaturated fatty acids C_{16:0} and C_{16:1}, which together made up approximately 80 % of the total phospholipid fatty acids. Other minor fatty acids were C_{14:0}, C_{15:0}, C_{16:1}ω9c, C_{18:1}ω9c, C_{18:1}ω7c and C_{18:0}. This distribution is similar to those of other members of the genus *Desulfosporosinus* (Ramamoorthy *et al.*, 2006); however, strain JW/YJL-B18^T showed a much higher content of C_{16:1} (57.7 %) compared with other *Desulfosporosinus* species (29–38 %). The G + C content of the genomic DNA was determined by the HPLC method as described previously (Mesbah *et al.*, 1989; Lee *et al.*,

2005). The G + C content was 36.6 mol% (mean of four replicates), which is lower than those reported for other species in the genus *Desulfosporosinus* (Table 1).

Based on evidence from this polyphasic study, including the inability to grow under chemolithoautotrophic conditions and the low genomic DNA G + C content, we propose to place strain JW/YJL-B18^T into the novel taxon *Desulfosporosinus youngiae* sp. nov., belonging to the family *Peptococcaceae* (Garrrity *et al.*, 2004; Spring & Rosenzweig, 2006).

Description of *Desulfosporosinus youngiae* sp. nov.

Desulfosporosinus youngiae (young'i. ae. N.L. fem. gen. n. *youngiae* of Young, in recognition of the contributions of Lily Y. Young to our understanding of the physiology and ecology of anaerobic bacteria).

Cells are curved rods, 0.7–1.2 µm in diameter and 3.0–7.0 µm long. Endospores are oval, central to subterminal. Cells stain Gram-negative but are phylogenetically Gram-type-positive (Wiegel, 1981), belonging to the phylum

Firmicutes. The temperature range for growth is 8–39 °C; optimum growth at 32–35 °C with no growth at 4 °C or below or at 42 °C or above. The pH^{25 °C} range for growth is 5.7–8.2; optimum growth at pH^{25 °C} 7.0–7.3, with no growth at or below pH^{25 °C} 5.2 or at or above pH^{25 °C} 8.4. The salinity range for growth is 0–3 % (w/v) (9:1 NaCl/KCl). In the presence of 0.02 % (w/v) yeast extract, beef extract (0.2 %), yeast extract (0.2 %), formate, succinate, lactate, pyruvate, ethanol (all at 20 mM) and toluene (0.1 mM) serve as carbon and energy sources. Fumarate, sulfate, sulfite and thiosulfate serve as electron acceptors. Arsenate, nitrate and iron (III) do not serve as electron acceptors. The predominant whole-cell fatty acids are C_{16:1} (~58 %) and C_{16:0} (~22 %). The genomic DNA G+C content of the type strain is 36.6 mol% (HPLC).

The type strain is JW/YJL-B18^T (=DSM 17734^T =ATCC BAA-1261^T).

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