Journal of Microbiology (2020) Vol. 58, No. 4, pp. 252–259
DOI 10.1007/s12275-020-9376-0
eISSN 1976-3794
pISSN 1225-8873

Paradesulfovibrio onnuriensis gen. nov., sp. nov., a chemolithoautotrophic sulfate-reducing bacterium isolated from the Onnuri vent field of the Indian Ocean and reclassification of *Desulfovibrio senegalensis* as *Paradesulfovibrio senegalensis* comb. nov.[§]

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(Received Aug 5, 2019 / Revised Oct 31, 2019 / Accepted Jan 21, 2020)

An anaerobic, rod-shaped, mesophilic, chemolithoautotrophic, sulfate-reducing bacterial strain IOR2^T was isolated from a newly found deep-sea hydrothermal vent (OVF, Onnuri Vent Field) area in the central Indian Ocean ridge (11°24′88″ S 66°25′42″ E, 2021 m water depth). The 16S rRNA gene sequence analysis revealed that the strain IOR2^T was most closely related to *Desulfovibrio senegalensis* BLaC1^T (96.7%). However, it showed low similarity with the members of the family *Desulfovibrionaceae*, such as *Desulfovibrio tunisiensis* RB22^T (94.0%), *D. brasiliensis* LVform1^T (93.9%), *D. halophilus* DSM 5663^T (93.7%), and *Pseudodesulfovibrio aespoeensis* Aspo-2^T (93.2%). The strain IOR2^T could grow at 23–42°C (optimum 37°C), pH 5.0–8.0 (optimum pH 7.0) and with 0.5–6.5% (optimum 3.0%) NaCl. The strain could use lactate, pyruvate, H_2, and glycerol as electron donors and sulfate, thiosulfate, and sulfite as electron acceptors. The major fatty acids of the strain IOR2^T were iso-$C_{15:0}$, iso-$C_{17:0}$, ante-iso-$C_{15:0}$, and summed feature 9 ($C_{16:0}$ methyl/iso-$C_{17:1}\omega9c$). Both the strains IOR2^T and BLaC1^T could grow with CO_2 and H_2 as the sole sources of carbon and energy, respectively. Genomic evidence for the Wood-Ljungdahl pathway in both the strains reflects chemolithoautotrophic growth. The DNA G + C content of the strain IOR2^T and BLaC1^T was 58.1–60.5 mol%. Based on the results of the phylogenetic and physiologic studies, *Paradesulfovibrio onnuriensis* gen. nov., sp. nov. with the type strain IOR2^T (= KCTC 15845^T = MCCC 1K04559^T) was proposed to be a member of the family *Desulfovibrionaceae*. We have also proposed the reclassification of *D. senegalensis* as *Paradesulfovibrio senegalensis* comb. nov.

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Keywords: *Paradesulfovibrio onnuriensis*, sulfate-reducing bacteria, deep-sea sediment

Introduction

Sulfate-reducing bacteria (SRB) are a group of bacteria that grows with the aid of anaerobic respiration using sulfate as an electron acceptor. They are especially present in marine environments where the concentration of sulfates is high (Matias *et al*., 2005). The genus *Desulfovibrio* Kluyver and van Niel 1936 is a known anaerobic SRB. The genus *Desulfovibrio*, affiliated to the family *Desulfovibrionaceae*, is one of the big and phylogenetically heterogeneous groups (Cao *et al*., 2016) with 69 valid published names as of June 5, 2019 (http://www.bacterio.net/-allnamesdl.html). It has been isolated from various environments, such as marine environments (deep-sea or marine sediments), subsurface aquifer, freshwater, waste digester, and gut of animals including humans (Gibson, 1990; van der Hoeven *et al*., 1995; Khelaifia *et al*., 2011; Thabet *et al*., 2011). The *Desulfovibrio* groups can be grown using organic or inorganic substrates in the presence of sulfate. Several members of the genus *Desulfovibrio* are known to be capable of hydrogenotrophic growth (using H_2 as an energy source) for sulfate reduction and most of these bacteria could grow lithoheterotrophically on acetate as the carbon source. The characteristics of hydrogenotrophic growth in this group play an important role in interspecies hydrogen transfer in the syntrophic communities (Morais-Silva *et al*., 2014). The genome size of the *Desulfovibrio* spp. group varies from 3.1 to 3.9 Mb except for *Desulfovibrio magneticus* (5.2 Mb), which has a particularly large genome size (Nakazawa *et al*., 2009; Morais-Silva *et al*., 2014; Sheik *et al*., 2017). The genes related to hydrogenotrophic growth, such as hydrogenases (Fe-only or NiFe hydrogenase) for hydrogen oxidation, and reductases (sulfate adenylyltransferase, adenylylsulfate reductase, and sulfite reductase) for sulfate reduction, have been identified from the genomic results of several *Desulfovibrio* species (Heidelberg *et al*., 2004; Nakazawa *et al*., 2009; Morais-Silva *et al*., 2014). Further, genes associated with chemoorganotrophic growth in lactate conditions, such as lactate dehydrogenase and pyruvate: ferredoxin oxidoreductase for lactate oxidation and hydrogenases (cytoplasmic hydrogenases for hydrogen evolving and periplasmic hydrogenases for hydrogen uptake) for hydrogen cycling, have been identified from the genomic results of several *Desulfovibrio* species (Heidelberg *et al*., 2004;

Nakazawa *et al*., 2009; Morais-Silva *et al*., 2014). Only a few strains have been reported to be capable of chemolithoautotrophic growth using H_2 as the energy source and CO_2 as the sole carbon source. These include *Desulfovibrio senegalensis* (Thioye *et al*., 2017), *D. tunisiensis* (Ben Ali Gam *et al*., 2009), and *Pseudodesulfovibrio profundus* (Bale *et al*., 1997; Cao *et al*., 2016). Phylogenetically, the members of the genus *Desulfovibrio* are too complex to be classified into only one genus. Hence, clades of different genera were intruded in the clade of the genus *Desulfovibrio* and, reclassification into

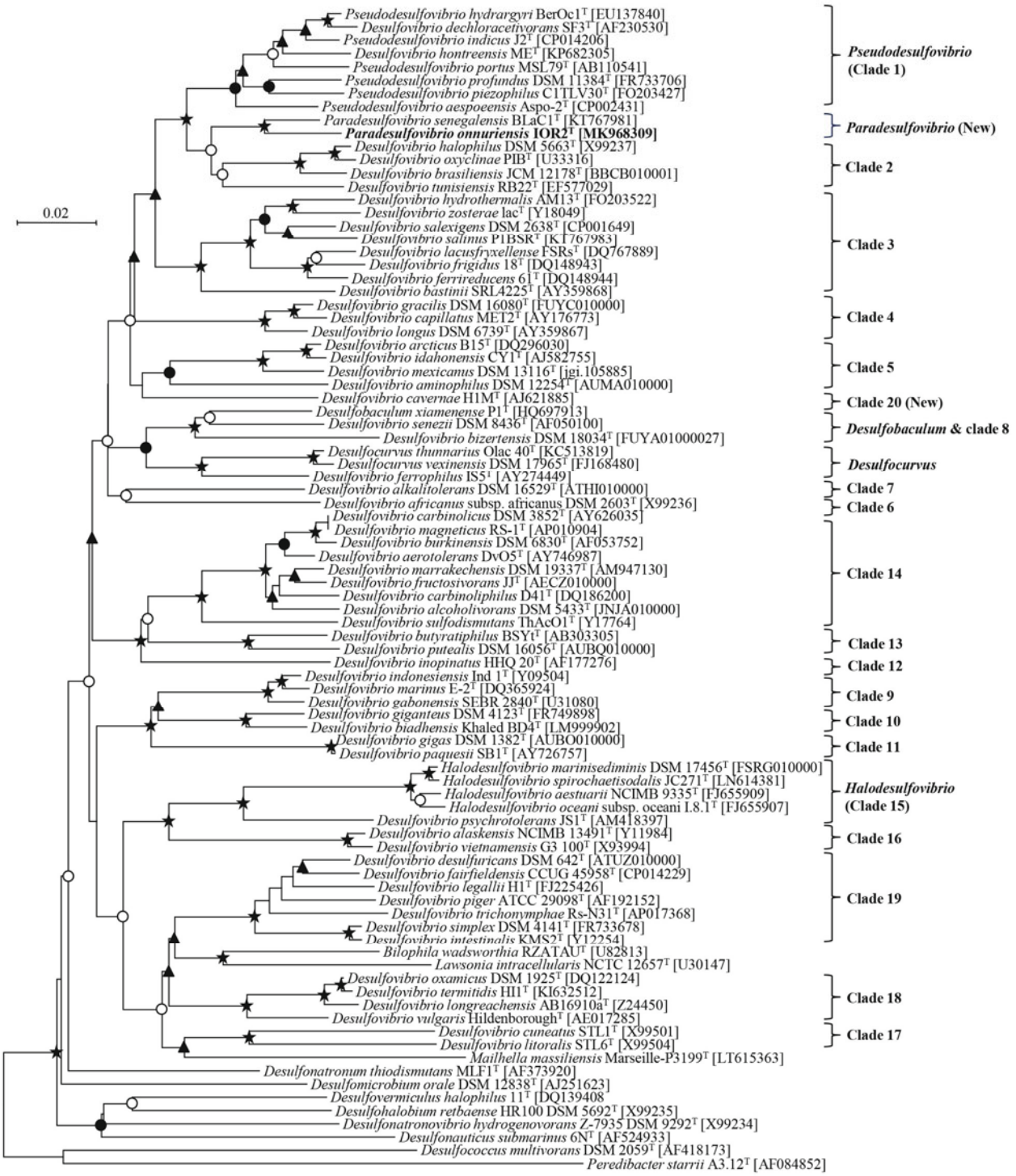

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain IOR2^T and other related taxa. The tree is based on the Juke and Cantor distances model and the neighbor-joining method with 1,000 bootstraps. The maximum-likelihood and the maximum-parsimony algorithms with bootstraps also supported the results. ★, Bootstrap values > 90% in all three methods. ●, Bootstrap values > 70% in all three methods. ○, Bootstrap values with three methods but at least one methods bootstrap values < 70%. ▲, Recovered with two different methods.

19 genera was recommended (Cao *et al.*, 2016). The median sequence identity of the 16S rRNA gene between the suggested groups ranged from 82.4 to 93.62% (Supplementary data Table S1) and these values were far lower than the taxonomic threshold of the genus as suggested by Yarza *et al.* (2014). Consequently, the genera *Pseudodesulfovibrio* (Cao *et al.*, 2016) and *Halodesulfovibrio* (Shivani *et al.*, 2017) were proposed for some novel isolates, as well as some species formerly classified as members of the genus *Desulfovibrio*. However, some remainders are not monophyletic and they vary in their physiological processes, and therefore, the current members of the genus *Desulfovibrio* should be reclassified into at least 17 genera by taxonomic studies (Cao *et al.*, 2016 and Fig. 1). In the present study, a novel strain IOR2^T, isolated from sediment sampled at the central Indian Ocean ridge and *Desulfovibrio senegalensis* BLaC1^T, a former member of the genus *Desulfovibrio* are proposed to belong to the members of the novel genus *Paradesulfovibrio.*

Materials and Methods

Sampling, isolation, and cultivation of the strains

During expedition of hydrothermal vents in the central Indian Ocean ridge with *R/V* ISABU in June 2018, a sediment sample was collected from the newly discovered hydrothermal vent area (Onnuri vent field, OVF) (11°24′88″ S, 66°25′42″ E, 2021 m water depth) by using video-guided grab sampler (Oktopus). Approximately 1.0 g of the sediment sample was suspended with 20 ml of modified acetogen (AT) medium (Kotsyurbenko *et al.*, 1995) in 50 ml serum vials while purging with N_2 gas onboard under nitrogen gas flow. The diluted subsample was inoculated into an artificial seawater-yeast extract/tryptone (ASW-YT) medium (Sato *et al.*, 2003) in the serum vials for the enrichment of anaerobic bacteria and incubated at 30°C for 7 days in anaerobic condition. Then, the enriched culture broths were transferred to a laboratory, and pure isolation was performed in an anaerobic chamber (Coy Laboratory Products). The strain IOR2^T was routinely cultivated with DSMZ no. 163 medium at 37°C after 16S rRNA gene-based identification and was stored at -80°C in a glycerol suspension (20%, w/v). A slightly modified basal (SMB) medium (lactate and sulfate as primary electron donor and acceptor, respectively) (Thioye *et al.*, 2017) was used for the substrate utilization test for the strain IOR2^T. For phenotypic comparisons, *Desulfovibrio senegalensis* DSM 101509^T (= BLaC1^T), which showed the highest 16S rRNA gene sequence similarity, was purchased from the Deutsche Sammlung von Mikroorganismenund Zellkulturen GmbH (DSMZ) and grown at 37°C with DSMZ no. 163 medium.

Phylogenetic and genomic analysis

Genomic DNA was extracted using a standard procedure (Robb *et al.*, 1995) and the 16S rRNA gene was amplified using the 27F and 1492R bacterial primer set (Giovannoni, 1991). The 16S rRNA gene sequencing was performed using an ABI 3730xl automatic sequencer. The obtained 16S rRNA gene sequence (1,416 bp) was compared by BLAST pair-wise alignment with sequences in the EzTaxon-e database (Yoon *et al.*, 2017a). Phylogenetic analysis based on the 16S rRNA gene sequence (1,327 sites) of the strain IOR2^T and the members of the *Desulfovibrionaceae* was conducted using MEGA version 6.06 (Tamura *et al.*, 2011) with partial deletion option (cutoff < 95%) after aligning with clustal W and manual correction. The phylogenetic tree with 1,000 replicated bootstrap analysis was reconstructed by using the neighbor-joining method (NJ) (Saitou and Nei, 1987) with the Jukes and Cantor distance model (Jukes and Cantor, 1969), and the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (MP) (Fitch, 1971) methods. The sequences of *Desulfococcus multivorans* and *Peredibacter starrii* were used as outgroups.

The whole genome sequences of strain IOR2^T was obtained using the PacBio RS II system and assembled by Pacific Biosciences SMRT tools and of strain BLaC1^T was obtained using the Illumina Miseq system and assembled by Spades tools from ChunLab Co. Ltd.. The obtained genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova *et al.*, 2016). A phylogenomic tree with members within the family *Desulfovibrionaceae* was produced using the bacterial core gene set (UBCG) (Na *et al.*, 2018). An average nucleotide identity (ANI) value was calculated using the OrthoANIu algorithm (Yoon *et al.*, 2017b).

Phenotypic, physiological, and biochemical properties

Gram reaction was tested using the Gram-staining kit (YD diagnostics). Oxygen sensitivity was determined by culturing in the air in the absence of Na_2S and cysteine. The morphological characteristics of the stain IOR2^T were determined by transmission electron microscopy (TEM) (JEM1010, JEOL) with fixed cells after negative staining with 2% (w/v) phosphotungstic acid reagent at pH 7.0. The temperature range for the growth of stain IOR2^T was tested on DSMZ no. 163 medium using a temperature gradient incubator (TVS126MA, Advantec) from 15 to 45°C (15.0, 17.7, 20.4, 23.1, 25.9, 28.6, 31.3, 34.0, 36.7, 39.5, 42.2, and 45.0°C). The pH range and NaCl requirement were also measured using a gradient incubator. The growth range for pH was tested (4.5–8.5) in DSMZ no. 163 medium with the pH adjusted using 100 mM HCl (pH 4.5–7.0, 0.5 intervals), 10% $NaHCO_3$ (pH 7.0–8.0, 0.5 intervals), and 8% Na_2CO_3 (pH 8.0 and 8.5). The NaCl requirement for growth was tested from 0–10.0% at 0.5% intervals with NaCl free DSMZ no. 163 medium.

The electron donors and acceptors of both the stains IOR2^T and DSM 101509^T were determined in the SMB medium in the presence of 3% NaCl. For the electron donor test, 20 mM each of lactate, malate, fumarate, formate, acetate, pyruvate, or propionate; 2 mM succinate; 0.1% (w/v) each of glycerol, methanol, ethanol, or butanol; and H_2/CO_2 (8:2) or 50% CO gas were added on the SMB medium instead of lactate with sulfate as an electron acceptor. Additionally, the potential electron acceptors were tested in the same medium with lactate as an electron donor and adding one of the followings; 20 mM of sulfate, 20 mM of thiosulfate, 2 mM of sulfite, 2 mM of nitrite, 20 mM of nitrate, 10 mM of fumarate and 0.1% of elemental sulfur. The OD_{600} > 0.1 was considered as positive and < 0.1 as negative according to a previous report (Sass *et al.*, 2009). Substrate utilization and enzyme properties of the stains IOR2^T and DSM 101509^T were tested by using Rapid

ID 32A and API ZYM systems (bioMérieux), respectively. For the preparation of the cell suspension, both the strains were colonized on SMB agar and the colonies were suspended in a 3.0% NaCl solution. The assays were performed according to the manufacturer's instructions.

Fatty acid profiles

Both the strains IOR2^T and DSM 101509^T were grown in the SMB medium with lactate and sulfate as an electron donor and acceptor, respectively. The cells were harvested at the end of the exponential phase of growth and transported to KCCM (Korea Culture Centre of Microorganisms) for fatty acid analysis. The cellular fatty acid profiles were performed by using the MIDI/Hewlett Packard Microbial Identification System (MIS) (Sasser, 1990) with Sherlock version 6.3 and the RTSBA6 database according to the manufacturer's instruction.

Nucleotide sequence accession numbers

The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene sequence of *Paradesulfovibrio onnuriensis* IOR2^T is MK968309. The genome sequences for IOR2^T and *Paradesulfovibrio senegalensis* DSM 101509^T are CP040751 and WAIE00000000, respectively.

Results and Discussion

Isolation and 16S rRNA gene sequence phylogeny of the strain IOR2^T

The culture was repeated three times in the same medium (ASW-YT) to obtain a single isolate by colonization on the ASW-YT agar plate in an anaerobic chamber (Coylab). The observed colonies were purified by re-plating on a solid medium. One of the colonies, strain IOR2^T, was selected for further study. The BLAST result revealed that the strain IOR2^T was most closely related to *Desulfovibrio senegalensis* BLaC1^T (96.7%), however, < 94.0% similarity was observed with the other members of the family *Desulfovibrionaceae*. In the phylogenetic analysis based on the 16S rRNA gene, the strain IOR2^T was tightly clustered with *Desulfovibrio senegalensis* BLaC1^T and the node was recovered by different methods with high bootstrap values (Fig. 1). The clade was located between the clade of the genus *Pseudodesulfovibrio* and the clade 2 which was defined by Cao *et al.* (2016). Additionally, two strains showed < 94.5% 16S rRNA gene sequence similarity from all validly reported species, and this was below the suggested threshold of the genus (Yarza *et al.*, 2014). This result implied that the strains IOR2^T and *D. senegalensis* BLaC1^T comprise of a new genus.

Phenotypic, physiological, and biochemical analysis of the strain IOR2^T

The strain IOR2^T was Gram-negative, anaerobic, and rod-shaped with 0.5 μm width and 2–5 μm length (Supplementary data Fig. S1). Growth of the strain IOR2^T was observed at 23–42°C (optimum 37°C), pH 5.0–8.0 (optimum pH 7.0), and in 0.5–6.5% (optimum 3.0%) NaCl (Table 1). The strain IOR2^T utilized lactate, formate, acetate, pyruvate, glycerol, and H_2/CO_2 (8:2) as electron donors; and sulfate, thiosulfate, and sulfite as electron acceptors (Table 1). The other tested materials could not serve as electron donors or acceptors. The range of the electron donors and acceptors was quite

Table 1. Comparison of the morphological, physiological, and chemotaxonomical properties between strain IOR2^T and *D. senegalensis* DSM 101509^T
Taxa: 1, IOR2^T; 2, *D. senegalensis* BLaC1^T (data obtained from this study and Thioye *et al.*, 2017†). +, Positive; -, negative. Both the strains could grow chemolithoautotrophically. Both the strains could utilize H_2, lactate, formate, acetate, and pyruvate as electron donors; and sulfate, thiosulfate, and sulfite as electron acceptors. Both the strains could not utilize malate, acetate, propionate, succinate, methanol, ethanol, butanol, and CO as electron donors; and nitrate, nitrite, fumarate, and elemental sulfur as electron acceptors. Both the strains could grow chemolithoautotrophically on H_2/CO_2. Both the strains showed alkaline phosphatase, esterase and leucine arylamidase activities. All other substrates or enzyme activities on API 32A and API ZYM kits showed negative results for both the strains.

Characteristics	1	2
Cell shape	Rod	Rod†
Cell size (μm)	0.5 × 2–5	0.5 × 2–4†
Isolation source	Deep-sea sediment	Marine sediment
Growth range of		
Temperature (optimum) (°C)	23–42 (37)	15–40 (40)†
pH (optimum)	5.0–8.0 (7.0)	6.3–8.5 (7.5)†
NaCl (optimum) (%, w/v)	0.5–6.5 (3.0)	0.5–6.5 (3.0)†
Electron donors (with sulfate)		
Glycerol	+	–
Substrate utilization		
Glutamic acid, L-leuine-β-naphthylamide, L-tyrosine-β-naphthylamide	–	+
L-Arginine, D-mannose, potassium nitrate, L-pyroglutamic acid-β-naphthylamide	+	–
Enzyme activities		
Acid-phosphatase, naphthol-AS-BI-phophohydrolase, N-acetyl-β-glucosaminidase	+	–
Major fatty acid (> 5%)	$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$, SF 9*	iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, anteiso-$C_{17:1}$ ω9c, SF 9*
DNA G + C (mol%)	60.5	58.1

*SF9: summed feature 9 comprised of $C_{16:0}$ methyl/iso-$C_{17:1}$ω9c.

similar to those of the strain DSM 101509^T except for that strain IOR2^T could utilize glycerol as the electron donor. Furthermore, the strain IOR2^T showed a broad range of substrate utilization and enzyme activities as compared to those of the strain DSM 101509^T (Table 1). The major fatty acids of the strain IOR2^T were $C_{16:0}$ (9.3%), iso-$C_{15:0}$ (28.7%), iso-$C_{17:0}$ (21.4%), anteiso-$C_{15:0}$ (11.1%), and summed feature 9 ($C_{16:0}$ methyl/iso-$C_{17:1}\omega9c$, 12.5%), which were similar to those of the strain DSM 101509^T but displayed difference in the proportion (Supplementary data Table S2).

Insight from the genome sequence of the new taxon

The genome size of the strain IOR2^T was 3.89 Mbp and the DNA G + C content was 60.5 mol%. The genome was comprised of 3,494 protein-coding genes, including 2,302 with assigned COG and 1,008 with unknown functions (Supplementary data Table S3). Like other *Desulfovibrio* members, the strain IOR2^T could use hydrogen or organic acids as electron donors for sulfate reduction. The genes related to sulfate-reducing metabolism were conserved in the genome contents of the strain IOR2^T. The gene for adenosine triphosphate (ATP) sulfate adenylyltransferase (FGL65_RS05430)

Table 2. Comparison of the morphological and physiological properties of the *Paradesulfovibrio* genus (including strain IOR2^T and BLaC1^T) and related 8 genera in the family *Desulfovibrionaceae*

Taxa: 1, *Paradesulfovibrio* (this study and Thioye *et al*., 2017); 2, *Pseudodesulfovibrio* (Bale *et al*., 1997; Motamedi and Pedersen, 1998; Suzuki *et al*., 2009; Khelaifia *et al*., 2011; Cao *et al*., 2016; Ranchou-Peyruse *et al*., 2018); 3, *Desulfocurvus* (Hamdi *et al*., 2013); 4, *Desulfobaculum* (Zhao *et al*., 2012); 5, *Halodesulfovibrio* (Postgate and Campbell, 1966; Takii *et al*., 2008; Finster and Kjeldsen, 2010; Shivani *et al*., 2017); 6, *Desulfovibrio* (Postgate and Campbell, 1966; Kuever *et al*., 2005; Thabet *et al*., 2011). +, Positive; –, negative; v, variable; nd, not determined.

Characteristics	1	2	3	4	5	6
Morphology						
Rod, vibroid, or both	R	V	V	R	B	B
Flagellum	–	–	SP	SP	nd	SP/LO
Optimum growth at;						
temperature (°C)	37–40	25–35	37–40	35–40	20–37	35–37
pH	7.0–7.5	6.0–7.5	6.9–7.1	7.0	7.0–8.0	7.0–7.5
NaCl (%, w/v)	3.0	0.7–8.0	0.2	0.5	1.5–3.5	2.0–3.0
Aerobic growth	–	–	–	–	–	v
Chemolithoautotrophic growth	+	v	–	–	–	v
Electron donors						
H_2	+	+	v	+	+	v
Lactate	+	+	+	+	+	+
Malate	–	v	–	+	+	v
Fumarate	–	v	–	+	+	v
Formate	v	v	+	–	+	v
Acetate	v	–	–	+	v	–
Pyruvate	+	+	+	+	+	+
Succinate	–	v	–	+	+	v
Propionate	–	–	–	–	–	–
Ethanol	–	v	–	–	v	+
Methanol	–	–	–	–	–	–
Butanol	–	–	–	nd	–	v
Glycerol	v	–	–	–	v	v
Electron acceptors						
Sulfate	+	+	+	+	+	+
Thiosulfate	+	+	+	–	+	+
Sulfite	+	+	+	+	v	+
Nitrite	–	–	–	–	–	–
Nitrate	–	v	–	–	–	v
Fumarate	–	v	nd	+	v	+
Elemental sulfur	–	v	–	–	–	v
Major fatty acids (> 10%)						
Saturated	i-$C_{15:0}$, a-$C_{15:0}$, i-$C_{17:0}$	i-$C_{15:0}$, i-$C_{17:0}$	$C_{16:0}$, i-$C_{15:0}$, a-$C_{15:0}$	$C_{16:0}$, i-$C_{15:0}$	i-$C_{15:0}$, i-$C_{17:0}$	$C_{16:0}$, i-$C_{15:0}$, i-$C_{14:0}$3OH
Unsaturated	–	–	–	i-$C_{17:1}\omega9c$	i-$C_{17:1}\omega9c$	–
Summed feature	SF9*	SF9*	–	–	–	–
DNA G+C (mol%)	58.1-60.5	50.0–63.5	67.2–70.0	64.5	45.1–47.1	47.5–64.0
Isolation Sources	Deep-sea or marine sediments	Various marine environments	Subsurface aquifer, wastewater digester	Mangrove sediments	Marine environments	Animals, waste digestor

*Summed feature 9 (SF9) is $C_{16:0}$ methyl/iso-$C_{17:1}\omega9c$. For fatty acids, i=isoform, a=anteisoform.

that converts sulfate to adenosine phosphosulfate (APS) with ATP was confirmed. The genes encoding APS reductase (FGL65_RS05435, RS05440) that converts APS to sulfite was confirmed. The genes for dissimilatory sulfite reductase (FGL65_RS00320, RS00325) that convert sulfite to sulfide were identified. For the hydrogenotrophic growth of IOR2^T strain, the genome content contained periplasmic hydrogenases, such as Fe-only hydrogenase (FGL65_RS09065 and FGL65_RS13825) or NiFe hydrogenase (FGL65_RS15290-15295 and FGL65_RS16430-RS16450) for hydrogen oxidation. As preferred organic acid substrates, lactate was oxidized to pyruvate by lactate dehydrogenase (FGL65_RS17810) and pyruvate was oxidized to acetate by pyruvate-ferredoxin oxidoreductase (FGL65_RS09305 or FGL65_RS13770). In addition, for hydrogen cycling, the genome contained a cytoplasmic membrane-bound Ech hydrogenase gene (FGL65_RS16430-RS16450) for hydrogen production using electrons from lactate and pyruvate oxidation, and the produced hydrogen was reoxidized by periplasmic hydrogenases. Interestingly, the strain IOR2^T was capable of chemolithoautotrophic growth with CO_2 and H_2 as carbon and energy sources, respectively. Although the genes associated with the reductive acetyl-CoA pathway were included in the genome contents, the key enzyme [ACS (acetyl-CoA synthase)] gene was not identified. Strain IOR2^T was also able to grow using glycerol, unlike *D. senegalensis* BLaC1^T. The genome of strain IOR2^T contains genes involved in the metabolism of glycerol to glyceraldehyde-3-phosphate including glycerol facilitator protein (GlpF; FGL65_RS00815), glycerol kinase (GlpK; FGL65_RS00810), glycerol 3-phophate dehydrogenases (GlpD; FGL65_RS09565-09570 and FGL65_RS16175) and triose-phosphate isomerase (TPI; FGL65_RS12145). The genome of the strain IOR2^T showed an ANI value of 73.7–75.4% as compared to *D. senegalensis* BLaC1^T, *D. oxyclinae* PIB^T (GCA_000375485.1), *D. halophilus* DSM5663^T (PRJNA234936), and *D. brsiliensis* JCM12178^T (GCA_001311825.1). This result suggested that stain IOR2^T belongs to a different species. A phylogenomic tree inferred by UBCG (Na *et al*., 2018) was similar to the 16S rRNA gene-based phylogenetic tree (Supplementary data Fig. S2).

Taxonomic conclusion

Considering the phylogenetic position (Fig. 1 and Supplementary data Fig. S2), ranges of electron donors and acceptors (Table 1), range of carbon sources and enzyme activities (Table 1), and fatty acids profile (Supplementary data Table S2), it was concluded that the strains IOR2^T and *D. senegalensis* DSM 101509^T comprise of different species of the same genus.

Additionally, the strains IOR2^T and *D. senegalensis* DSM 101509^T showed < 94% 16S rRNA gene sequence similarity with all the validly reported strains and this was satisfying the threshold of the genus. Considering the range of electron acceptors (sulfate, sulfite, and thiosulfate were generally utilized but not usual for nitrate, nitrite, and elemental sulfur) and types of major fatty acids (branched form) as common features of the family *Desulfovirionaceae*, both the strains differentiated from the other genera by habitats (from the genera *Bilophila*, *Lawsonia*, and *Mailhella*), cell shape (except the genera *Bilophila* and *Mailhella*), and DNA G + C ratio (from the genera *Desulfocurvus*, *Desulfobaculum, Halodesulfovibrio*, and *Lawsonia*) in terms of electron donors or the ability to grow chemolithoautotrophically (Table 2). These results also demonstrated that the strains IOR2^T and BLaC1^T should be identified as a novel genus. To conclude *Paradesulfovibrio onnuriensis* gen. nov. sp. nov. with type strain IOR2^T (= KCTC 15845^T = MCCC 1K04559^T) and the reclassification of *D. senegalensis* as *Paradesulfovibrio senegalensis* comb. nov. are proposed. Additionally, a recommendation on the G + C ratio of the genus *Halodesulfovibrio* by description error was also provided.

Description of *Paradesulfovibrio* gen. nov.

Paradefulfovibrio (Pa'ra.de.sul.fo.vi'bri.o. Gr. prep. *para* beside; N.L. masc. n. *Desulfovibrio* is the genus name; N.L. masc. n. *Paradesulfovibrio* phylogenetically adjacent to the genus *Desulfovibrio*). The cells are Gram-negative, anaerobic, and rod-shaped; and physiologically the cells are mesophilic, neutrophilic, and slightly halophilic. The cells could grow both chemolithoautotrophically and chemoorganotrophically. Lactate, formate, acetate, pyruvate, and H_2 are used as electron donors, while sulfate, thiosulfate, and sulfite are used as electron acceptors. The DNA G + C content is 58–61 mol%. The common major fatty acids are branched type fatty acids of 15–17 chain length. The type species is *Paradesulfovibrio onnuriensis*.

Description of *Paradesulfovibrio onnuriensis* sp. nov.

Paradefulfovibrio onnuriensis (on.nu.ri.en'sis. N.L. masc. adj. onnuriensis was derived from the newly found vent field Onnuri in the central Indian Ocean ridge). In addition to the characteristics described in the genus description, the following features were noted. The cells were rod-shaped (0.5 μm wide and 2–5 μm long). The ranges and optimal conditions of temperature, pH, and required NaCl concentration for the growth on DSMZ no. 163 medium were 23–42°C (optimum, 37°C), pH 5.0–8.0 (optimum, pH 7.0), and 0.5–6.5% (optimum, 3.0%), respectively. Glycerol was used as electron donors, but not malate, acetate, propionate, succinate, methanol, ethanol, butanol, and CO gas. Nitrate, nitrite, fumarate, and elemental sulfur could not serve as electron acceptors. Alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and N-acetyl-β-D-glucosaminidase activities on API ZYM were positive. The bacteria could utilize D-mannose, glutamic acid, L-tryptophan, 2-naphthyl-phosphate, L-arginine-β-naphthylamide, L-leucine-β-naphthylamide, and L-pyroglutamic acid-β-naphthylamide in API 32A kit. The major fatty acids were $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, and summed feature 9 ($C_{16:0}$ methyl/iso-$C_{17:1}\omega9c$). The DNA G + C content was 60.5 mol%.

The strain IOR2^T (= KCTC 15845^T = MCCC 1K04559^T) was isolated from a deep-sea hydrothermal vent (OVF, Onnuri Vent Field) area in central Indian Ocean ridge (11°24′88″ S, 66°25′42″ E, 2021 m water depth). The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is MK968309 and for the genome it is CP040751.

Description of *Paradesulfovibrio senegalensis* comb. nov.

Basonym : *Desulfovibrio senegalensis* (Thioye *et al*., 2017).

The description is identical to that of Thioye *et al.* (2017) with the following modifications. Pyruvate and hydrogen were used as electron donors in the presence of sulfate. The cells utilize L-arginine, glutamic acid, and L-leucine-β-naphthylamide in the API 32A kit. The enzymes of alkaline phosphatase, esterase and leucine-arylamidase activities were detected by the API ZYM kit. The major fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{17:0}$, anteiso-$C_{17:1}\omega9c$, and summed feature 9 ($C_{16:0}$ methyl/iso-$C_{17:1}\omega9c$). The DNA G + C ratio is 58.1 mol%. The type strain is BLaC1^T (=DSM 101509^T=JCM 31063^T) and the GenBank/DDBJ/EMBL accession number of the genome is WAIE00000000.

Emended description of the genus *Halodesulfovibrio* Shivani *et al.* 2017

The description is identical to that of Shivani *et al.* (2017) except that the DNA G + C ratio is changed to 45.1–47.1 mol%.

Acknowledgments

This work was supported by the KIOST In-house Program (PE99822) and the understanding of the deep-sea biosphere on seafloor hydrothermal vents in the Indian Ridge Program (No. 20170411) of the Ministry of Ocean and Fisheries of the Republic of Korea.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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