**Rhodohalobacter sulfatireducens** sp. nov., isolated from a marine solar saltern

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**Abstract**

A novel Gram-stain-negative, oxidase-positive, catalase-positive, non-motile, facultatively anaerobic, rod-shaped bacterium, designated WB101T, was isolated from a marine solar saltern located in Wendeng, PR China. Strain WB101T shared a high level of 16S rRNA gene sequence similarity with *Rhodohalobacter barkolensis* 15182T (93.5%), *R. halophilus* JZ3C29T (93.2%), and 'R. mucosus' 8A47T (92.1%). Strain WB101T formed a species-level branch within the genus *Rhodohalobacter* in both phylogenetic and phylogenomic topologies. The DNA G + C content was 42.0%. Strain WB101T was found to have menaquinone-7 as the only respiratory quinone. The dominant cellular fatty acid (≥ 10%) was iso-C₁₅:₀. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylcholine. Characterisation based on phylogenetic, physiological, and biochemical properties indicated that strain WB101T represents a novel species of the genus *Rhodohalobacter*, and the name *Rhodohalobacter sulfatireducens* sp. nov. is proposed. The type strain is WB101T (= KCTC 92204T = MCCC 1H00518T).

**Keywords** *Rhodohalobacter* · *Rhodohalobacter sulfatireducens* · 16S rRNA gene · Phylogenetic analysis · Novel species

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| AAI          | Average amino acid identity |
| ANI          | Average nucleotide identity |
| ASR          | Assimilatory sulphate reduction |
| BD           | Becton–Dickinson |
| dDDH         | Digital DNA–DNA hybridization |
| GTDB         | Genome Taxonomy Database |
| HPLC         | High-performance liquid chromatography |
| KCTC         | Korean Collection for Type Cultures |
| KEGG         | Kyoto Encyclopedia of Genes and Genomes |

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The GenBank accession number for the 16S rRNA gene sequence of *Rhodohalobacter sulfatireducens* WB101T is OM301685, and the draft genome has been deposited at DDBJ/ENA/GenBank under the accession JAKLWS000000000.

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**Introduction**

The genus *Rhodohalobacter*, belonging to the family *Balneolaceae* (Xia et al. 2016), was first established by Xia et al. (2017). At the time of writing, there were two valid species: *R. halophilus* (Xia et al. 2017) and *R. barkolensis* (Han et al. 2018), and one un-validated species: ‘*R. mucosus*’ (Wang...
et al. 2021), according to the List of Prokaryotic names with Standing in Nomenclature (LPSN) (https://lpsn.dsmz.de). All these strains were isolated from saline environments, including salterns and saline lakes.

Microorganisms can metabolise sulphur in both oxidised and reduced states (Carbonero et al. 2012). In assimilatory sulphate reduction (ASR), sulphate is reduced to sulphide, which then participates in the synthesis of organic compounds, such as sulphur amino acids. Compared to dissimilatory sulphate reduction, ASR is a more favourable metabolic pathway for bacteria because no toxic sulphide is produced during this process.

In this study, we proposed a novel species with the basis of the results of polyphasic taxonomy, named Rhodobacter aciducomitans sp. nov. Genome of the novel strain encodes a complete ASR pathway. This study increases the number of members of the genus Rhodobacter and enriches the research directions of studies on assimilatory sulphate-reducing bacteria.

Materials and methods

Isolation and maintenance conditions

Coastal sediment was used as a bacterial isolation source, and was sampled from a marine solar saltern in Wendeng, PR China (122° 1' 45'' E, 36° 59' 38'' N) in November 2020. The sample was serially diluted 10⁻⁴ times with sterilised seawater, and 0.1 mL aliquots of each dilution were spread onto marine agar 2216 (MA; BD). The plates were incubated at 33 °C for 2 weeks. Finally, tiny, reddish, convex, circular colonies were isolated, purified, and regular-edged colonies were isolated, purified, and subsequently designated as strain WB101ᵀ. For long-term storage, the strain was stored at -80 °C in sterile 15% (v/v) glycerol supplemented with 5.0% (w/v) NaCl. 'R. mucosus' 8A47ᵀ, 'R. halophilus' IZ3C29ᵀ (both isolated and studied in our laboratory), and 'R. barkolensis' MCCC 1K03442ᵀ (obtained from the Marine Culture Collection of China, MCCC) were collected as related type strains.

Phylogenetic and phylogenomic analyses

The 16S rRNA gene sequence was amplified by PCR using universal primers 27F and 1492R (Liu et al. 2014). After electrophoresis, the purified PCR product was recovered using an agarose gel DNA extraction kit (TaKaRa), ligated into the pMD19-T vector (TaKaRa), and cloned according to the manufacturer’s instructions. Sequencing was performed by Tsingke Biotechnology Co., Ltd. (Qingdao, PR China) using universal primers M13F and M13R. The 16S rRNA gene sequence annotated from the genome sequences was submitted to GenBank. Similar sequences were identified using BLAST (https://www.ncbi.nlm.nih.gov). The EzBioCloud identification service (https://eztaxon-e.ezbiocloud.net) (Kim et al. 2012) was used to determine similarity values amongst the sequences. Genomic DNA was extracted and purified using a bacterial genomic DNA extraction kit (TaKaRa) following the manufacturer’s instructions. The draft genome was sequenced by Novogene Biotechnology Co., PR China using the Illumina HiSeq platform (Illumina, San Diego, CA, USA). Raw sequencing reads were assembled using ABySS v. 2.0.2 (https://www.zebiocloud.net/tools/abys) (Simpson et al. 2009).

Phylogenetic trees were constructed using MEGA 7 (Kumar et al. 2016) with the neighbour-joining (NJ) (Saitou and Nei 1987) method based on the complete 16S rRNA gene retrieved from the genome sequences. Maximum-parsimony (MP) (Fitch 1971) and maximum-likelihood (ML) (Felsenstein 1981) methods were used to confirm the phylogenetic status of strain WB101ᵀ. Evolutionary distances were calculated using the Kimura two-parameter model (Kimura 1980). Bootstrap analysis with 1,000 replications was used to evaluate the tree topologies.

To detect the taxonomic status of strain WB101ᵀ at the genomic level, a phylogenomic tree based on the genome inferred bac120 marker set was constructed using the Genome Taxonomy Database (GTDB) (https://gtdb.ecogenomic.org) (Chaumeil et al. 2019). The average nucleotide identity (ANI) values (including OrthoANIu, ANIb, ANIm, and TETRA values) were obtained using the online calculators EzGenomes (https://www.zebiocloud.net/tools/ani) (Yoon et al. 2017) and JspeciesWS (https://jspecies.ribhost.com/jspeciesws) (Richter et al. 2016). The digital DNA–DNA hybridization (dDDH) values were calculated using GGDC (https://ggdc.dsmz.de) (Meier-Kolthoff et al. 2022). As additional taxonomic references, the average amino acid identity (AAI) (Rodriguez-R and Konstantinidis 2014) and the percentage of conserved proteins (POCP) (Qin et al. 2014) between related genomes were determined using an AAI and POCP calculator (https://github.com/2015qyliang/POCP).

Genomic analyses

Secondary metabolite clusters were identified using antiSMASH (https://antismash.secondarymetabolites.org) (Medema et al. 2011). Orthologous gene clusters were compared between strain WB101ᵀ and three related type strain genomes using OrthoVenn2 (Xu et al. 2019). Genome annotation was performed using the Rapid Annotation using Subsystem Technology (RAST) (https://rast.nmpdr.org) (Aziz et al. 2008) and Kyoto Encyclopedia of Genes and
Genomes (KEGG) pathway annotation (https://www.kegg.jp) (Kanehisa et al. 2016).

**Morphological, physiological, and biochemical analyses**

The morphological and physiological features of strain WB101^T^ were examined after incubation at 37 °C on MA for three days. A scanning electron microscope (Nova NanoSEM 450; FEI) was used to observe cell morphology. A late exponential phase culture (three days, according to the growth the curves in Supplementary Fig. S1) in marine broth 2216 (MB; BD) was collected and fixed with 2.5% glutaraldehyde solution preserved at 4 °C. Gradient dehydration was then performed with 50%, 70%, 80%, 90%, and 100% ethanol before observation. Gram staining was performed using a Gram stain kit (Hopebio), according to the manufacturer’s instructions. Motility was examined using a 3.0% (w/v) NaCl solution according to the hanging-drop method (Bowman 2000). Gliding was assessed using MB supplemented with 0.3% (w/v) agar, according to the method described by Bernardet et al. (2002). Oxidase activity was tested using an oxidase reagent kit (bioMérieux), according to the manufacturer’s instructions. Catalase activity was determined by visible bubble production in 3% (v/v) H_2O_2 solution.

The effects of different growth temperatures on MA were tested at 4, 15, 20, 25, 28, 30, 35, 37, 40, 42, and 45 °C. Growth at different NaCl concentrations was assessed using a modified MA (1 g yeast extract L^-1^, 5 g peptone L^-1^, 0.1 g ferric citrate L^-1^, and 18 g agar L^-1^), prepared with artificial seawater (0.32% MgSO_4, 0.12% CaCl_2, 0.07% KCl, and 0.02% NaHCO_3, all w/v). The NaCl concentrations were adjusted from 0 to 13.0% (w/v) at 1.0% intervals. To test the effect of pH on growth, MB was adjusted to different pH levels with additional buffers (Sangon): MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), and CAPSO (pH 9.0 and 9.5) at a concentration of 20 mM. The effects of temperature, NaCl tolerance, and pH on growth were recorded every 12 h in a 96-h-incubation (determined by growth curve) featuring visible colonies or optical density at 600 nm wavelength (OD_600). Anaerobic growth was determined at 37 °C for 3 weeks on modified MA and modified MA with the addition of 0.1% (w/v) KNO_3 in an anaerobic culture bag (Hopebio) with an oxygen indicator (Hopebio) and an AnaeroPack (MGC). Nitrate reduction ability was evaluated following the method described by Cowan and Steel (1974). H_2S production was tested according to lead acetate papers (Wang et al. 2012) and producing black precipitates in triple-sugar iron agar (TSI; BD). The hydrolysis of DNA, starch, casein, alginate, carboxymethyl cellulose (CMC), and esters, including Tweens 20, 40, 60, and 80, was investigated according to the methods of Smibert and Krieg (1994). Susceptibility to antibiotics was assessed as described by the Clinical and Laboratory Standards Institute (CLSI) (2012) using MA at 37 °C. The sizes of the inhibition zones generated by different drug-sensitive papers were measured until visible bacterial lawns were observed.

Other physiological and biochemical characteristics were tested using API 20NE, API ZYM, and API 50CH (bioMérieux). The oxidising potential of strain WB101^T^ for various carbon sources was assessed using the Biolog GEN III. All API and Biolog tests were performed with three related type strains, according to the manufacturer’s instructions, except that the NaCl concentration was adjusted to 5.0%, 3.0% and 10.0% (w/v), respectively, which were their optimal salinities.

**Chemotaxonomic analyses**

To determine the respiratory isoprenoid quinones and polar lipid composition, cells of strain WB101^T^ and related type strains were cultured in MB at 37 °C until the bacterial communities reached the late exponential growth stage (according to the growth curve). Respiratory isoprenoid quinones were extracted from 300 mg of freeze-dried cell material and separated into different classes using thin-layer chromatography (TLC) on silica gel. The effective components were removed from the plate by relying on the spots and analysed further using reverse-phase high-performance liquid chromatography (HPLC) according to the methods described by Kroppenstedt (1982). Polar lipids were extracted from 100 mg of fresh cell material and separated via TLC on silica gel plates (8 × 8 cm, no. 5554; Merck) according to the methods described by Tindall et al. (2007). Total lipid material was detected using molybdate phosphoric acid solution, and specific functional groups were tested using additional staining reagents (Sigma-Aldrich) on three other separate TLC plates, which included molybdenum blue solution (phosphates), a-naphthol sulfuric solution (carbohydrates), and ninhydrin solution (amines).

To determine the fatty acid composition, cells of strain WB101^T^, and related type strains were cultured on MA at 37 °C until the bacterial communities reached the late exponential growth stage (according to the four-quadrant streak method) (Sasser 1990). Fatty acids were extracted from 40 mg of fresh cell material, saponified, methylated, and extracted using the standard protocol of the Sherlock Microbial Identification System (MIDI) equipped with an Agilent model 6890 N gas chromatograph. Fatty acids with percentages were determined and calculated using MIS standard software with the TSBA40 database (Buyer 2002).
Results and discussion

Phylogenetic and phylogenomic analyses

The only complete 16S rRNA gene sequence (1,532 bp) extracted from the draft genome shared 99.9% similarity with nearly complete sequences (1,505 bp) obtained by conventional Sanger sequencing, which confirms their authenticity. Strain WB101T exhibited the highest similarity to R. barkolensis 15182T (93.5%), followed by R. halophilus JZ3C29T (93.2%), ‘R. mucosus’ 8A47T (92.1%), and other valid species of the family Balneolaceae (on the edge of or lower than 90.0%). The phylogenetic tree based on the NJ algorithm demonstrated that strain WB101T was distinct from R. halophilus JZ3C29T, R. barkolensis 15182T, and ‘R. mucosus’ 8A47T within the genus Rhodohalobacter at a high confidence level (Fig. 1), which was also emphasized by additional MP and ML phylogenetic trees.

The draft genome of strain WB101T was 5,104,032 bp in size, and the DNA G+C content was 42.0%. A total of 138 contigs were assembled from the raw sequences, and the N50 value and coverage depth were 123,641 and 275.0 ×, respectively. The detailed contents of the tRNAs coded by strain WB101T were reverse-complemented into codons and are shown in Supplementary Table S1. Strain WB101T had a significantly larger genome than members of the genus Rhodohalobacter, although they all shared a similar number of tRNAs and G+C content. Detailed genomic information on strain WB101T and related type strains is listed in Table 1.

The OrthoANIu, dDDH, AAI, POCP, and TETRA values between strains WB101T and ‘R. mucosus’ 8A47T, R. barkolensis 15182T, and R. halophilus JZ3C29T are shown in Table 2. The OrthoANIu values between each of these strains were far lower than 95.0–96.0%, the threshold for identifying potential novel species (Richter and Rosselló-Móra 2009). Moreover, the ANIb and ANIm values were below 90.0%. All dDDH values were below the threshold.
value (70.0%) for species delineation, demonstrating that each pair formed deep lineages (Li et al. 2010). The TETRA values were lower than 0.99, which is the threshold for novel species (Richter and Rosselló-Móra 2009). For the taxonomic boundaries for genera, all AAI and POCP values between strain WB101T and each of the three related type strains were over 60.0% and 50.0%, respectively, which were argued as genus boundaries (Rodriguez-R and Konstandtinidis 2014; Qin et al. 2014). Additionally, the same taxonomic status shown by the phylogenetic trees was also demonstrated by the phylogenomic tree based on the GTDB (Fig. 2).

**Table 1** Genomic data that differentiates strain WB101T from related type strains

| Genomic data          | 1                | 2\(^a\)           | 3\(^b\)           | 4\(^c\)           |
|-----------------------|------------------|--------------------|--------------------|--------------------|
| Genome size (bp)      | 5,104,032        | 3,908,029          | 3,597,295          | 3,122,722          |
| DNA G+C (%)           | 42.0             | 47.7               | 42.5               | 44.4               |
| Number of coding genes| 4233             | 3219               | 3015               | 2727               |
| Number of total pseudo genes | 24              | 9                  | 5                  | 26                 |
| Number of tRNAs       | 38               | 40                 | 39                 | 38                 |
| Number of rRNAs (5S, 16S, 23S) | 3, 1, 1         | 1, 1, 1            | 1, 2, 1            | 1, 2, 1            |
| Number of contigs     | 138              | 17                 | 7                  | 92                 |
| N50 values            | 123,641          | 417,940            | 1,807,017          | 84,288             |
| Coverage depth        | 275.0×           | 198.0×             | 544.0×             | 137.9×             |

1, Strain WB101T; 2, ‘R. mucosus’ 8A47T; 3, R. barkolensis 15182T; 4, R. halophilus JZ3C29T

Data from: aWang et al. (2021)  
b Han et al. (2018)  
c Xia et al. (2017)

**Table 2** Phylogenomic value between strain WB101T and related type strains

| Phylogenomic value | 1 | 2 | 3 |
|--------------------|---|---|---|
| OrthoANIu          | 69.2% | 69.5% | 69.4% |
| dDDH               | 18.5%  | 17.6%  | 18.1%  |
| TETRA              | 0.81   | 0.93   | 0.92   |
| AAI                | 68.0%  | 68.6%  | 68.6%  |
| POCP               | 56.0%  | 53.4%  | 52.1%  |

1, Strain WB101T vs ‘R. mucosus’ 8A47T; 2, Strain WB101T vs R. barkolensis 15182T; 3, Strain WB101T vs R. halophilus JZ3C29T

GenBank assembly accession: Strain WB101T, GCA_022012475; ‘R. mucosus’ 8A47T, GCA_003150675; R. barkolensis 15182T, GCA_002834295; R. halophilus JZ3C29T, GCA_001715195

**Fig. 2** Maximum-likelihood phylogenomic trees based on genomes inferred the bac120 marker set of strain WB101T and related taxa within the phylum Balneolota and others. GenBank accession numbers of assemblies are given in parentheses. *Escherichia coli* JCM 1649T (GCA_000613265) was used as an outgroup. Bar, 0.100 substitutions per nucleotide position.
Genomic analyses

No known secondary metabolite clusters were identified with high similarity according to the antiSMASH. Compared to other genus Rhodohalobacter members, strain WB101T uniquely has a non-ribosomal peptide synthetase cluster (NRPS), suggesting that an unknown peptide synthesis pathway is encoded by its genome. Unclassified hserlac-tone, terpene, and T3PKS were encoded by strain WB101T and three related type strains.

OrthoVenn2 analysis clustered 7211 protein entries into 1789 clusters shared by strain WB101T and the three related type strains. Figure 3 shows the overlapping cluster relationships. Strain WB101T contained 442 unique protein entries, clustered into 151 clusters. Furthermore, 103 entries of these unique proteins were annotated by KEGG and were mainly responsible for carbohydrate metabolism, glycan biosynthesis and metabolism, and unclassified metabolism.

Based on the RAST programme, the galactosylceramide and sulphatide metabolism pathways encoded by the strain WB101T genome were more abundant than those of the three related type strains. The results of analyses by KEGG Orthology and Links Annotation (KOALA) indicated that the genome contained enzymes involved in ASR. The sulphate reduction occurs within cells of strain WB101T with adenylylsulphate kinase (cysC), sulphate adenylyltransferase subunit 1 (cysN), sulphate adenylyltransferase subunit 2 (cysD), phosphoadenosine phosphosulphate reductase (cysH), and sulphite reductase (sir). As an intermediate during this process, 3'-phosphoadenosine-5'-phosphosulphate (PAPS) is reduced to sulphite. Then, sulphite is transformed to l-cysteine by O-acetyl serine-(thiol)-lyase (Schiff 1979). The sulphate/thiosulphate transport system encoded by the cysPUWA operon, an ABC-type transporter, mediates high-affinity sulphate and thiosulphate uptake (Kushkevych et al. 2020). Thus, the lack of cysPUWA operon suggests that strain WB101T might not utilise extracellular sulphates. According to the genomic data from GenBank, the assimilatory sulphate reduction pathways were incomplete in ‘R. mucusus’ 8A47T and R. barkolensis 15182T (both lacking cysH and sir) and were even absent in R. halophilus JZ3C29T (lacking cysC, cysN, cysD, cysH and sir). Conversion amongst polysulphides can be achieved by the sulphhydrogenase subunits gamma, beta, alpha, and delta (hydG, hydB, hydA, and hydD) (Ma et al. 2000). Dissimilatory or assimilatory nitrate reduction was incomplete within the genus Rhodohalobacter, as confirmed by the experimental results. Ribokinase (rbsK) allows cells of strain WB101T to transform d-ribose 5-phosphate to d-ribose. Phosphatidylcholine (lecithin) can be synthesised using cdp-diacylglycerol and phosphatidylcholine synthase (pcs). In summary, compared to three related type strains, strain WB101T had more numerous metabolic pathways, which explains why it had a relatively larger genome and demonstrates its potential application value.

Morphological, physiological, and biochemical characteristics

The scanning electron micrograph of strain WB101T is shown in Supplementary Fig. S2. Strain WB101T formed visible colonies on MA with or without 0.1% (w/v) KNO3 in an anaerobic bag after 3 weeks. Nitrate reduction was negative, and no H2S was detected using lead acetate paper or TSI. The strain was susceptible to penicillin (10 μg), ampicillin (10 μg), carbenicillin (100 μg), erythromycin (15 μg), vancomycin (30 μg), rifampicin (5 μg), chloramphenicol (30 μg), norfloxacin (30 μg), ofloxacin (5 μg), cefotaxime (30 μg), clarithromycin (15 μg), lincomycin (2 μg), ceftriaxone (30 μg), and polymyxin B (300 μg), but resistant to tobramycin (10 μg), tetracycline (30 μg), neomycin (30 μg), gentamicin (10 μg), streptomycin (10 μg), and kanamycin (30 μg). Remarkably, according to available data on related taxa, including Alitofodinibius salicampi KHM44T (Cho et al. 2017), ‘A. salipaludis’ WN023T (Zhao et al. 2020), A. salipilhus ECH52T (Cho and Whang 2020), R. barkolensis 15182T (Han et al. 2018), and ‘R. mucusus’ 8A47T, members of the phylum Balneolota showed the same sensitivity to vancomycin as strain WB101T. However, vancomycin, a glycopeptide antibiotic, is inherently inactive against Gram-stain-negative bacteria because it cannot cross the bacterial outer membrane (Yarlagadda et al. 2016). Thus, it is suggested that the cells of these taxa differ from typical Gram-stain-negative bacteria.

According to API 20NE, strain WB101T and the three related type strains were negative for nitrate reduction and indole production. Compared to R. barkolensis MCCC 1K03442T and R. halophilus JZ3C29T, strain WB101T had a stronger positive effect on the assimilation of capric
acid. Positive reactions for β-galactosidase and N-acetyl-β-glucosaminidase differentiated strain WB101T from the three related type strains, and negative reactions for lipase (C14) and α-fucosidase were consistent in the genus Rhodo-halobacter. Strain WB101T oxidised d-glucuronic acid and glucuronamide more strongly than the other three related type strains.

The integrated morphological, physiological, and biochemical characteristics of strain WB101T are provided in the species description. The details that distinguish strain WB101T from the related type strains are summarised in Table 3. Additional Supplementary Table S2 lists the traits of strain WB101T and the generic traits of neighbouring genera of the family Balneolaceae.

| Table 3 Characteristics that differentiate strain WB101T from related type strains |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Properties                                      | 1               | 2               | 3               | 4               |
| Oxygen requirement                              | Facultatively anaerobic | Aerobic | Aerobic | Facultatively anaerobic |
| Temperature (°C)                                |                 |                 |                 |                 |
| Growth range                                    | 25–42           | 20–42           | 10–40           | 20–50           |
| Optimum                                         | 35–37           | 37–40           | 37            | 40              |
| NaCl concentration (% w/v)                      |                 |                 |                 |                 |
| Growth range                                    | 1.0–11.0        | 2.6–14.0        | 0.5–4.0         | 2.0–16.0        |
| Optimum                                         | 5.0–6.0         | 4.0–6.0         | 2.0–3.0         | 8.0–16.0        |
| pH                                              |                 |                 |                 |                 |
| Growth range                                    | 7.0–8.5         | 7.0–9.0         | 7.0–8.0         | 7.0–9.0         |
| Optimum                                         | 7.5–8.0         | 7.5–8.0         | 7.5             | 7.5–8.5         |
| Starch                                          | −               | +               | +               | +               |
| Casein                                          | +               | +               | −               | +               |
| Alginate                                        | −               | +               | +               | −               |
| Tweens 20                                       | +               | +               | −               | −               |
| Tweens 40                                       | +               | −               | −               | −               |
| Tweens 60                                       | +               | −               | ND             | ND             |
| Tweens 80                                       | +               | −               | −               | −               |
| Acid production from d-Arabinose                 | +               | −               | −               | −               |
| Inositol                                        | +               | −               | −               | W               |
| d-Sorbitol                                      | +               | −               | −               | −               |
| Oxidase                                         | +               | +               | +               | −               |
| β-Galactosidase                                 | +               | −               | −               | −               |
| β-Glucosidase                                   | +               | +               | −               | −               |
| N-Acetyl-β-glucosaminidase                      | +               | −               | −               | −               |
| Oxidation of d-Galactose                        | +               | −               | −               | −               |
| d-Fucose                                        | +               | −               | −               | −               |
| Glycerol                                        | −               | −               | −               | +               |
| Pectin                                          | +               | −               | −               | −               |
| d-Glucuronic acid                               | +               | −               | −               | W               |

The data without particular indications are from this study. 1, Strain WB101T; 2, ‘R. mucosus’ 8A47T; 3, R. barkolensis MCCC 1K03442T; 4, R. halophilus IZ3C29T. + positive; w weakly positive; − negative; ND no data available.

Data from: Wang et al. (2021)
 Han et al. (2018)
 Xia et al. (2017)
Chemotaxonomic analyses

The sole respiratory quinone of strain WB101<sup>T</sup> was menaquinone-7 (MK-7), which was consistent with species of the genus *Rhodohalobacter*. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylcholine (PC) were the major polar lipids detected in strain WB101<sup>T</sup>. Additionally, minor amounts of an unidentified phospholipid (PL1), three glycolipids (GL1, GL2, and GL3), and four unidentified lipids (L1, L2, L3, and L4) were present. Compared to the three related type strains, cells of strain WB101<sup>T</sup> could synthesise lecitin, which was also identified in genomic analyses. The absence of aminolipids (AL1, AL2, and AL3) could differentiate strain WB101<sup>T</sup> from *R. mucosus* sp. nov. *R. barkolensis* MCCC 1K03442<sup>T</sup>. Further details of the polar lipid content of strain WB101<sup>T</sup> and the three related type strains are shown in Supplementary Fig. S3. Detailed discrepancies between strain WB101<sup>T</sup> and related type strains are listed in Supplementary Table S3.

Conclusion

Combined with the results of the genotypic, phenotypic, and chemotaxonomic analyses, the similarities and differences between strain WB101<sup>T</sup> and other related taxa were explicitly demonstrated. Based on the phylogenetic and phylogenomic tree topologies, we concluded that strain WB101<sup>T</sup> belongs to the genus *Rhodohalobacter* but differs from *R. mucosus*, *R. barkolensis*, and *R. halophilus* at a novel species level. Therefore, *Rhodohalobacter sulfatireducens* sp. nov. was proposed, with strain WB101<sup>T</sup> as the type strain.

Description of *Rhodohalobacter sulfatireducens* sp. nov.

*Rhodohalobacter sulfatireducens* (sul.fa.ti.re.du’cens. N.L. n. sulfas -atis, sulphate; L. v. reducere to lead back, bring back; N.L. part. adj. sulfatireducens reducing sulphate).

Cells are Gram-stain-negative, oxidase-positive, catalase-positive, non-motile, facultatively anaerobic, and rod-shaped (0.2–0.5 μm in wide and 0.6–3.7 μm in length). The colonies are reddish, convex, circular, and regularly edged on the MA plate. Optimal growth occurs at 35–37 °C (range, 25–42 °C), 5.0–6.0% (w/v) NaCl (range, 1.0–11.0%), and pH 7.5–8.0 (range, 7.0–8.5). The hydrolysis of casein and Tweens 20, 40, 60, and 80 was positive. Negative for H<sub>2</sub>S and indole production. The complete assimilatory sulphate reduction pathway is encoded by the genome. The activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase (weakly), β-galactosidase, β-glucuronidase (weakly), α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, and α-mannosidase (weakly) were positive. Acids were produced from D-arabinose, L-arabinose (weakly), D-ribose, D-xylose, L-xylose (weakly), D-glucose (weakly), D-fructose (weakly), D-mannose (weakly), L-sorbitose, inositol, D-sorbitol, methyl-α-D-mannopyranoside, N-acetyl glucosamine, arbutin, esculin ferric citrate, D-lyxose (weakly), D-tagatose, potassium 2-ketogluconate (weakly), and potassium 5-ketogluconate. Dextrin, D-maltose, D-trehalose, D-cellulose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, β-D-mannose, D-galactose, D-fucose, L-histidine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, glucuronamide, α-keto-glutaric acid, and acetooacetic acid were oxidised. MK-7 was the only respiratory quinone. The polar lipid pattern consisted of DPG, PG, PC, PL, GL, and L. The major fatty acid was iso-C<sub>15:0</sub>. The genomic DNA G+C content was 42.0%.

The type strain WB101<sup>T</sup> (= KCTC 92204<sup>T</sup> = MCCC 1H00518<sup>T</sup>) was isolated from a marine solar saltern in Weihai, PR China.

The GenBank accession numbers are OM301685 for the 16S rRNA gene and JAKLWS00000000 for the draft genome.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical statements This article does not contain any studies with animals performed by any authors.
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