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Rhodopirellula rosea sp. nov., a Novel Bacterium Isolated from an Ark Clam *Scapharca broughtonii*

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A novel Gram-negative, motile, and ovoid-shaped strain, LHWP3^T, which belonged to the family *Planctomycetaceae* in the phylum *Planctomycetes*, was isolated from a dead ark clam *Scapharca broughtonii* collected during a mass mortality event on the south coast of Korea. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that the isolate was most closely related to the type strain of *Rhodopirellula baltica*, with a shared 16S rRNA gene sequence similarity of 94.8%. The isolate grew optimally at 30°C in 4–6% (w/v) NaCl, and at pH 7. The major isoprenoid quinone was menaquinone-6 (MK-6). The dominant polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and unidentified polar lipids. The predominant cellular fatty acids were $C_{16:0}$, $C_{18:1}$ *ω9c*, and $C_{18:0}$. The genomic DNA G+C content of strain LHWP3^T was 53.0 mol%. Based on polyphasic taxonomic analyses, strain LHWP3^T should be classified as a novel species in the genus *Rhodopirellula* in the family *Planctomycetaceae*, for which the name *Rhodopirellula rosea* sp. nov. is proposed. The type strain is LHWP3^T (=KACC 15560^T =JCM 17759^T).

Keywords: bacterial taxonomy, *Planctomycetaceae* family, *Rhodopirellula rosea* sp. nov.

Introduction

The genus *Rhodopirellula* is affiliated with the family *Planctomycetaceae* in the phylum *Planctomycetes* that is a budding and peptidoglycan-free bacterial group (Fuerst, 2005). This genus was proposed based on taxonomic heterogeneity by Schlesner *et al.* (2004). Currently, this genus comprises only one species, *Rhodopirellula baltica* (www.bacterio.cict.fr). The annual mass mortality events of cage-cultured ark clam *Scapharca broughtonii* have damaged the aquaculture industry in Korea. When we investigated tentative bacterial pathogens that can influence the mass mortality of the ark clams, a novel *Planctomycetes*-like bacterial strain, designated LHWP3^T was isolated in an ark clam farm. The aim of the present study was to establish the taxonomic position of strain LHWP3^T and to elucidate a novel species in the genus *Rhodopirellula* based on a polyphasic approach.

Materials and Methods

Bacterial strains

Strain LHWP3^T was isolated from a dead ark clam *Scapharca broughtonii*, which was collected from an ark clam farm in Gangjin Bay in the south coastal region of Korea during a mass mortality event. The dead ark clam was homogenized with sterilized phosphate-buffered saline (PBS) and incubated at 25°C after spreading on marine agar 2216 (MA; Difco) plates supplemented with penicillin G (3 mg/ml). A colony was subcultured repeatedly to isolate a pure culture. Strain LHWP3^T was suspended in marine broth 2216 (MB; Difco) containing 40% glycerol and frozen at -80°C to facilitate its long-term storage. For comparative taxonomic analyses, the type strain *Rhodopirellula baltica* SH 1^T (DSM 10527) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Phylogenetic and genomic analyses

The 16S rRNA gene of strain LHWP3^T was amplified from colonies of the isolate by PCR and the PCR products were purified and sequenced as described previously (Roh *et al.*, 2008). The sequence fragments were assembled using the SeqMan program and the almost full length 16S rRNA gene sequence of strain LHWP3^T was compared with those from validly published species using the EzTaxon-e server (Kim *et al.*, 2012). The 16S rRNA gene sequences of strain LHWP3^T and related taxa were aligned using the multiple sequence alignment program CLUSTAL_X. Phylogenetic trees were constructed by MEGA5 (Tamura *et al.*, 2011) using the neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-parsimony (MP) (Kluge and Farris, 1969), and maximum-likelihood (ML) methods (Felsenstein, 1981) with 1000 randomly selected bootstrap replicates. The genomic DNA was extracted from strain LHWP3^T using a G-spin^{TM} Genomic DNA Extraction Kit (iNtRON Biotechnology, Korea). The genomic DNA G+C content was assessed using the fluorimetric method described by Gonzalez and Saiz-Jimenez (2002) where the calibration references were genomic DNAs from *Escherichia coli* K12, *Ruegeria pomeroyi*

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DSS-3^T, and *Ruminococcus obeum* ATCC 29174^T.

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LHWP3^T is JF748734.

Morphological, physiological, and biochemical characterization

The growth of strain LHWP3^T was examined at different temperatures (4, 15, 20, 25, 30, 37, 45, and 65°C) on MA. The effects of a range of pH values (pH 4–10, with intervals of 1.0 pH unit) on growth were examined in MB, which was adjusted using 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) (for pH 4, 5, and 6) or 10 mM N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) (for pH 7, 8, 9, and 10). Growth with various NaCl concentrations was tested in a medium that contained all of the constituents of MB except NaCl, which was supplemented at the appropriate concentrations of NaCl (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 15% w/v). Gram staining was determined using a Gram staining kit (bioMérieux, France), according to the manufacturer's instructions. The morphologies of the colonies and cells were studied after incubation for 7 days on MA. The morphologies and Gram staining of cells were observed using a light microscope (ECLIPSE 50*i*, Nikon, Japan). A motility test was conducted using semi-solid MA, according to the method of Tittsler and Sandholzer (1936). Growth on MA in anaerobic condition was tested in an anaerobic chamber filled with mixed gases (N_2:CO_2:H_2, 90:5:5) for 1 week. The catalase and oxidase activities of strain LHWP3^T were assessed using 3% (w/v) hydrogen peroxide and 1% (v/v) p-tetramethyl phenylenediamine (bioMérieux), respectively. API ZYM strip (bioMérieux) was used to determine the activities of various enzymes, according to the manufacturer's instructions. Acid production was assessed using API 50CH strips with 50 CHB/E medium (bioMérieux), according to the manufacturer's instructions.

Chemotaxonomy

The isoprenoid quinones were extracted with chloroform-methanol (2:1, v/v), purified by one-dimensional thin layer chromatography (TLC) on a silica gel 60 F254 plate (Merck, Germany), and identified using reverse phase-high performance liquid chromatography (TSP-0299, Thermo Scientific, USA), according to the method of Collins and Jones (1981). The polar lipids were analyzed using TLC, according to method of Minnikin *et al.* (1984). The separated polar lipid spots were identified using specific appropriate detection reagents as follows: 5% ethanolic molybdophosphoric acid for total lipids, ninhydrin for amino-containing lipids, molybdenum blue for phospholipids, and α-naphthol-sulfuric acid for glycolipids. For the cellular fatty acid analysis, strain LHWP3^T was cultivated on MA supplemented with 2% NaCl at 30°C for 7 days. For the analysis of cellular fatty acid composition, strain LHWP3^T and *Rhodopirellula baltica* SH 1^T were cultivated on MA at 30°C and harvested at the same physiological age, i.e. after 117 and 194 h, respectively. The fatty acid analysis was performed according to the standard protocol for the Sherlock Microbial Identification System (version 6.2) using gas chromatography (Hewlett Packard 6890, USA) and the Microbial Identification software package with the TSBA6 database (Sasser, 1990).

Results and Discussion

Phylogenetic and genomic analysis

The phylogenetic analyses showed that strain LHWP3^T was closely related to *Rhodopirellula baltica* SH 1^T and shared a 16S rRNA gene sequence similarity of 94.8%. Other type strains in the family *Planctomycetaceae* shared lower 16S rRNA gene sequence similarities with the isolate: *Blastopirellula marina* SH 106^T (88.7%), *Planctomyces maris* 534-30^T (85.1%), and *Pirellula staleyi* DSM 6068^T (84.5%). The low 16S rRNA gene sequence similarities between the isolate and the other type strains suggest that strain LHWP3^T should be considered as a distinct genospecies (Wayne *et al.*, 1987). The phylogenetic trees constructed using the 16S rRNA gene sequences indicated that strain LHWP3^T formed a tight phyletic lineage with *Rhodopirellula baltica* SH 1^T based on 100% bootstrap values, irrespective of whether

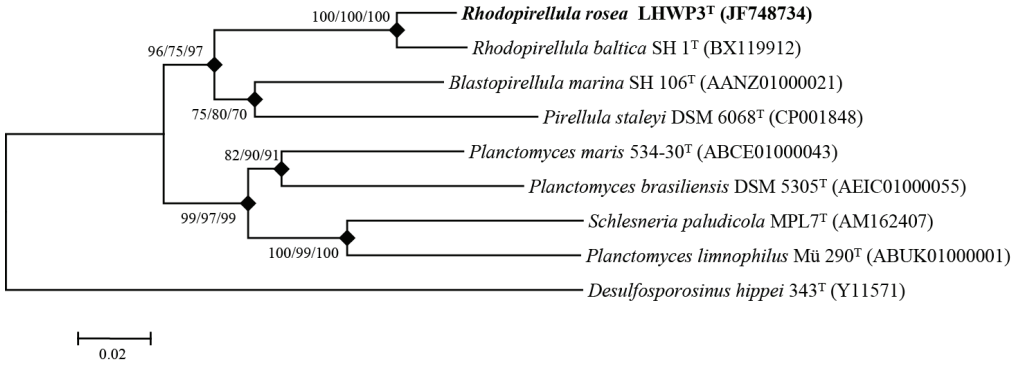

Fig. 1. Phylogenetic tree of the 16S rRNA gene sequences constructed using the neighbor-joining method. The taxonomic position of strain LHWP3^T was compared to closely related taxa in the family *Planctomycetaceae*. The numbers (percentages of 1,000 replicates) on the nodes indicate the bootstrap values (>70%) calculated using the neighbor-joining/maximum-parsimony/maximum-likelihood probabilities. The filled diamonds on the nodes indicate branches that were also recovered using the maximum-parsimony and maximum-likelihood algorithms. *Desulfosporosinus hippei* 343^T served as the outgroup. Bar=0.02 substitutions per nucleotide position.

Table 1. Characteristics of strain LHWP3^T and *Rhodopirellula baltica* SH 1^T. All data were obtained from the present study, except where indicated. Based on API ZYM, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Based on API 50CH to test acid production, all strains were positive for D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-mannitol, N-acetylglucosamine, esculin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, gentiobiose, D-lyxose, and L-fucose, but negative for glycerol, erythritol, D-adonitol, methyl-β-D-xyloside, dulcitol, inositol, D-sorbitol, inulin, starch, glycogen, xylitol, D-tagatose, D-arabitol, gluconate, and 2-keto-gluconate.
Taxa: 1, *Rhodopirellula rosea* sp. nov. LHWP3^T; 2, *Rhodopirellula baltica* SH 1^T. Symbols: +, positive; -, negative.

Characteristics	1	2
Growth at 37°C	+	-[a]
API ZYM:		
Cystine arylamidase	-	+
α-Glucosidase	-	+
β-Glucosidase	-	+
API 50CH (acid production):		
Methyl-α-D-mannoside	-	+
Methyl-α-D-glucoside	-	+
Amygdalin	-	+
Arbutin	-	+
Salicin	-	+
D-Melezitose	-	+
D-Raffinose	-	+
D-Turanose	-	+
D-Fucose	-	+
L-Arabitol	-	+
5-Keto-gluconate	-	+
G+C content (mol%)	53	55[a]

[a] Data from Schlesner *et al.* (2004).

Table 2. Fatty acid compositions of strain LHWP3^T and *Rhodopirellula baltica* SH 1^T. Strains: 1, *Rhodopirellula rosea* LHWP3^T sp. nov.; 2, *R. baltica* SH 1^T. All data from this study. Fatty acids amounting to <1% of the total fatty acids in two strains are not shown. -, Not detected.

Fatty acid	1	2
$C_{10:0}$	7.2	4.9
$C_{11:0}$ 2-OH	1.9	1.3
$C_{11:0}$ 3-OH	1.3	-
iso-$C_{12:0}$	1.4	-
$C_{16:0}$	37.3	37.6
$C_{16:1}$ *ω11c*	2.9	-
$C_{17:1}$ *ω8c*	3.5	3.9
$C_{18:0}$	16.2	11.3
$C_{18:1}$ *ω9c*	22.2	28.7
Summed feature 1[a]	1.9	1.5
Summed feature 3[a]	3.6	7.3
Summed feature 8[a]	-	1.9

[a] Summed feature 1 comprised $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H; summed feature 3 comprised $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*; summed feature 8 comprised $C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*.

NJ, MP, or ML was used to construct the trees (Fig. 1). The DNA G+C content of strain LHWP3^T was 53.0 mol%, which is within the range reported for strains of *Rhodopirellula baltica* (53–57 mol%) (Schlesner *et al.*, 2004). The results based on the phylogenetic and genomic analyses suggest that strain LHWP3^T is associated with the genus *Rhodopirellula* in the family *Planctomycetaceae*.

Morphological, physiological, and biochemical characteristics

Strain LHWP3^T was Gram-negative, motile, ovoid-shaped, strictly aerobic, and catalase and oxidase positive, and these characteristics were shared with the strains of *Rhodopirellula baltica* (Schlesner *et al.*, 2004). Optimal growth of the novel strain occurred at 30°C and pH 7, and in the presence of 4–6% (w/v) NaCl. Strain LHWP3^T formed pink to red-colored, round, smooth colonies after 7 days on MA at 25°C. A detailed description of strain LHWP3^T is given in the species description below. Table 1 provides the comparison of the characteristics between strain LHWP3^T and the closely related strain *Rhodopirellula baltica* SH 1^T, which shows that strain LHWP3^T can be distinguished from *Rhodopirellula baltica* SH 1^T.

Chemotaxonomy

Strain LHWP3^T contained menaquinone-6 (MK-6) as the predominant menaquinone, which is also the case in strains of *Rhodopirellula baltica* (Schlesner *et al.*, 2004). The polar lipids of strain LHWP3^T were phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and unidentified polar lipids (Fig. 2). Glycolipids were not detected. Schlesner *et al.* (2004) reported that members of the genus *Rhodopirellula* possessed the polar lipids PC and PG, and unidentified polar lipids, whereas the present study also identified PE in strain LHWP3^T. The fatty acid com-

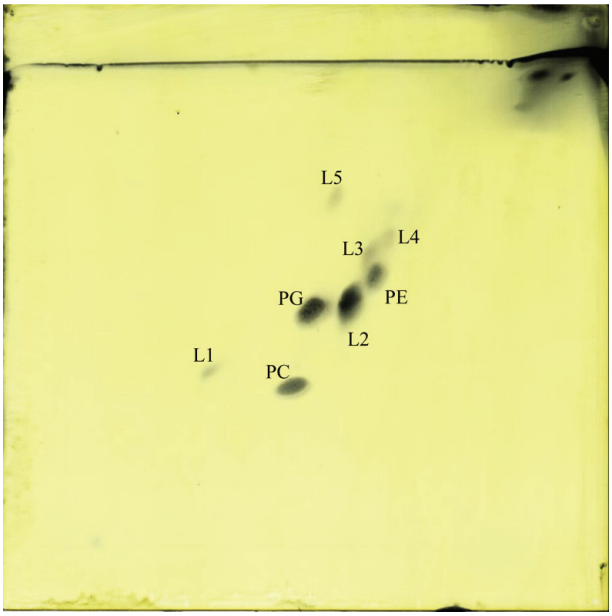

Fig. 2. Two-dimensional thin layer chromatogram of the total polar lipids from strain LHWP3^T, which were detected using 5% ethanolic molybdophosphoric acid. Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; L1-5, unidentified polar lipids.

positions of strain LHWP3^T and the reference strain are shown in Table 2. Major amounts (>10%) of $C_{16:0}$, $C_{18:1}$ $\omega 9c$, and $C_{18:0}$ were present in the two strains. Strain LHWP3^T differed from the reference strain by presence of $C_{11:0}$ 3-OH and iso-$C_{12:0}$ and absence of summed feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$).

Taxonomic conclusion

In conclusion, strain LHWP3^T differed from *Rhodopirellula baltica* SH 1^T by the low 16S rRNA gene sequence similarity, different phenotypic characteristics such as growth at 37°C, enzyme activity of cystine arylamidase and α-, β-glucosidase, acid production from several carbon sources. Based on the phylogenetic, genomic, phenotypic, and chemotaxonomic data, strain LHWP3^T is closely related to members of the genus *Rhodopirellula* in the family *Planctomycetaceae* but it can be distinguished from the type strain of *Rhodopirellula baltica*. Thus, the isolate represents a novel species in the genus *Rhodopirellula*, for which the name *Rhodopirellula rosea* sp. nov. is proposed.

Description of *Rhodopirellula rosea* sp. nov.

Rhodopirellula rosea (ro'se.a. L. fem. adj. *rosea*, pink).
The cells are Gram-negative, strictly aerobic, ovoid-shaped (0.6–1.5 μm × 0.6–1.4 μm), and motile. Colonies are pink to red-colored, smooth, round, and 0.1–0.4 mm in diameter after incubation for 7 days on MA. Growth occurs at 20–37°C (optimum, 30°C), pH 6–8 (optimum, 7), and in the presence of 0–7% (w/v) NaCl (optimum, 4–6%). Positive for catalase and oxidase. In the API ZYM strip, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. In the API 50CH strips to the test of acid production, positive for D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-mannitol, N-acetyl-glucosamine, esculin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, gentiobiose, D-lyxose, and L-fucose, but negative for glycerol, erythritol, D-adonitol, methyl-β-D-xyloside, dulcitol, inositol, D-sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, arbutin, salicin, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-turanose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate, and 5-keto-gluconate. The major isoprenoid quinone is menaquinone-6 (MK-6). The polar lipids comprise phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and unidentified polar lipids. The major cellular fatty acids are $C_{16:0}$, $C_{18:1}$ $\omega 9c$, and $C_{18:0}$. The DNA G+C content of strain LHWP3^T is 53.0 mol%. Strain LHWP3^T (=KACC 15560^T =JCM 17759^T) was isolated from a dead ark clam in the southern coastal region of Korea.

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