Full Paper

Psychrosphaera saromensis gen. nov., sp. nov., within the family Pseudoalteromonadaceae, isolated from Lake Saroma, Japan

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Three Gram-negative, motile, coccoid- and ellipsoidal-shaped, non-pigmented, chemoheterotrophic bacteria, designated strains SA4-31, SA4-46 and SA4-48T, were isolated from Lake Saroma in Japan and subjected to a polyphasic taxonomical study. 16S rRNA gene sequence analysis revealed that the novel isolates could be affiliated to the family Pseudoalteromonadaceae of the order Alteromonadales. The strains shared approximately 99.7–100% sequence similarity with each other and showed 89.5–93.2% similarity with members of the family Pseudoalteromonadaceae with validly published names. The DNA–DNA relatedness among the strains SA 4-31, SA 4-46 and SA 4-48T was higher than 80%, a value that is accepted as a phylogenetic definition of one species. The DNA G+C contents of the three strains were 38.7–39.6 mol%. The major isoprenoid quinone was Q-8 and C16:0, C16:1ω7c, C18:1ω7c and C12:1 3OH were the major fatty acids. Based on the evidence from the polyphasic taxonomical study, it was concluded that the three strains should be classified as representing a new genus and species of the family Pseudoalteromonadaceae, for which the name Psychrosphaera saromensis gen. nov., sp. nov. (type strain SA4-48T = NBRC 107123T = KCTC 23240T) is proposed.

Key Words—marine environment; Psychrosphaera saromensis gen. nov., sp. nov.

Introduction

The family Pseudoalteromonadaceae currently comprised only two genera, Pseudoalteromonas and Algicola (Ivanova et al., 2004). The genus Pseudoalteromonas (type genus of the family Pseudoalteromonadaceae) (Gauthier et al., 1995; Ivanova et al., 2004) currently comprises 34 recognized species, with Pseudoalteromonas haloplanktis as the type species. These bacteria play an important role in marine environments owing to their abundance and high metabolic activities. Pseudoalteromonas are highly capable of surviving in nutrient-poor marine environments by adjustment of their biochemical pathways and production of a wide variety of metabolites, including biologically active compounds and enzymes (Ivanova et al., 2003). On the other hand, the genus Algicola has been proposed to resolve the phylogenetic relationships among the marine Alteromonas-like proteobacteria (Ivanova et al., 2004) and currently comprised only two species, Algicola bacteriolytica and Algicola sagamiensis.

In this study, three novel aerobic bacteria were isolated from Lake Saroma and were found to belong to the family Pseudoalteromonadaceae. Morphological, physiological and phylogenetic analyses of these isolates demonstrated that they represent a novel genus and a novel species as Psychrosphaera saromensis gen. nov., sp. nov. within the family Pseudoaltero-
monadaceae.

Materials and Methods

Isolation of bacterial strains and cultivation. In this study, three novel aerobic bacteria were isolated from brackish water samples just below the ice. The samples were collected from Lake Saroma in Hokkaido, Japan on 22 February 2009 (44°07’ N, 143°58’ E; temperature, −1.5°C; salinity, 34.0 PSU; surface brackish water below sea ice). Lake Saroma is a brackish lake and the surface layer is iced over in winter. The seawater sample (100 μl) was inoculated on 1/2 strength ZoBell agar containing 80% seawater and incubated at 4°C for 30 days. Isolated strains were maintained on 1/2 Marine agar 2216 (MA; Difco) containing 2% NaCl under 15°C. The temperature (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 37°C and 45°C) and pH (5–10) ranges for growth were determined by incubating the isolates on 1/2 MA. The NaCl concentration for growth was determined on 1/2 MA (agar 15 g, MgCl₂ 4.4 g, peptone 2.5 g, Na₂SO₄ 1.62 g, CaCl₂ 0.9 g, yeast extract 0.5 g, KCl 0.27 g, NaHCO₃ 0.8 g, ferric citrate 0.5 g, KBr 0.04 g, SrCl₂ 0.015 g, H₃BO₃ 0.01 g, Na₂HPO₄ 4 mg, Na₂SiO₃ 2 mg, NaF 1.2 mg, NH₄NO₃ 0.8 mg/L) containing 0–15% (w/v) NaCl. Gram-staining was performed as described by Murray et al. (1994).

Morphological, physiological and biochemical tests. Cell morphology and motility were observed using light microscopy (BX60; Olympus) and transmission electron microscopy (TEM). Growth under anaerobic conditions was determined after incubation for 4 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co.) on 1/2 MA. Catalase activity was determined by bubble formation in a 3% H₂O₂ solution. Oxidase activity was determined using cytochrome oxidase test paper (Nissui Pharmaceutical Co.). API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) were used to determine the physiological and biochemical characteristics. All suspension media for the API test strips were supplemented with 2% (w/v) NaCl solution (final concentration). API 20E, API 20NE, API 50CH and API ZYM test strips were read after incubation for 5 days and 2 days at 15°C.

16S rRNA gene sequencing, phylogenetic analysis and DNA–DNA hybridization. DNA was prepared according to the method of Marmur (1961) from cells grown on 1/2 MA containing 2% NaCl and the DNA base composition was determined by using the HPLC method of Mesbah et al. (1989). DNA–DNA hybridizations were carried out with photobiotin-labeled probes in microplate wells as described by Ezaki et al. (1989). The hybridization temperature was set at 41°C. Hybridization was performed using five replications for each. Of the values obtained, the highest and lowest for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

A fragment of approximately 1,450 bp from the 16S rRNA gene was amplified from the extracted DNA by using bacterial universal primers specific to the 16S rRNA gene: 27F and 1492R (Weisburg et al., 1991). To ascertain the phylogenetic position of the novel isolates, the 16S rRNA gene sequences of strains SA4-31, SA4-46 and SA4-48T were compared with sequences obtained from GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). Multiple alignments of the sequences were performed using CLUSTAL_X (version 1.83) (Thompson et al., 1997). Alignment gaps and ambiguous bases were not taken into consideration when the 1,392 bases of 16S rRNA gene nucleotides were compared. Aligned sequences were analyzed by using MEGA 4 (Tamura et al., 2007). The evolutionary distances [distance options according to the Kimura two-parameter model (Kimura, 1983)] and clustering with the neighbor-joining (Kimura and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods were determined by bootstrap values based on 1,000 replications (Felsenstein, 1985). The similarities were calculated using the same software.

Chemotaxonomic investigation. Determination of the respiratory isoprenoid quinone system was carried out as described previously (Xie and Yokota, 2003). Bacterial strains grown on 1/2 MA for 15 days at 15°C were used for the analysis of fatty acid methyl esters. Fatty acid methyl esters were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990).

Nucleotide sequence accession numbers. The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequence of strain SA4-31, SA4-46 and SA4-48T are AB545805, AB545806 and AB545807, respectively.
Results and Discussion

Phylogenetic analysis

The phylogenetic tree based on neighbor-joining (NJ) and maximum-parsimony (MP) generated comparisons of the 16S rRNA gene sequences revealed that the strains studied formed a monophyletic clade within the family *Pseudoalteromonadaceae* with bootstrap confidence values of 99% in the NJ method (Fig. 1) and 96% in the MP method (data not shown). Strains SA4-30, SA4-46 and SA4-48$^T$ formed a single cluster with sequence similarities ranging from 99.7% to 100% among the strains (Fig. 1) and showed 89.5–93.2% similarities with strains in the family *Pseudoalteromonadaceae* with validly published names. They showed 92.9%, 92.9% and 92.7% similarity with *Pseudoalteromonas haloplanktis* (type species of the genus) and 89.5%, 89.5% and 89.8% similarity with *Algicola bacteriolytica*, respectively. Most closely related species in the family *Pseudoalteromonadaceae* were *Pseudoalteromonas atlantica* (93.2%, 93.2% and 93.1% respectively) and *Pseudoalteromonas espejiana* (93.2%, 93.2% and 93.1%). The DNA hybridization values among strains SA4-31, SA4-46 and SA4-48$^T$ were higher than 80% (data not shown). This result strongly suggests that strains SA4-31, SA4-46 and SA4-48$^T$ should be classified as representing one species (Wayne et al., 1987).

Morphological, physiological and biochemical analysis

Strain SA4-48$^T$ grown on 1/2 MA agar containing 2% NaCl at 15°C for 14 days was coccoid- or ellipsoidal-shaped, approximately 0.5–0.7 μm wide and 0.75–1 μm long. Cells were motile by means of a polar flagellum. Spores were not observed under the microscope (Fig. 2). Cultural, physiological and biochemical characteristics of SA4-31, SA4-46 and SA4-48$^T$ were com-

![phylogenetic tree]

Fig. 1. Neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains SA4-31, SA4-46 and SA4-48$^T$ among the currently known and cultivated species of the family *Pseudoalteromonadaceae*. Numbers at nodes are bootstrap percentages derived from 1,000 replications (frequencies less than 50% are not shown). The sequence of *Bacillus halodurans* LB 35 (EF113325) was used as an outgroup. Bar, 2% sequence divergence.
pared to related genera in the family *Pseudoalteromonadaceae* (Table 1). No growth was observed under anaerobic conditions in 1/2 MA containing 2% NaCl. Growth occurred only under aerobic conditions at between 4 and 30°C. Optimal temperature and pH for strain SA4-31, SA4-46 and SA4-48<sup>T</sup> growth were 15–20°C and pH 6–9, respectively. The strains required NaCl for growth and grew in 1–5%.

**Chemotaxonomic analysis**

As shown in Table 2, the predominant cellular fatty acids of strains SA4-31, SA4-46 and SA4-48<sup>T</sup> were C16:0 (19.4%, 19.8% and 21% respectively), C18:1ω7c (25.3%, 20.9% and 20.4%) and C16:1ω7c (16.76%, 16.4% and 18.8%). They contained C12:1 3OH (4.52%, 5.71% and 6.2%) as a 3-hydroxy fatty acid which distinguishes them from cultivated members of the family *Pseudoalteromonadaceae*. Based on the detailed profiles of fatty acids, these strains could be separated into an independent group.

**In conclusion**

Based on the results of the phylogenetic analysis and their biochemical and physiological properties, these three novel strains, SA4-31, SA4-46 and SA4-48<sup>T</sup>, isolated from Lake Saroma in Hokkaido, Japan,
represent a novel genus and a novel species within the family *Pseudoalteromonadaceae*, for which the name *Psychrosphaera saromensis* gen. nov., sp. nov., is proposed.

**Description of *Psychrosphaera* gen. nov.**

*Psychrosphaera* (Psy.chro.sphae-ra. Gr. adj. psychros cold; M.L. fem. n. sphaera sphere; N.L. fem. n. Psychrosphaera a cold sphere).

Cells are non pigmented, coccoid and ellipsoid in shape, Gram-negative and obligately aerobic. Cells are motile by means one polar flagellum. Non-spore-forming. The major respiratory quinone is isoprenoid quinone 8. The G+C content of the genomic DNA is 38.7–39.6 mol%. Predominant cellular fatty acids are C16:0, C16:1 ω7c, C18:1 ω7c and C12:1 3OH. The type species is *Psychrosphaera saromensis*.

**Description of *Psychrosphaera saromensis* sp. nov.**

*Psychrosphaera saromensis* (sa.ro.men’sis N. L. fem. adj. saro-mensis, pertaining to Lake Saroma, where organisms were collected).

Exhibits the following properties in addition to those given in the genus description. Cells are approximately 0.5–0.7 μm wide and 0.75–1 μm long. Cells are motile by means of a polar flagellum and are non-spore-forming. Temperature range for growth is 4–30°C. Optimal temperature for growth is 15–20°C. No growth occurs at above 37°C. pH range for growth is 6.0–9.0. NaCl is required for growth and can be tolerated up to 5% (w/v). Catalase-negative but oxidase-positive. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive, but N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, cystine arylamidase, chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase are negative. Gelatin is hydrolyzed. Acid is produced from glycerol, L-arabinose, D-xylose, β-methyl-D-xylopyranoside, galactose, glucose, fructose, mannose, amygdalin, esculin, salicin, cellobiose, maltose and gentiobiose. Major fatty acid components (>5.0%) include C16:0, C16:1 ω7c, C18:1 ω7c, C12:1 3OH and C15:1 ω8c. The DNA G+C content of the type strain is 38.7 mol%. The type strain, SA4-48T (=NBRC 107123T = KCTC 23240T), was isolated from Lake Saroma in Hokkaido, Japan.

Table 2. Comparative fatty acid content (%) of strains SA 4-31, SA 4-46 and SA 4-48T and related strains belonging to the family *Pseudoalteromonadaceae*.

<table>
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<td>2.2</td>
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<td>2.7</td>
<td>2.4</td>
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<td>30.1</td>
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<td>1.9</td>
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<tr>
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<td>6.4</td>
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<td>27.5</td>
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Strains: 1, SA 4-31; 2, SA 4-46; 3, SA 4-48T; 4, A. bacteriolytica IAM14595T; 5, A. sagamiensis B-10-31T; 6, P. haloplanktis CECT 4188T (Khudary et al., 2008); 7, P. arctica A 37-1-2T (Khudary et al., 2008); 8, P. tetraodonis IAM 14160T (Ivanova et al., 2001); 9, P. atlantica IAM 14164 (Bozal et al., 1997); 10, P. lipolytica LMEB 39T (Xu et al., 2009); 11, P. byunsanensis JCM 12483T (Xu et al., 2009); 12, P. undina DSM 6065T (Xu et al., 2009). —, Not detected.

Summed feature 3 comprises C16:1 ω7c and/or C15:0 ISO 2OH.
Acknowledgments

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Reference


