Sporosarcina saromensis sp. nov., an aerobic endospore-forming bacterium

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Two Gram-positive, endospore-forming, rod-shaped bacterial strains, HG645^T and HG711, were respectively isolated from surface water of a brackish lake and sediment of a fishery harbour in Japan and were subsequently characterized taxonomically using a polyphasic approach. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains HG645^T and HG711 are affiliated phylogenetically to the genus *Sporosarcina*, and they exhibit sequence similarities of 95.7–97.3% to the type strains of *Sporosarcina* species. DNA–DNA relatedness between strain HG645^T and the type strain of the phylogenetically related species *Sporosarcina aquimarina* was less than 10%. The DNA G+C content of strains HG645^T and HG711 were respectively 46.0 and 45.2 mol%. Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The cell-wall peptidoglycan type (Lys–Glu), major cellular fatty acids (iso-C_{15:0} and anteiso-C_{15:0}) and quinone type (MK-7) of the isolates support their affiliation to the genus *Sporosarcina*. On the basis of phylogenetic analysis and physiological and chemotaxonomic data, the isolates represent a novel species of the genus *Sporosarcina*, for which the name *Sporosarcina saromensis* sp. nov. is proposed. The type strain is strain HG645^T (=MBIC08270^T=IAM 15429^T=KCTC 13119^T).

The genus *Sporosarcina* was first described by Kluyver & van Niel (1936). At present, the genus *Sporosarcina* contains six recognized species (Claus & Fahmy, 1986; Larkin & Stokes, 1967; Miquel, 1889; Nakamura, 1984; Reddy *et al.*, 2003; Yoon *et al.*, 2001), with *Sporosarcina ureae* as the type species. Members of the genus *Sporosarcina* are Gram-positive or Gram-variable, endospore-forming, rod-shaped or spherical bacteria. They have L-lysine as the diagnostic amino acid at position 3 of the peptide subunit of the peptidoglycan and MK-7 as the predominant menaquinone. In this study, we report two strains, HG645^T and HG711, respectively isolated from surface water of Lake Saroma (Hokkaido, Japan) and from sediment of Nagasaka fishery harbour (Miyagi, Japan). On the basis of phenotypic characteristics, chemotaxonomic data and phylogenetic analysis of the 16S rRNA gene sequence, these isolates represent a novel species of *Sporosarcina*.

Strain HG645^T was isolated by incubation for 1 month at 25 °C on JCM57 medium (JCM online catalogue; [http://](http://www.jcm.riken.go.jp/)

www.jcm.riken.go.jp/). Strain HG711 was isolated by incubation for 1 month at 25 °C on 1/10-diluted marine agar 2216 (BD). Isolates HG645^T and HG711 were routinely cultivated on trypticase soy agar (TSA; BD BBL) containing 50% Herbst's artificial seawater at 25 °C. Herbst's artificial seawater contains the following (per l distilled water): NaCl, 30 g; KCl, 0.7 g; MgSO₄ · 7H₂O, 5.3 g; CaSO₄ · 2H₂O, 1.3 g; and MgCl₂ · 6H₂O, 10.8 g. Cell morphology was observed by scanning electron microscopy (JSM-6700F apparatus; JEOL). Cell motility was examined by the hanging drop method using phase-contrast microscopy (BX60 microscope; Olympus). The temperature for growth was measured over the range 5 to 50 °C. The pH range for growth was assessed at pH 5.0–10.0 at intervals of 0.5 pH units. Growth under anaerobic conditions was determined after 2 weeks of incubation in an AnaeroPack (Mitsubishi Gas Chemical Co., Inc.). Catalase activity was tested by adding a drop of 3% H₂O₂ to a single colony and was recorded as positive when development of bubbles was observed. Oxidase activity was determined by using cytochrome oxidase paper (Nissui Pharmaceutical Co., Inc.). Hydrolysis of casein and starch was tested according to the method of Smibert & Krieg (1994). API 20E and API 50CH

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains HG645^T and HG711 are AB243859 and AB243864.

microtest galleries (bioMérieux) were used to determine physiological and biochemical characteristics according to the manufacturer's instructions. The API strips were incubated for 2 days at 30 °C. The isolates were Gram-positive, endospore-forming, strictly aerobic, motile and rod-shaped. Morphological and physiological characteristics of the isolates are given in the species description. The isolates showed differences from other species of *Sporosarcina* in some physiological properties (Table 1).

Based on partial 16S rRNA gene sequence analyses (Goto *et al.*, 2000, 2002), the strains were grouped into the *Sporosarcina* cluster. However, they were found to be distinct from previously described species of the genus. 16S rRNA gene sequences were determined using a 16S rRNA Gene kit following the protocols of the manufacturer (Applied Biosystems). The 16S rRNA gene sequences of the strains HG645^T and HG711 were used for a BLAST search via NCBI (National Centre for Biotechnology Information). The sequences obtained were aligned using the CLUSTAL W software package (Thompson *et al.*, 1994) and evolutionary distances and K_{nuc} values (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when 1303 bases of the 16S rRNA gene nucleotide were compared. The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987), which was obtained with MEGA3. The topology

of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Similarity values were calculated using MEGA3 (Kumar *et al.*, 2004).

Almost-complete 16S rRNA gene sequences of the strains HG645^T and HG711 shared 100 % similarity. The isolates showed the highest degree of 16S rRNA gene sequence similarity with the type strain of *Sporosarcina aquimarina* (97.3 %), followed by the type strains of *Sporosarcina globispora* (96.9 %), *Sporosarcina psychrophila* (96.8 %), *S. ureae* (96.8 %), *Sporosarcina pasteurii* (96.0 %) and *Sporosarcina macmurdoensis* (95.9 %). The phylogenetic tree indicated that strains HG645^T and HG711 were closely related to the genus *Sporosarcina* (Fig. 1).

Genomic DNA was prepared according to the method of Marmur (1961). DNA–DNA hybridization was performed by the photobiotin-labelling method of Ezaki *et al.* (1989) using a multi-well plate reader (CytoFluoR; PerSeptive Biosystems). The values of DNA–DNA hybridization between strains HG645^T and HG711 were 79–86 %. The two strains should therefore be considered to be members of a single species (Stackebrandt *et al.*, 2002). The level of DNA–DNA relatedness between strain HG645^T and *S. aquimarina* JCM 10887^T was less than 10 %, i.e. much less than the threshold value of 70 % suggested for separate

Table 1. Differential characteristics of *Sporosarcina* species

Species: 1, strains HG645^T and HG711 (*S. saromensis* sp. nov.); 2, *S. ureae* (data from Claus & Fahmy, 1986); 3, *S. globispora* (Nakamura, 1984; Rüger, 1983); 4, *S. psychrophila* (Nakamura, 1984); 5, *S. aquimarina* (Yoon *et al.*, 2001); 6, *S. pasteurii* (Yoon *et al.*, 2001); 7, *S. macmurdoensis* (Reddy *et al.*, 2003). +, Positive; –, negative; NA, data not available.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Rods	Spherical	Rods	Rods	Rods	Spherical	Rods
NaCl tolerance (%)	9	3	5	4	13	10	3
Optimum pH for growth	6.5	7	7	7	6.5–7.5	9	7
Optimum temperature for growth (°C)	27	25	20	25	25	30	20
Presence of:							
Urease	+	+	+	+	+	+	–
Gelatinase	+	–	+	+	+	+	+
Starch hydrolysis	+	–	+	–	–	–	+
Nitrate reduction	–	–	–	+	+	+	+
Acid production from:							
D-Fructose	–	NA	–	+	+	NA	–
D-Galactose	–	NA	–	+	–	NA	–
D-Glucose	–	NA	+	+	–	NA	–
Lactose	–	NA	+	–	–	NA	–
D-Mannitol	–	NA	–	+	–	NA	–
Sucrose	–	NA	+	+	–	NA	–
D-Xylose	–	NA	–	+	–	NA	–
Peptidoglycan type	Lys–Glu	Lys–Gly–Glu	Lys–Glu	Lys–Glu	Lys–Ala–Asp	Lys–Asp	Lys–Glu
Major fatty acid(s)	iso-C _{15:0} ; anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0} ; iso-C _{16:1}
DNA G + C content (mol%)	46	40–42	40	44	40	39	44

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains HG645^T and HG711 and other related taxa. Numbers at nodes indicate percentages of occurrence in 1000 bootstrapped trees; only values greater than 50 % are shown. Bar, 1 substitution per 100 nucleotide positions.

species delineation by Wayne *et al.* (1987). The G+C content of the total DNA was measured by HPLC according to the method described by Mesbah *et al.* (1989). The DNA G+C contents of HG645^T and HG711 were 46.0 and 45.2 mol%, which is slightly higher than those reported for other species of genus *Sporosarcina* (Table 1). Cellular fatty acids of strains HG645^T and HG711 were prepared from cell biomass grown on TSA medium for 48 h at 27 °C and then separated and identified with the Microbial Identification System (MIDI Inc.). The fatty acids (>1 % of the total fatty acids in at least one strain) of strains HG645^T and HG711 were iso-C_{15:0} (49.5/45.6 %, respectively), anteiso-C_{15:0} (33.3/35.2 %), iso-C_{14:0} (5.6/5.3 %), iso-C_{16:0} (4.2/2.1 %), anteiso-C_{17:0} (2.4/3.01 %), C_{16:0} (1.4/0 %), C_{16:0}ω7c alcohol (0.4/2.0 %) and summed feature 4 (0/1.5 %). Summed feature 4 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7t. Analysis of cell-wall peptidoglycan of strains HG645^T and HG711 was carried out by the methods of Schleifer & Kandler (1972); strains HG645^T and HG711 possessed a cell-wall type of Lys–Glu. Respiratory quinone analyses of strains HG645^T and HG711 were performed by the method of Collins & Jones (1981); the major isoprenoid quinone was MK-7. Polar lipids were analysed according to the method of Tindall (1990a, b); the predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine.

On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics, we conclude that strains HG645^T and HG711 belong to a novel species of genus *Sporosarcina*, for which the name *Sporosarcina saromensis* sp. nov. is proposed.

Description of *Sporosarcina saromensis* sp. nov.

Sporosarcina saromensis (sa.ro.men'sis. N.L. fem. adj. *saromensis* pertaining to Lake Saroma, where the type strain was collected).

Cells are Gram-positive, strictly aerobic rods (0.8–1.0 × 2.0–3.2 μm), motile by means of lateral flagella. Spherical endospores are formed in a terminal position. Colonies grown on TSA medium containing 50 % Herbst's artificial seawater are circular, convex and beige. Optimal temperature for growth is 27 °C; growth occurs at 5–40 °C but not

at 45 °C. Optimal pH for growth is 6.5; growth occurs at pH 5.5–9.0. NaCl is not required for growth but can be tolerated up to 9 % (w/v). Tests for catalase and oxidase activities are positive. Starch is hydrolysed but casein is not. H₂S, indole and acetoin are not produced. Nitrate is not reduced. Tests for urease, gelatinase and β-galactosidase activities are positive. Tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and citrate utilization are negative. Acid is not produced from carbohydrates in the API 50CH gallery. The cell wall contains peptidoglycans of the Lys–Glu type. The major isoprenoid quinone system is MK-7. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. Predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The genomic DNA G+C content of the type strain is 46.0 mol%.

The type strain, HG645^T (=MBIC08270^T =IAM 15429^T =KCTC 13119^T), was isolated from surface water in Lake Saroma (Hokkaido, Japan). Strain HG711, isolated from sediment in Nagasuka fishery harbour (Miyagi, Japan), is a reference strain.

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