Sporosarcina koreensis sp. nov. and *Sporosarcina soli* sp. nov., isolated from soil in Korea

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Two Gram-positive, aerobic, spore-forming rods, F73^T and I80^T, were isolated from upland soil. A phylogenetic analysis of 16S rRNA gene sequences placed both isolates within the genus *Sporosarcina*, and showed a sequence similarity of 98.9% between the two strains and a similarity of approximately 94.6–97.3% with respect to *Sporosarcina* species with validly published names. The values for DNA–DNA relatedness between the two isolates and related type strains of the genus *Sporosarcina* were below 28.0%. For both strains, the major cellular fatty acids were anteiso-C_{15:0} and iso-C_{15:0}. In both cases, the cell-wall peptidoglycan was of the A4 α type (L-Lys–D-Glu) and the major menaquinone was MK-7. Diaminopimelic acid was absent from both strains. The genomic DNA G + C contents of strains F73^T and I80^T were 46.5 and 44.5 mol%, respectively. On the basis of the phylogenetic analysis and physiological and chemotaxonomic data, the isolates represent two novel species of the genus *Sporosarcina*, for which the names *Sporosarcina koreensis* sp. nov. (type strain F73^T = KACC 11299^T = DSM 16921^T) and *Sporosarcina soli* sp. nov. (type strain I80^T = KACC 11300^T = DSM 16920^T) are proposed.

The genus *Sporosarcina*, which belongs to the family *Bacillaceae*, was established by Kluyver & van Niel (1936) for strains that have spherical or oval-shaped cells. The first species of this genus to be described was *Sporosarcina ureae*, the type species. The second species, *Sporosarcina halophila*, was described by Claus *et al.* (1983) but was reclassified within the newly described genus *Halobacillus* as *Halobacillus halophilus* (Spring *et al.*, 1996). The species *Sporosarcina globispora*, *Sporosarcina psychrophila* and *Sporosarcina pasteurii* were created by reclassification of *Bacillus globisporus*, *Bacillus psychrophilus* and *Bacillus pasteurii*, respectively, and *Sporosarcina aquimarina*, isolated from seawater, was also added to the genus (Yoon *et al.*, 2001). More recently, *Sporosarcina macmurdoensis*, a

psychrophilic bacterium isolated from a pond in Antarctica, was described by Reddy *et al.* (2003). In this study, we describe two spore-forming bacteria, F73^T and I80^T, that were isolated from soil in Suwon, Korea, and subjected to detailed phylogenetic, phenotypic and chemotaxonomic analyses.

The two strains were isolated from soil samples by using a dilution-plating technique on trypticase soy agar (TSA; BBL) and cultured at 30 °C for 2 days. Yellowish colonies were produced on TSA and on nutrient agar (Difco). Gram staining was performed using a Gram-stain kit (Difco) according to the manufacturer's protocol. After incubation for 2 days on TSA, cells were fixed using a glass slide coated with 0.8% agar (to keep the cells in focus) and observed under a phase-contrast microscope (Zeiss). The following physiological tests were carried out according to Gordon *et al.* (1973) and Claus & Berkeley (1986): catalase and oxidase reactions, Voges–Proskauer test, growth temperature determinations (5–45 °C, using increments of 5 °C), determinations of growth in the presence of NaCl (0, 5, 7, 10 and 15%) and at pH 5.7, formation of acid from

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains F73^T and I80^T are DQ073393 and DQ073394, respectively.

Two-dimensional thin-layer chromatograms of the polar lipids of strains F73^T and I80^T are available as supplementary material with the online version of this paper.

carbohydrates (D-glucose, L-arabinose, D-xylose and D-mannitol), hydrolysis of starch, casein, gelatin and Tween 80, utilization of citrate and propionate, reduction of nitrate, production of indole, deamination of phenylalanine and the urease test. Motility tests were performed on motility medium (0.1 % yeast extract, 0.01 % K₂HPO₄ and 0.2 % agar). Growth under anaerobic conditions was tested by incubation in an anaerobic chamber (BBL) for 2 weeks.

The cells of strains F73^T and I80^T were Gram-positive and rod-shaped, occurring singly or in filamentous chains. Single cells were 0.5–0.7 (F73^T) or 0.7–1.0 (I80^T) µm in diameter and 2.5–3.0 (F73^T) or 2.0–3.0 (I80^T) µm in length. They did not grow anaerobically. In both cases, spores were spherical or oval. However, the cellular positions of the sporangia differed: like spores in described

Sporosarcina species, those of F73^T were positioned terminally, whereas the spores of I80^T were positioned centrally (Table 1). Cells of strain F73^T were motile, but those of strain I80^T were non-motile on the medium used in this study. The growth temperature range for strain F73^T was 15–40 °C, while that for strain I80^T was 15–37 °C. Isolate F73^T grew at pH 6.0–9.0 (optimum, pH 7.0), whereas isolate I80^T grew at pH 7.0–9.0 (optimum, pH 8.0). Strain F73^T grew in the presence of 7 % NaCl whereas I80^T grew in the presence of up to 5 % NaCl. These data indicate that the two isolates are very different physiologically. The two strains were positive for catalase, urease and oxidase and negative for phenylalanine deamination, the formation of indole and dihydroxyacetone, in the Voges–Proskauer test, for acid production from D-glucose, L-arabinose, D-xylose and D-mannitol, and

Table 1. Differential phenotypic and physiological characteristics of strains F73^T and I80^T and type strains of *Sporosarcina* species

Taxa: 1, strain F73^T; 2, strain I80^T; 3, *S. macmurdoensis* (data from Reddy *et al.*, 2003); 4, *S. aquimarina* (Yoon *et al.*, 2001); 5, *S. globispora* (Nakamura, 1984; Rüger, 1983); 6, *S. psychrophila* (Nakamura, 1984); 7, *S. pasteurii* (Yoon *et al.*, 2001); 8, *S. ureae* (Claus & Fahmy, 1986). All of the taxa shown are positive for catalase activity. +, Positive; –, negative; w, weak; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8
Colony colour*	LO	LO	WH	LO	WH	WH	WH	WH
Cell shape†	R	R	R	R	R	R	S	S
Spore position‡	T	C	ST	T	NA	T	T	NA
Motility	+	–	–	+	+	+	+	+
NaCl (%) tolerance	7	5	3	13	5	2	10	3
Optimum pH	7	8	7	6.5–7.0	7	7	9	7
Growth at:								
pH 5.7	–	–	–	+	–	–	–	–
5 and 10 °C	–	–	+	+	+	+	NA	NA
30 °C	+	+	–	+	+	w	+	+
37 °C	+	+	–	+	–	–	+	w
40 °C	+	–	–	–	–	–	w	–
Optimum growth temperature (°C)	30	30	20	25	25	20	30	25
Anaerobic growth	–	–	+	+	+	–	+	–
Presence of:								
Oxidase	+	+	–	+	+	+	NA	+
Urease	+	+	–	+	+	+	+	+
Hydrolysis of:								
Casein	–	–	NA	–	–	w	w	–
Gelatin	+	–	+	+	+	+	+	–
Starch	–	–	+	–	–	w	–	–
Tween 80	–	–	NA	–	–	NA	NA	w
Tyrosine	–	–	NA	+	NA	–	NA	+
Nitrate reduction	–	+	–	+	+	–	+	+
Deamination of phenylalanine	–	+	NA	NA	NA	+	NA	w
Acid production from:								
D-Glucose	–	–	–	–	+	+	NA	–
D-Mannitol	–	–	–	–	+	–	NA	NA
D-Xylose	–	–	–	–	+	–	NA	–
DNA G + C content (mol%)	46.5	44.5	44	40	40	44.1	39	40–41.5§

*LO, Light orange; WH, white.

†R, Rod; S, spherical.

‡C, Central; ST, subterminal; T, terminal.

§Data for 11 strains.

for hydrolysis of starch, casein and Tween 80. The detailed results of the physiological characterizations are given in the species descriptions; features that serve to distinguish between the two strains and related *Sporosarcina* species are listed in Table 1.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene have been described previously (Song *et al.*, 2004). The 16S rRNA gene sequences were analysed using an Applied Biosystems DNA sequencer (ABI 3100). Phylogenetic analyses were performed by using MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data by CLUSTAL W (Thompson *et al.*, 1994). Distances were obtained using Kimura's two-parameter model (Kimura, 1980) and clustering was performed using the neighbour-joining algorithm (Saitou & Nei, 1987). The stability of relationships was evaluated by performing a bootstrap analysis based on 1000 resamplings. The phylogenetic tree indicated that our isolates belong to the genus *Sporosarcina* (at a high bootstrap value) (Fig. 1). Strain F73^T showed the highest sequence similarity with strain I80^T (98.9%), followed by the type strains of *S. globispora* (97.3%), *S. aquimarina* (97.2%) and *S. psychrophila* (97.2%). Strain I80^T showed the closest relationships with strain F73^T (98.9%) and the type strains of *S. globispora* (97.2%), *S. aquimarina* (97.2%) and *S. psychrophila* (96.9%). Lower levels of sequence similarity (<96.0%) were found with species from other genera. Furthermore, five of the *Sporosarcina* type strains plus F73^T and I80^T formed a compact cluster with 97% bootstrap support. Thus, it is clear that these two isolates belong to the genus *Sporosarcina*.

DNA–DNA hybridization was carried out by using the filter hybridization method (Seldin & Dubnau, 1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime system (Roche), and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). The levels of DNA–DNA hybridization between the isolates and related

type strains of *Sporosarcina* species were less than the 70% threshold value suggested by Wayne *et al.* (1987) for separate species delineation. Isolate F73^T showed low values for DNA relatedness with respect to isolate I80^T (28.0%), *S. globispora* DSM 4^T (20.0%), *S. aquimarina* KCCM 41039^T (15.2%) and *S. psychrophila* DSM 3^T (18.5%). Likewise, isolate I80^T showed low values for DNA relatedness with respect to *S. globispora* DSM 4^T (19.3%), *S. aquimarina* KCCM 41039^T (15.2%) and *S. psychrophila* DSM 3^T (10.0%).

Analysis of the peptidoglycan structure was carried out as described previously (Groth *et al.*, 1996; Schleifer & Kandler, 1972). Respiratory lipoquinones and polar lipids were extracted from freeze-dried cell material (100 mg) by using a two-stage method (Tindall, 1990a, b). Biomass for most of the chemotaxonomic studies was prepared following growth of the isolates and standard strains in shake flasks of trypticase soy broth for 3 days at 30 °C; after a purity check, biomass was harvested by centrifugation, washed twice in distilled water and freeze-dried. Whole-cell fatty acid profiles were determined using the Microbial Identification system (MIDI; Microbial ID), from biomass grown on TSA for 2 days at 30 °C. The G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989).

The peptidoglycan of the two isolates contained lysine, glutamic acid, ornithine and alanine [i.e. a variant of the A4 α type, with L-Lys–D-Glu (A11.33; <http://www.dsmz.de/species/murein.htm>), as described by Schleifer & Kandler (1972)]. Neither strain contained diaminopimelic acid as the diagnostic amino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone was an unsaturated menaquinone with seven isoprene units (MK-7). The polar lipids of strain F73^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified phospholipid, an unidentified aminolipid and two unidentified aminophospholipids. Meanwhile, the polar lipids of strain I80^T were diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid (see

Fig. 1. Phylogenetic tree showing the relationship between isolates F73^T and I80^T and other strains of the genera *Sporosarcina* and *Bacillus* and related genera. The tree was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987). Bootstrap percentages (based on 1000 replications) above 50% are shown. Bar, 1 nucleotide substitution per 100 nt.

Supplementary Fig. S1 available in IJSEM Online). The major fatty acids of the two isolates were anteiso-C_{15:0}, iso-C_{15:0} and iso-C_{14:0}, being similar to those present in species of the genus *Sporosarcina*. However, the proportion of iso-C_{15:0} was much higher in the isolates, differentiating them from recognized species in the genus *Sporosarcina* (Table 2). The G+C contents of the DNAs of strains F73^T and I80^T were 44.5 and 46.5 mol%, which are slightly higher than those of *Sporosarcina* species (Table 1). These chemotaxonomic characteristics, together with the morphological features, also support the affiliation of the isolates to the genus *Sporosarcina*.

In conclusion, on the basis of the results from this polyphasic study, strains F73^T and I80^T represent two novel species of the genus *Sporosarcina*, for which the names

Table 2. Comparison of the cellular fatty acids of strains F73^T and I80^T and type strains of members of the genus *Sporosarcina*

Strains: 1, F73^T; 2, I80^T; 3, *S. macmurdoensis* DSM 15428^T; 4, *S. aquimarina* KCCM 41039^T; 5, *S. globispora* DSM 4^T; 6, *S. psychrophila* KCTC 3446^T; 7, *S. pasteurii* KCTC 3558^T; 8, *S. ureae* DSM 2281^T. Data are from Yoon *et al.* (2001), Reddy *et al.* (2003) and this study. Only fatty acids accounting for at least 0.5% of total fatty acid content are listed.

Fatty acid	1	2	3	4	5	6	7	8
Saturated fatty acids								
C _{13:0}	—	—	0.5	—	—	—	—	—
C _{14:0}	0.8	2.1	0.5	1.9	0.6	0.9	1.1	0.8
C _{15:0}	—	—	1.1	—	0.4	0.5	3.3	0.7
C _{16:0}	1.1	3.6	0.7	3.8	0.9	1.5	4.7	2.1
C _{18:0}	—	—	—	0.5	—	0.2	—	0.6
Unsaturated fatty acids								
C _{15:1}	—	—	1.5	—	—	—	—	—
C _{16:1} ω7c	—	—	1.1	—	—	—	—	—
C _{16:1} ω7c alcohol	1.2	—	—	0.2	5.5	2.4	2.8	1.4
C _{16:1} ω11c	0.7	0.6	—	0.6	3.1	1.8	3.2	2.1
C _{18:1} ω9c	—	—	—	0.4	—	0.3	0.8	0.5
Branched fatty acids								
anteiso-C _{13:0}	—	—	—	0.3	—	0.1	—	0.7
iso-C _{14:0}	6.5	6.1	11.1	3.6	3.4	4.1	15.4	2.1
iso-C _{15:0}	44.4	36.8	4.0	5.4	4.0	6.4	6.9	7.0
anteiso-C _{15:0}	38.1	44.2	37.4	77.3	61.8	68.4	48.6	68.7
iso-C _{16:0}	3.4	2.7	3.2	1.9	1.5	2.1	7.5	1.1
iso-C _{16:1}	—	—	22.6	—	—	—	—	—
iso-C _{17:0}	0.7	0.9	3.0	—	—	0.2	—	0.5
anteiso-C _{17:0}	1.9	2.2	13.0	4.1	6.9	6.6	4.0	8.8
Summed feature 4*	0.5	—	—	—	10.6	3.9	0.7	2.5
Unknown fatty acid	—	—	—	—	0.7	0.5	—	—

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Sporosarcina koreensis sp. nov. and *Sporosarcina soli* sp. nov., respectively, are proposed.

Description of *Sporosarcina koreensis* sp. nov.

Sporosarcina koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* referring to Korea, where the isolates were collected).

Cells are Gram-positive, aerobic, motile, spore-forming rods (0.5–0.7 × 2.5–3.0 μm) that occur singly or in short chains. Endospores are mainly oval and positioned terminally in swollen sporangia. Light-orange-coloured colonies are formed after incubation for 2 days on nutrient agar or TSA at 30 °C. Growth occurs at temperatures ranging from 15 to 40 °C, with optimum growth occurring at 30 °C. Growth does not occur in the presence of >7% NaCl. The pH range for growth is 6.0–9.0, the optimum being pH 7.0; growth is not observed at pH 5.7 or 10.0. Positive for catalase, oxidase, urease and gelatinase. Negative for anaerobic growth, formation of indole and dihydroxyacetone, in the Voges–Proskauer test, for phenylalanine deamination, nitrate reduction, acid production from D-glucose, L-arabinose, D-xylose and D-mannitol and for hydrolysis of starch, casein and Tween 80. Does not utilize citrate or propionate. The peptidoglycan is of the L-Lys-D-Glu type (variation A4α). The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. The major menaquinone is MK-7. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified phospholipid and two unidentified aminophospholipids. The G+C content of the DNA is 46.5 mol%.

The type strain, F73^T (=KACC 11299^T =DSM 16921^T), was isolated from upland soil in Suwon, Korea.

Description of *Sporosarcina soli* sp. nov.

Sporosarcina soli (so'li. L. neut. gen. n. *soli* of soil, the source of the organism).

Cells are Gram-positive, aerobic, non-motile, spore-forming rods (0.7–1.0 × 2.0–3.0 μm) that occur singly or in pairs and occasionally in short chains. Endospores are round and are positioned centrally in non-swollen sporangia. Light-orange-coloured colonies are formed after incubation for 2 days on nutrient agar or TSA at 30 °C. Growth occurs at temperatures ranging from 15 to 37 °C, with optimum growth occurring at 30 °C. Growth does not occur in the presence of >5% NaCl. The pH range for growth is 7.0–9.0, the optimum being pH 8.0; growth is not observed at pH 5.7 or 10.0. Positive for catalase, oxidase, urease, phenylalanine deamination and nitrate reduction. Negative for anaerobic growth, formation of indole and dihydroxyacetone, in the Voges–Proskauer test, for acid production from D-glucose, L-arabinose, D-xylose and D-mannitol and for hydrolysis of starch, casein, gelatin and Tween 80. Does not utilize citrate or propionate. The peptidoglycan is of the L-Lys-D-Glu type (variation A4α). The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0}. The major menaquinone is MK-7. The major polar

lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid. The G+C content of the DNA is 44.5 mol%.

The type strain, I80^T (=KACC 11300^T =DSM 16920^T), was isolated from upland soil in Suwon, Korea.

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