J. Gen. Appl. Microbiol., **55**, 217–223 (2009)

Full Paper

Sporosarcina luteola sp. nov. isolated from soy sauce production equipment in Japan

Tatsuya Tominaga,[1,*] Sun-Young An,[2] Hiroshi Oyaizu,[3] and Akira Yokota[2]

[1] *Saitama Industrial Technology Center North Institute, Kumagaya, Saitama 360–0031, Japan*
[2] *Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–0032, Japan*
[3] *Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo 113–8657, Japan*

(Received January 19, 2009; Accepted March 6, 2009)

A Gram-variable, spore-forming, motile rod, designated strain Y1^T, was isolated from the hopper surface of equipment used for soy sauce production. Phylogenetic analysis based on 16S rRNA gene sequence revealed that Y1^T is affiliated phylogenetically to the genus *Sporosarcina*, and the strain showed sequence similarities of 95.8–99.2% to those of *Sporosarcina* species with validly published names. The values of DNA-DNA relatedness between strain Y1^T and related type strains of the genus *Sporosarcina* were below 27%. The major cellular fatty acids were iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The cell-wall peptidoglycan was of the A4α type (Lys-Glu) and the major isoprenoid quinone was MK-7. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. The genomic DNA G+C content of the strain was 43.6 mol%. On the basis of phylogenetic analysis and physiological and chemotaxonomic data, the isolate represents a novel species of the genus *Sporosarcina*, for which the name *Sporosarcina luteola* sp. nov. is proposed. The type strain is strain Y1^T (=JCM 15791^T=NRRL B-59180^T =NBRC 105378^T=CIP 109917^T=NCIMB 14541^T).

Key Words——soy sauce; spore; *Sporosarcina luteola* gen. nov., sp. nov.

Introduction

Soy sauce is a traditional fermented seasoning. Steamed soybeans, roasted wheat, and mold starter (*Aspergillus oryzae* or *Aspergillus soja*) are mixed and fermented for 2 days. Next, the fermenting material (termed *koji*) is mixed with salt water and matured for more than a year. If bacterial spore contaminants are present when the soybeans and wheat are mixed, such spores germinate and proliferate, resulting in inhibition of *Aspergillus* growth and spoiling of the fermentation (Takazane et al., 1998). Bacterial spores are resistant to dried conditions and routine cleaning detergents, and thus survive on the surface of soy sauce production equipment. However, the nature of spore-forming bacteria contaminating such equipment is unknown. Therefore, we isolated spore-forming bacteria from various items of equipment and investigated bacterial distribution, with the aim of effectively preventing such contamination. In our research, an unknown *Sporosarcina* strain was isolated from the surface of a hopper (an item of equipment used to receive steamed soybeans from the factory autoclave).

The genus *Sporosarcina*, which belongs to the family *Bacillaceae*, was proposed by Kluyver and van Niel (1936) to accommodate bacteria that have spherical or oval-shaped cells and contain MK-7 as the major menaquinone. Recently, *S. antarctica*, isolated from

* Address reprint requests to: Tatsuya Tominaga, Saitama Industrial Technology Center North Institute, 2–133 Suehiro, Kumagaya, Saitama 360–0031, Japan.
Tel: +81(485)210614 Fax: +81(485)256052
E-mail: tatsuyatominaga@gmail.com

soil samples in the Antarctic, was reported by Yu et al. (2008), so the genus *Sporosarcina* now contains 10 recognized species; *Sporosarcina ureae* (Claus and Fahmy, 1986; Claus et al., 1983), *S. globispora* (Larkin and Stokes, 1967; Yoon et al., 2001), *S. psychrophila* (Nakamura, 1984; Yoon et al., 2001), *S. pasteurii* (Chester, 1898; Yoon et al., 2001), *S. aquimarina* (Yoon et al., 2001), *S. macmurdoensis* (Reddy et al., 2003), *S. koreensis* and *S. soli* (Kwon et al., 2007) and *S. saromensis* (An et al., 2007).

Here, we report on the characterization of a novel bacterium of the genus *Sporosarcina*, isolated from a hopper used in the soy sauce production process.

Materials and Methods

Bacterial strains and isolation. The surfaces of soy sauce production equipment were swabbed with cotton-tipped swabs (Fukifuki-check, Eiken Chemical, Tokyo, Japan). Swabbed samples were incubated for 10 min at 80°C, and spread on Brain Heart Infusion (BHI) (Oxoid, Hampshire, United Kingdom) agar. After 1 day incubation at 30°C, strain Y1^T was isolated from the grown colonies.

Physiological and biochemical test. The cell morphology of strain Y1^T was examined using a BX50F4 microscope (Olympus, Tokyo, Japan). Colony morphology was observed following incubation on LB agar (Becton Dickinson, MD, USA) at 30°C. Gram-staining was performed using Favor G "Nissui" (Nissui Pharmaceutical, Tokyo, Japan). Growth temperature was varied between 5−45°C at 5°C intervals. Growth in the presence of NaCl was examined in BHI broth containing NaCl at 0, 5, 7.5 or 10% (all w/v). The pH range for growth was determined using BHI broth adjusted to various pH values with HCl or NaOH. Anaerobic growth was investigated by use of an AnaeroPack (Mitsubishi Gas Chemical, Tokyo, Japan) on LB agar at 30°C. The motility was observed on BHI agar by microscope. General physiological tests were achieved using conventional methods (Barrow and Feltham, 1993). API strips (API 20E and API 50CHB; bioMérieux, Lyon, France) were used, according to the manufacturer's instructions, to determine the physiological and biochemical characteristics of strain Y1^T. The API strips were recorded after 1 day at 30°C.

16S rRNA gene sequencing, phylogenetic analysis and DNA-DNA hybridization. Genomic DNA was isolated using an ISOPLANT kit (Nippongene, Tokyo, Japan), according to the manufacturer's instructions. The 16S rRNA gene was sequenced as described previously (Klijn et al., 1991; Mori et al., 1997). Multiple alignment was performed using CLUSTAL W (Thompson et al., 1994) followed by a phylogenetic tree construction using Kimura's two-parameter model (Kimura, 1980). Clustering was performed employing the neighbor-joining algorithm (Saitou and Nei, 1987) implemented in the program MEGA version 4.0 (Tamura et al., 2007). Resultant tree topologies were evaluated by a bootstrap analysis based on 1,000 resamplings. DNA-DNA hybridization was performed using the photobiotin-labeling method (Ezaki et al., 1989) and employing a multi-well plate reader (Cytofluof; PerSeptive Biosystems, MA, USA).

Chemotaxonomic investigation. Respiratory quinones were extracted and purified according to a published protocol (Nishijima et al., 1997), and were analyzed by HPLC (Waters 600 series; Waters, MA, USA). Cell-wall peptidoglycan was prepared and analyzed by HPLC (Waters). For analysis of fatty acid methyl esters, strain Y1^T was harvested from Trypticase Soy (Becton Dickinson (BD), NJ, USA) plates after incubation at 27°C for 2 days. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System Version 5.0 (MIDI, DE, USA). Polar lipids were analyzed as described previously (Tindall, 1990a, b). G+C content of DNA was determined by HPLC (Mesbah et al., 1989).

Nucleotide sequence accession numbers. The nucleotide sequence determined in this study has been deposited with DDBJ under the accession number AB473560.

Results and Discussion

Phylogenetic analysis

Pairwise analysis revealed that strain Y1^T showed the highest 16S rRNA gene sequence similarity with the type strain of *S. koreensis* (99.2%), followed by the type strains of *S. saromensis* (99.1%), *S. soli* (98.2%), *S. aquimarina* (97.8%), *S. psychrophila* (97.4%), *S. globispora* (97.3%), *S. ureae* (96.9%), *S. pasteurii* (96.2%), *S. macmurdoensis* (95.9%) and *S. antarctica* (95.8%). On the other hand, 16S rRNA gene sequence similarities of less than 96.0% were obtained with species of other related genera. The phylogenetic tree showed that strain Y1^T was positioned in the genus *Sporosarci-*

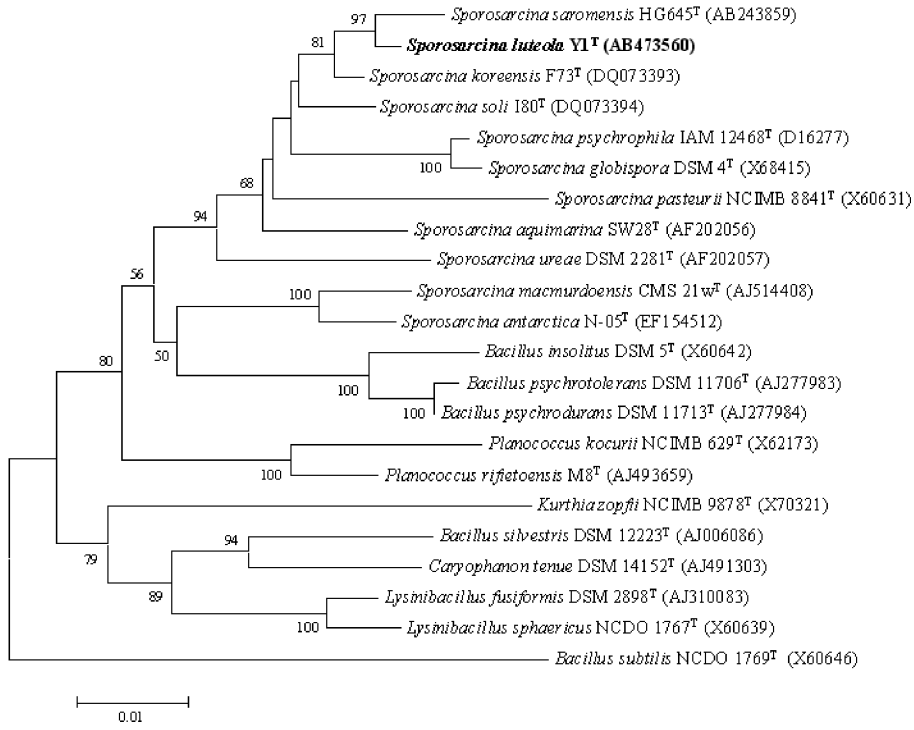

Fig.1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain Y1^T and other related taxa.

Alignment gaps and ambiguous bases were not taken into consideration so that 1,271 bases of the 16S rRNA gene nucleotide were compared. Numbers at nodes represent levels of bootstrap support (%) based on 1,000 resampled datasets. Bootstrap percentages more than 50% are shown. DDBJ accession numbers are given in parentheses. Bar, 1% sequence divergence.

na (Fig. 1). Strain Y1^T and *S. ureae* formed a cluster with 94% bootstrap support, indicating that strain Y1^T is a member of the genus *Sporosarcina*.

Strain Y1^T, *S. saromensis* JCM 23205^T, and *S. koreensis* DSM 16921^T formed a cluster with 81% bootstrap support indicating that DNA relatedness between Y1^T and the other two strains should be compared. The levels of DNA-DNA relatedness between Y1^T and *S. saromensis* JCM 23205^T (27%), *S. koreensis* DSM 16921^T (24%), and *S. soli* 16920^T (17%), were all much less than the 70% threshold value suggested by Wayne et al. (1987) for species differentiation. Therefore, strain Y1^T is suggested to be a novel species distinct from other *Sporosarcina* species.

Morphological, physiological and biochemical analysis

Morphological, physiological, and biochemical characteristics of strain Y1^T are given in the species description below or are shown in Table 1. Strain Y1^T could grow under anaerobic conditions, showed no urease activity, and could produce acid from D-glucose; these are differences between strain Y1^T on the one hand, and *S. saromensis*, *S. koreensis,* and *S. soli*, on the other hand; the latter three species are those most closely related to strain Y1^T. In addition, strain Y1^T produced acid from glycerol, D-ribose, D-galactose, D-glucose, D-fructose, dulcitol, α-methyl-D-glucoside, maltose, and L-fucose, whereas *S. saromensis* produced no acid from any of the sugars in the API 50CHB gallery (An et al., 2007). These data established that strain Y1^T showed phenotypic characteristics distinct from those of *S. saromensis*, *S. koreensis,* and *S. soli*.

Chemotaxonomic analysis

Strain Y1^T contained MK-7 as the major menaquinone, in agreement with other *Sporosarcina* species. The peptidoglycan contained lysine, glutamic acid, and alanine at a ratio of 1 : 2.5 : 2.2, which is of the Lys-Glu type (variation A4α) (Schleifer and Kandler, 1972). The predominant cellular fatty acids of strain Y1^T were iso-$C_{15:0}$ and anteiso-$C_{15:0}$, as for *S. saromensis*, *S. koreensis*, and *S. soli*, the closely related

Table 1. Differential phenotypic characteristics between strain Y1^T and recognized *Sporosarcina* species.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Cell size (μm)	0.7–0.8× 1.5–2.5	0.8–1.0× 2.0–3.2	0.5–0.7× 2.5–3.0	0.7–1.0× 2.0–3.0	NA	0.9–1.2× 2.0–3.5	0.9–1.0× 2.5–4.0	NA	NA	NA	0.3–0.6× 1.1–2.6
Colony color[a]	Y	B	LO	LO	WH	LO	WH	WH	WH	B	LY
Cell shape[b]	R	R	R	R	R	R	R	R	S	S	R
Spore position[c]	T	T	T	C	ST	T	ST, T	T	T	NA	NA
Motility	+	+	+	−	−	+	+	+	+	+	−
NaCl (%) tolerance	7.5	9	7	5	3	13	5	2	10	7.5	9
Optimum pH	7	6.5	7	8	7	6.5–7.0	7	7	9	8.8	6.0–8.0
Growth at:											
pH 5.7	−	+	−	−	−	+	−	−	−	−	+
5°C	−	+	−	−	+	+	+	+	NA	NA	+
40°C	w	+	+	−	−	−	−	−	w	−	−
Growth temperature range (°C)	10–40	5–40	15–40	15–37	4–25	4–37	NA	NA	NA	10–37	0–23
Optimum growth temperature (°C)	30	27	30	30	18–20	25	25	20	30	25	17–18
Anaerobic growth	+	−	−	−	+	+	+	−	+	−	+
Oxidase	+	+	+	+	−	+	+	+	NA	−	+
Catalase	+	+	+	+	+	+	+	NA	NA	+	+
Urease	−	+	+	+	−	+	+	+	+	+	−
Nitrate reduction	+	−	−	+	−	+	+	−	+	+	−
Deamination of phenylalanine	−	NA	−	+	NA	NA	−	+	NA	w	NA
Hydrolysis of:											
Casein	−	−	−	−	NA	−	−	w	w	−	−
Gelatin	+	+	+	−	+	+	+	+	+	−	−
Starch	−	+	−	−	+	−	−	w	−	−	−
Tween 80	+	NA	−	−	NA	−	−	NA	NA	w	NA
Acid production from:											
D-Glucose	+	−	−	−	−	−	+	+	NA	NA	−
D-Xylose	−	−	−	−	−	−	−	+	NA	NA	−
D-Fructose	+	−	NA	NA	−	+	−	+	NA	NA	−
D-Galactose	+	−	NA	NA	−	−	−	+	NA	NA	−
D-Mannitol	−	−	−	−	−	−	−	+	NA	NA	−
DNA G+C content (mol%)	43.6	46.0	46.5	44.5	44	40	40	44.1	39	40–41.5	39.2

Strains: 1, Y1^T (*Sporosarcina luteola* sp. nov.); 2, *S. saromensis* HG645^T (data from An et al., 2007); 3, *S. koreensis* F73^T (Kwon et al., 2007); 4, *S. soli* I80^T (Kwon et al., 2007); 5, *S. macmurdoensis* CMS 21w^T (Reddy et al., 2003); 6, *S. aquimarina* SW28^T (Yoon et al., 2001); 7, *S. globispora* DSM 4^T (Nakamura, 1984; Rüger, 1983); 8, *S. psychrophila* IAM 12468^T (Nakamura, 1984); 9, *S. pasteurii* NCIMB 8841^T (Yoon et al., 2001); 10, *S. ureae* DSM 2281^T (Claus and Fahmy, 1986); 11, *S. antarctica* N-05 ^{T} (Yu et al., 2008). +, Positive; −, negative; w, weak; NA, no data available. [a]LO, Light orange; WH, white; Y, yellow; B, beige; LY, light yellow. [b]R, Rod; S, spherical. [c]C, Central; ST, subterminal; T, terminal.

Table 2. Comparison of the cellular fatty acids of strain Y1^T and type strains of the genus *Sporosarcina*.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids											
$C_{13:0}$	–	–	–	–	0.5	–	–	–	–	–	–
$C_{14:0}$	0.8	–	0.8	2.1	0.5	1.9	0.6	0.9	1.1	0.8	–
$C_{15:0}$	0.5	–	–	–	1.1	–	0.4	0.5	3.3	0.7	–
$C_{16:0}$	1.1	1.4	1.1	3.6	0.7	3.8	0.9	1.5	4.7	2.1	–
$C_{18:0}$	0.2	–	–	–	–	0.5	–	0.2	–	0.6	–
Unsaturated fatty acids											
$C_{15:1}$	–	–	–	–	1.5	–	–	–	–	–	–
$C_{16:1}\omega7c$	–	–	–	–	1.1	–	–	–	–	–	–
$C_{16:1}\omega7c$ alcohol	1.9	0.4	1.2	–	–	0.2	5.5	2.4	2.8	1.4	18.89
$C_{16:1}\omega11c$	0.7	–	0.7	0.6	–	0.6	3.1	1.8	3.2	2.1	3.67
$C_{18:1}\omega9c$	–	–	–	–	–	0.4	–	0.3	0.8	0.5	–
Branched fatty acids											
iso-$C_{13:0}$	0.2	–	–	–	–	–	–	–	–	–	–
anteiso-$C_{13:0}$	–	–	–	–	–	0.3	–	0.1	–	0.7	–
iso-$C_{14:0}$	9.0	5.6	6.5	6.1	11.1	3.6	3.4	4.1	15.4	2.1	7.55
iso-$C_{15:0}$	45.6	49.5	44.4	36.8	4.0	5.4	4.0	6.4	6.9	7.0	–
anteiso-$C_{15:0}$	31.8	33.3	38.1	44.2	37.4	77.3	61.8	68.4	48.6	68.7	39.75
iso-$C_{16:0}$	4.5	4.2	3.4	2.7	3.2	1.9	1.5	2.1	7.5	1.1	7.01
iso-$C_{16:1}$	–	–	–	–	22.6	–	–	–	–	–	–
iso-$C_{17:0}$	1.1	–	0.7	0.9	3.0	–	–	0.2	–	0.5	–
anteiso-$C_{17:0}$	2.1	2.4	1.9	2.2	13.0	4.1	6.9	6.6	4.0	8.8	4.23
iso-$C_{17:1}\omega10c$	0.4	–	–	–	–	–	–	–	–	–	–
Summed feature 4[a]	0.4	0.0	0.5	–	–	–	10.6	3.9	0.7	2.5	11.91
Unkown fatty acid	–	–	–	–	–	–	0.7	0.5	–	–	–

Strains: 1, Y1^T (*Sporosarcina luteola* sp. nov.); 2, *S. saromensis* HG645^T; 3, *S. koreensis* F73^T; 4, *S. soli* I80^T; 5, *S. macmurdoensis* CMS 21w^T; 6, *S. aquimarina* SW28^T; 7, *S. globispora* DSM 4^T; 8, *S. psychrophila* IAM 12468^T; 9, *S. pasteurii* NCIMB 8841^T; 10, *S. ureae* DSM 2281^T; 11, *S. antarctica* N-05 ^{T}. Data are from Yoon et al. (2001), Reddy et al. (2003), Kwon et al. (2007), An et al. (2007), Yu et al. (2008) and this study. [a]Summed feature 4: iso-$C_{17:1}$ I + anteiso-$C_{17:1}$ B.

species (An et al., 2007; Kwon et al., 2007). The fatty acid profiles of strain Y1^T and the other recognized *Sporosarcina* species are detailed in Table 2. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, as for *S. saromensis* and *S. koreensis* (An et al., 2007; Kwon et al., 2007). The DNA G+C content of strain Y1^T was 43.6 mol%.

Based on the phenotypic, phylogenetic, and genomic evidence presented, strain Y1^T is considered to represent a novel species of the genus *Sporosarcina*, for which the name *Sporosarcina luteola* sp. nov. is proposed.

Description of *Sporosarcina luteola* sp. nov.

Sporosarcina luteola (lu.te.o' la. L. fem. adj. *luteola* associated with the colony color seen on LB agar).

Cells are Gram-variable, spore-forming, motile rods, 0.7–0.8×1.5–2.5 μm in size. Colonies formed on LB agar are yellow, smooth, circular, and convex, with en-

tire margins after 48 h incubation at 30°C. Endospores are positioned terminally. Produces catalase and oxidase. Growth occurs under anaerobic conditions on LB medium. Growth occurs at 10–40°C and pH 6.0–10.0, with optimum growth at 25–30°C and approximately pH 7.0. Growth occurs in the presence of 0–7.5% (w/v) NaCl. Nitrate is reduced and hydrogen sulfide is not produced. Negative for indole production and the Voges-Proskauer reaction. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, β-galactosidase, tryptophan deaminase, phenylalanine deaminase, DNase, citrate utilization and hydrolysis of casein, Tween 20 and Tween 40. Positive for gelatinase and for hydrolysis of Tween 60 and Tween 80. Acid is produced from glycerol, D-ribose, D-galactose, D-glucose, D-fructose, dulcitol, α-methyl-D-glucoside, maltose and L-fucose, but not from the other carbohydrates of the API 50CHB test suite. The major isoprenoid quinone is MK-7. The cell wall contains peptidoglycans of the Lys-Glu type (variation A4α). The predominant cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content of the type strain is 43.6 mol%.

The type strain is strain Y1^T (=JCM 15791^T=NRRL B-59180^T=NBRC 105378^T=CIP 109917^T=NCIMB 14541^T), which was isolated from the surface of soy sauce production equipment in Japan.

Acknowledgments

We would like to thank Mr. Y. Okuzawa for useful discussions.

References

An, S.-Y., Haga, T., Kasai, H., Goto, K., and Yokota, A. (2007) *Sporosarcina saromensis* sp. nov., an aerobic endospore-forming bacterium. *Int. J. Syst. Evol. Microbiol.*, **57**, 1868–1871.

Barrow, G. I. and Feltham, R. K. A. (1993) Cowan and Steel's Manual for the Identification of Medical Bacteria, 3rd ed., Cambridge University Press, Cambridge.

Chester, F. D. (1898) Report of the mycologist: Bacteriological work. *Del. Agric. Exp. Stn. Annu. Rep.*, **10**, 47–137.

Claus, D. and Fahmy, F. (1986) Genus *Sporosarcina* Kluyver and van Niel 1936, 401^{AL}. *In* Bergey's Manual of Systematic Bacteriology, Vol. 2, ed. by Sneath, P. H. A., Mair, N. S., Sharpe, M. E., and Holt, J. G., Williams & Wilkins, Baltimore, pp. 1202–1206.

Claus, D., Fahmy, F., Rolf, H. J., and Tosunoglu, N. (1983) *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst. Appl. Microbiol.*, **4**, 496–506.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.*, **39**, 224–229.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**, 111–120.

Klijn, N., Weerkamp, A. H., and de Vos, W. M. (1991) Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. *Appl. Environ. Microbiol.*, **57**, 3390–3393.

Kluyver, A. J. and van Niel, C. B. (1936) Prospects for a natural classification of bacteria. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. II*, **94**, 369–403.

Kwon, S.-W., Kim, B.-Y., Song, J., Weon, H.-Y., Schumann, P., Tindall, B. J., Stackebrandt, E., and Fritze, D. (2007) *Sporosarcina koreensis* sp. nov. and *Sporosarcina soli* sp. nov., isolated from soil in Korea. *Int. J. Syst. Evol. Microbiol.*, **57**, 1694–1698.

Larkin, J. M. and Stokes, J. L. (1967) Taxonomy of psychrophilic strains of *Bacillus*. *J. Bacteriol.*, **94**, 889–895.

Mesbah, M., Premachandran, U., and Whitman, W. B. (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.*, **39**, 159–167.

Mori, K., Yamazaki, K., Ishiyama, T., Katsumata, M., Kobayashi, K., Kawai, Y., Inoue, N., and Shinano, H. (1997) Comparative sequence analyses of the genes coding for 16S rRNA of *Lactobacillus casei*-related taxa. *Int. J. Syst. Bacteriol.*, **47**, 54–57.

Nakamura, L. K. (1984) *Bacillus psychrophilus* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.*, **34**, 121–123.

Nishijima, M., Araki-Sakai, M., and Sano, H. (1997) Identification of isoprenoid quinones by frit-FAB liquid chromatography-mass spectrometry for the chemotaxonomy of microorganisms. *J. Microbiol. Methods*, **28**, 113–122.

Reddy, G. S., Matsumoto, G. I., and Shivaji, S. (2003) *Sporosarcina macmurdoensis* sp. nov., from a cyanobacterial mat sample from a pond in the McMurdo Dry Valleys, Antarctica. *Int. J. Syst. Evol. Microbiol.*, **53**, 1363–1367.

Rüger, H.-J. (1983) Differentiation of *Bacillus globisporus*, *Bacillus marinus* comb. nov., *Bacillus aminovorans*, and *Bacillus insolitus*. *Int. J. Syst. Bacteriol.*, **33**, 157–161.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.

Schleifer, K. H. and Kandler, O. (1972) Peptidoglycan types of

bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.*, **36**, 407–477.

Takazane, S., Endo, T., Shida, O., Tagami, H., Takagi, H., and Kadowaki, K. (1998) Influences of *Bacillus* species in shoyu koji on shoyu brewing (Part 1). *J. Soy Sauce Res. Technol.*, **24**, 77–82.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.*, **24**, 1596–1599.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Tindall, B. J. (1990a) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst. Appl. Microbiol.*, **13**, 128–130.

Tindall, B. J. (1990b) Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol. Lett.*, **66**, 199–202.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P., and Trüper, H. G. (1987) International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.*, **37**, 463–464.

Yoon, J.-H., Lee, K.-C., Weiss, N., Kho, Y. H., Kang, K. H., and Park, Y.-H. (2001) *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of the genus *Sporosarcina*. *Int. J. Syst. Evol. Microbiol.*, **51**, 1079–1086.

Yu, Y., Xin, Y.-H., Liu, H.-C., Chen, B., Sheng, J., Chi, Z.-M., Zhou, P.-J., and Zhang, D.-C. (2008) *Sporosarcina antarctica* sp. nov., a psychrophilic bacterium isolated from the Antarctic. *Int. J. Syst. Evol. Microbiol.*, **58**, 2114–2117.