Sporosarcina newyorkensis sp. nov. from clinical specimens and raw cow's milk

William J. Wolfgang,^{1,2} An Coorevits,^{3,4} Jocelyn A. Cole,¹ Paul De Vos,⁴ Michelle C. Dickinson,¹ George E. Hannett,¹ Reashma Jose,⁵ Elizabeth J. Nazarian,¹ Peter Schumann,⁶ Anita Van Landschoot,^{3,4} Samantha E. Wirth¹ and Kimberlee A. Musser^{1,2}

Correspondence

William J. Wolfgang
wwolfgan@wadsworth.org

¹Wadsworth Center, Bacteriology Laboratory, New York State Department of Health, PO Box 22002, Albany, NY 12201, USA

²Department of Biomedical Sciences, State University of New York, Albany, NY 12201, USA

³Department of Applied Engineering Sciences, Laboratory of Biochemistry and Brewing, University College Ghent, Schoonmeersstraat 52, 9000 Ghent, Belgium

⁴Laboratory of Microbiology (LM-UGent), Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

⁵Department of Biomedical Technology, Albany College of Pharmacy, 106 New Scotland Ave, Albany, NY 12208, USA

⁶DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, 38124 Braunschweig, Germany

Twelve independent isolates of a Gram-positive, endospore-forming rod were recovered from clinical specimens in New York State, USA, and from raw milk in Flanders, Belgium. The 16S rRNA gene sequences for all isolates were identical. The closest species with a validly published name, based on 16S rRNA gene sequence, is *Sporosarcina koreensis* (97.13 % similarity). DNA–DNA hybridization studies demonstrate that the new isolates belong to a species distinct from their nearest phylogenetic neighbours. The partial sequences of the 23S rRNA gene for the novel strains and their nearest neighbours also provide support for the novel species designation. Maximum-likelihood phylogenetic analysis of the 16S rRNA gene sequences confirmed that the new isolates are in the genus *Sporosarcina*. The predominant menaquinone is MK-7, the peptidoglycan has the type A4 α L-Lys–Gly–D-Glu, and the polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The predominant fatty acids are iso-C_{14:0}, iso-C_{15:0} and anteiso-C_{15:0}. In addition, biochemical and morphological analyses support designation of the twelve isolates as representatives of a single new species within the genus *Sporosarcina*, for which the name *Sporosarcina newyorkensis* sp. nov. (type strain 6062^T = DSM 23544^T = CCUG 59649^T = LMG 26022^T) is proposed.

In August 2008, the Wadsworth Center Bacteriology Laboratory, New York, USA, received an isolate from

human blood that was most closely related to *Sporosarcina koreensis* with a 16S rRNA gene sequence similarity of 97.13 % (EzTaxon, Chun *et al.*, 2007). The laboratory subsequently received ten additional isolates, all from different patients residing in New York State, having 100 % 16S rRNA gene sequence similarity to the first strain (strain list, GenBank accession numbers and stock centre accession numbers are listed in Table 1). In addition, strain R-31323 was acquired, which has 100 % 16S rRNA gene sequence similarity to the New York strains, and was isolated from cow's milk in Belgium (Coorevits *et al.*, 2008). Based on the polyphasic study below, we report the identification of twelve strains of a novel species of *Sporosarcina* isolated from human clinical samples and cow's milk.

Abbreviation: CFA, cellular fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the partial 16S and 23S rRNA gene sequences of strain 6062^T are GU994085 and GU994086, respectively. The accession numbers for the partial 23S rRNA gene sequences of *Sporosarcina ureae* DSM 2281^T, *S. koreensis* DSM 16921^T, *S. soli* DSM 16920^T, *S. aquimarina* DSM 14554^T, *S. contaminans* CCUG 53915^T and *S. thermotolerans* CCUG 53480 are HM245314–HM245319, respectively. Additional accession numbers are given in Table 1.

Four supplementary tables and a supplementary figure are available with the online version of this paper.

Table 1. Strain information for *Sporosarcina newyorkensis* sp. nov.

Strain designation	Source	Date isolated	GenBank accession no.	
			16S rRNA	23S rRNA
6062 ^T (=DSM 23544 ^T = CCUG 59649 ^T =LMG 26022 ^T)	Blood, 57-year-old male; Erie County, New York, USA	17 Oct. 2008	GU994085	GU994086
4331 (=DSM 23542 = CCUG 59647 =LMG 26020)	Blood, 28-year-old female; Albany County, New York, USA	5 Aug. 2008	GU994083	GU994084
5868 (=DSM 23543 = CCUG 59650 =LMG 26021)	Eye, 30-year-old female; Erie County, New York, USA	2 Oct. 2008	GU994087	GU994088
1655 (=DSM 23541 = CCUG 59648 =LMG 26023)	Blood, 93-year-old female; Broome County, New York, USA	31 Mar. 2009	GU994079	GU994080
2681 (=DSM 23540 = CCUG 59651 =LMG 26024)	Blood, 87-year-old male; Erie County, New York, USA	5 Jun. 2009	GU994081	GU994082
R-31323 (=DSM 23966 = CCUG 60203 =LMG 26025)	Raw milk, region of Flanders, Belgium	7 Mar. 2005	AM910326	HQ246159
3418	Blood, 48-year-old male; Suffolk County, New York, USA	29 Jun. 2009	HM245297	
4469	Blood, 85-year-old female; Erie County, New York, USA	8 Aug. 2008	HM245298	
57	Blood, female; Albany County, New York, USA	9 Sept. 2008	HM245299	
4984	Blood, 54-year-old female; Albany County, New York, USA	10 Oct. 2009	HM245300	
4974	Blood, 67-year-old male; Nassau County, New York, USA	3 Oct. 2009	HM245302	
5353	Blood, 87-year-old male; Nassau County, New York, USA	1 Nov. 2009	HM245301	

Ten of the twelve strains were isolated from human blood, one from a human eye, and one from cow's milk (Table 1). All were cultured at 37 °C on trypticase soy agar (TSA) plates (Becton Dickinson) supplemented with 5 % sheep blood. For cellular fatty acid (CFA) studies, strains were grown at 28 °C on trypticase soy broth agar plates without sheep blood (TSBA). Strain 6062^T was selected as the type strain and, along with five additional strains, was deposited at the DSMZ, CCUG and BCCM/LMG stock centres (Table 1).

The near full-length sequence of the 16S rRNA gene (1445–1474 nt) for eight of the isolates was determined as previously described (Wolfgang *et al.*, 2011) with the addition of one sequencing primer pF 5'-GAGGAAG-GTGGGGATGACGT-3' (corresponding to *Escherichia coli* positions 1175–1194). For strain R-31323, 16S rRNA gene sequence (1514 nt) was determined as previously described (Coorevits *et al.*, 2008). For the three remaining isolates, the 16S rRNA gene sequence lengths ranged from 435–473 nt and were amplified and sequenced with primers 16S1 and rpD (Wolfgang *et al.*, 2011). Each sequence contained from one to nine polymorphic sites, which were denoted using the IUPAC code. An alignment of all the sequences revealed that polymorphic sites were often conserved. However, when a strain contained a single nucleotide at a polymorphic site, that nucleotide was always concordant with one of the polymorphic residues. Therefore, these sites were considered to be matches. Using these criteria, all sequences had 100 % similarity (for GenBank accession numbers refer to Table 1).

The identification of closely related species was carried out by using the BLAST (Altschul *et al.*, 1997) and megablast (Zhang *et al.*, 2000) programs to compare near full-length 16S rRNA gene sequences (1474 nt) of strain 6062^T against

the database of 16S rRNA gene sequences for type strains with validly published prokaryotic names (Chun *et al.*, 2007). The 50 sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Previous studies indicate that for organisms with greater than 97.0 % 16S rRNA gene sequence similarity, alternative methods must be employed, such as DNA–DNA hybridization, for determining inclusion or exclusion of the organism in closely related species (discussed in Tindall *et al.*, 2010). Therefore, type strains for all organisms with greater than 97.0 % similarity to strain 6062^T (these ranged between 97.07 % and 97.13 % over 1454 to 1465 nt) were acquired to include in the polyphasic analysis. The closest species with validly published names were: *Sporosarcina koreensis* (F73^T, DQ073393) (Kwon *et al.*, 2007) (DSM 16921^T acquired for this study), *Sporosarcina contaminans* (CCUG 53915^T, FN298444) (Kämpfer *et al.*, 2010), *Sporosarcina ureae* (DSM 2281^T, AF202057) (Claus & Fahmy, 1986) and *Sporosarcina aquimarina* (SW28^T, AF202056) (Yoon *et al.*, 2001) (DSM 14554^T acquired for this study). Also included in some of the analyses are the type strains of *Sporosarcina thermotolerans* (CCUG 53480^T, FN298445) (Kämpfer *et al.*, 2010), *Sporosarcina soli* (I80^T, DQ073394) (Kwon *et al.*, 2007) (DSM 16920^T acquired for this study) and *Sporosarcina luteola* (Y1^T, AB473560) (Tominaga *et al.*, 2009) (DSM 23150^T acquired for this study), as they match just below the 97.0 % level of sequence similarity.

In order to support the proposed novel species designation, DNA–DNA hybridization was performed by the DSMZ Identification Service (Braunschweig, Germany) and by us between strain 6062^T and the type strains with a 16S rRNA

gene sequence similarity greater than 97.0 % (Tindall *et al.*, 2010). Spectroscopic DNA–DNA hybridization was performed in duplicate on DNA that was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). For comparisons to strain R-31323, DNA was purified as described by Logan *et al.* (2000) and hybridization was performed using a modification of the microplate method described by Ezaki *et al.* (1989), as described by Willems *et al.* (2001). Hybridization values for strain 6062^T against strain 4331, strain R-31323, *S. koreensis* DSM 16921^T, *S. ureae* DSM 2281^T, *S. aquimarina* DSM 14554^T, *S. soli* DSM 16920^T and *S. contaminans* CCUG 53915^T are shown in Supplementary Table S1 (available in IJSEM Online). Based on a cut-off of 70.0 % hybridization for defining species (Wayne *et al.*, 1987), strain 6062^T belongs to the same species as strains 4331 and R-31323 and furthermore does not belong to any of the other species tested.

To obtain further support for the new species designation, we partially sequenced the 23S rRNA gene for the six isolates deposited at CCUG, DSMZ and BCCM/LMG and six of the most closely related species. Amplification and sequencing were performed as previously described (Wolfgang *et al.*, 2011) except that sequencing primer 1602F was replaced with 1602F' (5'-TACCGCAAACCGACACAGGTA-3'). The 23S rRNA gene sequences for the six novel strains, 6062^T (GU994086), 4331 (GU994084), 5868 (GU994088), 1655 (GU994080), 2681 (GU994082), and R-31323 (HQ246159) harboured two to thirteen polymorphisms. Using the criteria established for 16S rRNA polymorphisms, all polymorphic sites were matches and all six sequences were identical over 2139 nt. By contrast, for the most closely related species, *S. koreensis* (HM245315), *S. ureae* (HM245314), *S. aquimarina* (HM245317), *S. soli* (HM245316), *S. contaminans* (HM245318), and *S. thermotolerans* (HM245319), the 23S rRNA gene sequence similarity ranged from 94.65 % to 97.97 % over 2142 to 2147 nt (see Supplementary Table S2). Thus, 23S rRNA gene sequence analysis provides additional support that the six deposited strains analysed are members of the same species.

CFA analysis was performed on a loop-full of growth from isolates cultured for 24–48 h at 28 °C in air, on TSBA plates. Fatty acid methyl esters were extracted using the Sherlock Microbial Identification System version 4.5 according to the manufacturer's instructions (MIDI) and were identified using an Agilent Technologies 6890N gas chromatograph and method TSBA 50. For the twelve novel strains, the predominant fatty acids were iso-C_{14:0}, iso-C_{15:0} and anteiso-C_{15:0} (Supplementary Table S3). This is consistent with other species of the genus *Sporosarcina*; however, the

relative proportions were significantly different (Table 2). Notably, the novel *Sporosarcina* strains generally have higher levels of iso-C_{14:0} and lower levels of anteiso-C_{15:0}. For the closely related genera *Paenisporosarcina* and *Psychrobacillus*, some but not all of the predominant CFAs are the same (Krishnamurthi *et al.*, 2009, 2010).

Polar lipids of *S. ureae* LMG 17366^T and *S. newyorkensis* sp. nov. strains 6062^T, 4331 and R-31323 were extracted after aerobic growth on TSA (Oxoid) for 24 h at 28 °C. The lipid fraction was subsequently separated by two-dimensional thin-layer chromatography according to Tindall (1990a, b). The total lipid profiles were visualized by spraying with molybdotophosphoric acid and further characterized by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates) and α -naphthol (specific for sugars). For the *S. ureae* and *S. newyorkensis* strains examined, the polar lipid profiles were identical, with diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids, along with minor amounts of two phospholipids (Supplementary Figure S1a, b). Furthermore, this profile is in agreement with other polar lipid profiles described for *S. contaminans* and *S. thermotolerans* (Kämpfer *et al.*, 2010). The closely related genera, *Paenisporosarcina* and *Psychrobacillus* have lipid profiles that overlap with those of the novel strains (Krishnamurthi *et al.*, 2009, 2010).

Isoprenoid quinones were extracted from lyophilized cells of strains 6062^T (cultivated for one day at 37 °C in medium 220, <http://www.dsmz.de>) and R-31323 (cultivated for one day at 37 °C in tryptone soy agar, Oxoid) and analysed by HPLC (Groth *et al.*, 1996). The major menaquinone of both strains was MK-7 (>90 %) with MK-6 (approx. 2 %) found as a minor component. Additionally strain R-31323 contained MK-8 (approx. 1 %). The occurrence of MK-7 as a major menaquinone is a characteristic feature of members of the genus *Sporosarcina* (Yoon *et al.*, 2001).

Peptidoglycan preparations of strains 6062^T and R-31323 were purified according to the method of Schleifer (1985) after disruption of cells by shaking with glass beads and subsequent trypsin digestion. The amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional ascending thin-layer chromatography (2D-TLC) on cellulose plates using previously described methods and solvent systems (Schleifer, 1985). Amino acid enantiomers were determined from the mobilities of the diastereomeric dipeptides on 2D-TLC plates analogously to data reported for paper chromatography by Schleifer (1985). The amino-terminal amino acid of the interpeptide bridge was detected by dinitrophenylation (Schleifer, 1985). The molar ratios of amino acids were determined by gas chromatography (GC 14A, Shimadzu) and gas chromatography-mass spectrometry (320 Singlequad, Varian) of *N*-heptafluorobutyl amino acid isobutyl esters (Groth *et al.*, 1996; MacKenzie, 1987). The hydrolysates (4 M HCl, 100 °C, 16 h) of the peptidoglycan preparations of both strains contained lysine, alanine, glycine and glutamic acid in a molar ratio of

Table 2. Per cent fatty acid composition of strain 6062^T and closely related taxa

Taxa: 1, strain 6062^T; 2, *S. ureae* DSM 2281^T; 3, *S. aquimarina* DSM 14554^T; 4, *S. soli* DSM 16920^T; 5, *S. koreensis* DSM 16921^T; 6, *S. thermotolerans* CCUG 53480^T; 7, *S. contaminans* CCUG 53915^T; 8, *S. luteola* DSM 23150^T; 9, *S. antarctica* N-05^T; 10, *S. globispora* DSM 4^T; 11, *S. psychrophila* KCTC 3446^T; 12, *S. pasteurii* KCTC 3558^T; 13, *S. saromensis* HG645^T. Fatty acid methyl esters for which no values were greater than 0.1 % for any organism have been omitted from the table. The three most prevalent fatty acids for each isolate are in bold text.

Fatty acid	1	2	3	4	5	6	7	8	9 ^{a*}	10 ^b	11 ^b	12 ^b	13 ^c
Saturated													
C _{14:0}	0.6	0.3	2.4	2.5	0.5	9.0	0.4	1.2	—	0.6	0.9	1.1	—
C _{15:0}	—	—	—	—	—	—	—	—	—	0.4	0.5	3.3	—
C _{16:0}	0.8	0.6	3.0	2.2	0.2	10.6	0.5	0.5	1.2	0.9	1.5	4.7	1.4
Unsaturated													
C _{16:1} ω7c alcohol	6.6	2.7	1.0	0.5	3.9	—	3.6	2.2	18.9	5.5	2.4	2.8	—
C _{16:1} ω11c	3.4	3.5	2.4	1.5	1.2	5.6	1.4	1.1	3.7	3.1	1.8	3.2	—
iso-C _{17:1} ω10c	1.6	1.5	—	—	0.5	—	1.3	—	—	—	—	—	—
Branched													
iso-C _{14:0}	26.1	1.8	4.3	5.5	20.9	7.5	4.7	11.0	7.6	3.4	4.1	15.4	5.6
iso-C _{15:0}	32.3	7.6	4.3	45.5	34.6	27.7	55.8	37.6	1.4	4.0	6.4	6.9	49.5
anteiso-C _{15:0}	15.2	62.7	76.8	37.7	30.8	39.6	23.5	41.1	39.8	61.8	68.4	48.6	33.3
iso-C _{16:0}	10.0	0.6	1.6	1.1	4.4	—	3.6	2.6	7.0	1.5	2.1	7.5	4.2
iso-C _{16:1}	—	—	—	—	—	—	—	—	1.3	—	—	—	—
iso-C _{17:0}	1.3	0.5	—	1.3	0.4	—	1.1	0.4	—	—	0.2	—	—
anteiso-C _{17:0}	1.4	11.3	3.2	1.1	1.0	—	2.8	1.6	4.2	6.9	6.6	4.0	2.4
Summed feature 4†	0.8	6.7	0.7	—	1.0	—	1.1	0.4	11.9	10.6	3.9	0.7	—

*Data from: a, Yu *et al.* (2008); b, Yoon *et al.* (2001); c, An *et al.* (2007).

†Summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

approximately 1.0:1.5:1.0:2.0. Dinitrophenylation revealed that glutamic acid represents the *N*-terminus of the inter-peptide bridge. From these data and the appearance of the peptides L-Ala-D-Glu, D-Ala-D-Glu, L-Lys-Gly and L-Lys-D-Ala in the partial hydrolysates of the peptidoglycan (4 M HCl, 100 °C, 0.75 h), the peptidoglycan type A4x L-Lys-Gly-D-Glu (Schleifer & Kandler, 1972) was concluded for both strains 6062^T and R-31323.

DNA G+C content, determined by HPLC, for strains 6062^T, 4331 and R-31323 was 42.4, 42.4 and 42.2 mol%, respectively [for method see (Logan *et al.*, 2000; Mesbah *et al.*, 1989)], and is concordant with the emended description for the genus *Sporosarcina* (Yoon *et al.*, 2001).

Phylogenetic analysis was performed using near full-length 16S rRNA gene sequences from the six novel *Sporosarcina* strains deposited and the top 50 BLAST hits returned for type strain 6062^T from the EzTaxon (Chun *et al.*, 2007) database. These sequences were aligned using the CLUSTAL W utility in MEGA 4.0.2 (Tamura *et al.*, 2007) and exported to PhyML 3.0 (Guindon & Gascuel, 2003). An unrooted maximum-likelihood tree was recreated using the general time reversal model, six substitution rate categories and 5 random starting trees (Guindon & Gascuel, 2003). Tree reliability was evaluated using approximate likelihood ratio test (aLRT) (Anisimova & Gascuel, 2006) (Fig. 1). This analysis shows strong support for the novel strains of *S. newyorkensis* sp. nov. residing basally in

the clade that harbours *S. koreensis*, *S. luteola*, *S. thermotolerans*, *S. saromensis*, *S. aquimarina* and *S. ureae*.

Characteristics described in the emended description for the genus *Sporosarcina* (Yoon *et al.*, 2001) that support inclusion of our strains within the genus are: (i) Gram-positive rods, (ii) formation of round endospores, (iii) motile, (iv) facultatively anaerobic, (v) oxidase- and catalase-positive, (vi) hydrolysis of urea but not starch, (vii) CFA composition (Table 2 and species description), (viii) MK-7 as the major menaquinone, (ix) L-lysine as the diamino acid of the peptidoglycan and (x) the DNA G+C content. Additional support is provided by the phylogenetic analysis (see above).

The following characteristics support exclusion from the neighbouring genera: (i) the single species of the genus *Filibacter* is a Gram-negative filamentous rod that shows gliding motility and does not form endospores (Clausen *et al.*, 1985; Maiden & Jones, 1984); (ii) the two species of the genus *Paenisporosarcina* are non-motile and have MK-8 as well as MK-7 as a major menaquinone (Krishnamurthi *et al.*, 2009); (iii) the three species of the genus *Psychrobacillus* have MK-8 as the major menaquinone, are urease-negative, and the cell wall peptidoglycan is of the A4β type (Krishnamurthi *et al.*, 2010).

Characteristics that aid in distinguishing the novel *Sporosarcina* strains from other species of the genus *Sporosarcina* are: (i) for *S. soli*, *Sporosarcina globispora* and

Fig. 1. An unrooted, maximum-likelihood phylogenetic tree, for *Sporosarcina newyorkensis* sp. nov. and the 50 closest taxa returned from a BLAST search of EzTaxon (see text). Branch probability is indicated at the nodes and determined by aLRT. Probabilities below 50 % are not shown and the branches are collapsed. Bar, expected changes per site.

Sporosarcina psychrophila, the absence of acid production from various carbohydrates in Gordon base medium; (ii) for *S. ureae*, the presence of DNase activity and rod-shaped cells; (iii) for *S. contaminans*, *S. luteola* and *Sporosarcina antarctica*, the presence of urease activity; (iv) for *S. koreensis*, *S. aquimarina*, *S. luteola* and *S. thermotolerans*, the absence of lysozyme resistance; (v) for *S. saromensis*, absence of hydrolysis of starch; (vi) other characteristics including the

absence of trypsin activity (API ZYM, bioMérieux) and the relative abundance of major CFA (Tables 2 and 3). Additionally, strains 6062^T and R-31323 share the peptidoglycan structure A4α L-Lys-Gly-D-Glu with *S. ureae* (Stackebrandt *et al.*, 1987) but differ in this feature from all other type strains of the genus *Sporosarcina* which do not contain glycine in the interpeptide bridge of the peptidoglycan D-Ala-D-Glu.

Table 3. Characteristics useful in distinguishing *S. newyorkensis* sp. nov. from other species of the genus *Sporosarcina*

Taxa: 1, strain 6062^T; 2, *S. ureae* DSM 2281^T; 3, *S. aquimarina* DSM 14554^T; 4, *S. soli* DSM 16920^T; 5, *S. koreensis* DSM 16921^T; 6, *S. thermotolerans* CCUG 53480^T; 7, *S. contaminans* CCUG 53915^T; 8, *S. luteola* DSM 23150^T; 9, *S. saromensis* HG645^T; 10, *S. globispora* DSM 4^T; 11, *S. psychrophila* KCTC 3446^T; 12, *S. pasteurii* KCTC 3558^T; 13, *S. antarctica* N-05^T. T, Terminal; ST, subterminal; C, central; R, rod; S, spherical; NA, not available.

Characteristic	1	2	3	4	5	6	7	8	9 ^{a*}	10 ^b	11 ^b	12 ^b	13 ^c
Acid production from CHO (Gordon base)													
D-Fructose	—	—	—	—	—	—	—	—	—	+	+	NA	—
D-Galactose	—	—	—	—	—	—	—	—	—	+	+	NA	—
D-Glucose	—	—	—	—	—	—	—	—	—	+	+	NA	—
Maltose	—	—	—	—	—	—	—	—	NA	+	+	NA	—
D-Mannitol	—	—	—	—	—	—	—	—	—	—	+	NA	—
Sucrose	—	—	—	—	—	—	—	—	—	—	+	NA	—
Xylose	—	—	—	+	—	—	—	—	—	—	+	NA	—
Inositol	—	—	—	+	—	—	—	—	NA	NA	NA	NA	NA
Glycerol	—	—	—	+	—	—	—	—	NA	NA	NA	NA	—
Biochemical reaction													
Urease	+	+	+	+	+	+	—	—	+	+	+	+	—
Arginine dihydrolase	—	—	—	—	+	—	—	—	NA	NA	NA	NA	—
Acetate utilization	—	—	—	+	—	—	—	—	NA	NA	NA	NA	NA
Lysozyme resistance	+	+	—	+	—	—	+	—	NA	NA	NA	NA	NA
DNase	+	—	+	+	+	+	+	+	NA	NA	NA	NA	NA
Hydrolysis of:													
Tyrosine	—	—	—	+	+	—	+	—	NA	NA	NA	NA	NA
Starch	—	—	—	—	—	—	—	—	+	—	—	—	—
Morphology													
Cell	R	S	R	R	R	R	R	R	R	R	R	S	R
Spore position	ST	NA	ST	C ^d	T ^d	T ^e	ST, T	NA	T	NA	NA	T	NA
Growth at:													
37 °C	+	+	+	+	+	+	+	+	+	—	—	+	—
42 °C	+	—	—	+	+	—	+	+	NA	NA	—	—	—
NaCl tolerance													
2 %	+	+	+	+	+	+	+	+	+	NA	NA	NA	+
5 %	+	+	+	+	+	—	+	+	+	—	—	NA	+
Motility at 28 °C	+	+	+	+	+	+	+	+	+	+	+	+	—
Trypsin (API ZYM)	—	—	—	—	+	+	+	+	NA	NA	NA	NA	NA

*Data from; a, An *et al.* (2007); b, Yoon *et al.* (2001); c, Yu *et al.* (2008); d, Kwon *et al.* (2007); e, Kämpfer *et al.* (2010).

Based on 16S and 23S rRNA gene sequences, DNA–DNA hybridization, CFA composition, peptidoglycan type, polar lipid content, DNA G+C content and phenotypic and phylogenetic analyses, we propose that the twelve novel strains (Table 1) all represent a single species, *Sporosarcina newyorkensis* sp. nov.

Description of *Sporosarcina newyorkensis* sp. nov.

Sporosarcina newyorkensis (new.york.en'sis. N.L. fem. adj. *newyorkensis* of or belonging to the state of New York in the USA, where the majority of isolates were obtained).

Cells are Gram-positive rods (1.5 × 0.6 µm), arranged singly or in palisades, are facultative anaerobes, and are motile at 28 °C. Five of the twelve strains from this study display motility at 37 °C, whereas the other strains are non-motile at this temperature (for all variable characters see

Supplementary Table S4). Round to slightly oval subterminal spores form in swollen sporangia. Colonies are 3.1 to 4.1 mm in diameter after 48 h, moist, grey, circular, raised and have entire margins with no haemolysis at 24 h, but slight beta-haemolysis at 48 h of incubation (37 °C in air). A strong odour is present. Growth occurs from 10 to 42 °C with optimal growth between 22 and 28 °C determined by turbidity in TSB (9 of 12 and 8 of 12 strains can grow at 45 °C and 55 °C, respectively). The pH optimum is between pH 7.2 and 9.5 determined by turbidity in TSB adjusted to the appropriate pH. Growth is observed in 2 to 13 % NaCl (2 of 12 and 4 of 12 strains unable to grow above 8 and 10 % NaCl, respectively). Oxidase, catalase, urease and DNase are present and cells are resistant to lysozyme. Cells are negative for hydrolysis of casein, tyrosine, aesculin, adenine, xanthine, hypoxanthine and starch. Cells are negative for the methyl red/Voges–Proskauer tests. Cells are negative for decarboxylation of arginine, lysine and ornithine using

Moeller's decarboxylase medium and the utilization of acetate. Cells are variable for gelatinase (2 of 12 strains positive), phenylalanine deaminase (1 of 12 strains positive), nitrate reduction (7 of 12 strains positive) and utilization of Simmons' citrate (2 of 12 strains positive) (Atlas, 1993; Forbes *et al.*, 1998; Priest *et al.*, 1988). Cells are negative for acid production (Gordon sugar base containing 10% carbohydrate) from adonitol, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, D-rhamnose, D-salicin, D-sorbitol, sucrose, trehalose and D-xylose (Gordon *et al.*, 1973). API ZYM reactions were variable for esterase (C4) (3 of 12 strains positive), esterase lipase (C8) (8 of 12 strains positive), α -chymotrypsin (8 of 12 strains positive), leucine arylamidase (2 of 12 strains positive), cystine arylamidase (1 of 12 strains positive), naphthol-AS-BI-phosphohydrolase (1 of 12 strains positive) alkaline phosphatase (1 of 12 strains positive), α -galactosidase (1 of 12 strains positive) and β -glucuronidase (2 of 12 strains positive). Negative for valine arylamidase, acid phosphatase, lipase (C14), trypsin, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. No reaction to the carbohydrates in the API 50CH test strip. The predominant fatty acids are iso-C_{14:0}, iso-C_{15:0} and anteiso-C_{15:0}. The peptidoglycan type is A4 α L-Lys-Gly-D-Glu. DNA G+C content is 42.4 mol%. The major menaquinone is MK-7.

The type strain, 6062^T (=DSM 23544^T =CCUG 59649^T =LMG 26022^T) was isolated from a patient's blood in New York State, USA.

Acknowledgements

We thank the Wadsworth Center Applied Genomic Technologies Core for sequencing, the Wadsworth Center Orphan Bacterium Laboratory, the Wadsworth Biodefense Laboratory and the Institute for Agricultural and Fisheries Research for the *S. newyorkensis* strains, Anika Wasner (DSMZ), Leah Nazarian (WC) and Amy Saylor (WC) for assistance, Donna Kohlerschmidt (WC) for helpful discussions, and Dr Andrea Habura (WC) for assistance with the phylogenetic analysis.

References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- An, S. Y., Haga, T., Kasai, H., Goto, K. & Yokota, A. (2007). *Sporosarcina saromensis* sp. nov., an aerobic endospore-forming bacterium. *Int J Syst Evol Microbiol* **57**, 1868–1871.
- Anisimova, M. & Gascuel, O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst Biol* **55**, 539–552.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Claus, D. & Fahmy, F. (1986). Genus *Sporosarcina* Kluyver and van Niel 1936, 401^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1202–1206. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Clausen, V., Jones, J. G. & Stackebrandt, E. (1985). 16S ribosomal RNA analysis of *Filibacter limicola* indicates a close relationship to the genus *Bacillus*. *J Gen Microbiol* **131**, 2659–2663.
- Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., De Vos, P. & Heyndrickx, M. (2008). Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. *Syst Appl Microbiol* **31**, 126–140.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Ezaki, T., Hashimoto, Y. & 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.
- Forbes, B. A., Sahm, D. F. & Weissfeld, A. S. (1998). Overview of bacterial identification methods and strategies. In *Bailey and Scott's Diagnostic Microbiology*, 10th edn, pp. 424–446. Edited by J. Roche. St. Louis, MO: Mosby.
- Gordon, R. E., Haynes, W. C. & Pang, C. H.-N. (1973). Media and methods. In *The Genus Bacillus*, US Department of Agriculture Handbook no. 427, pp. 2–14. Washington, DC: US. Government Printing Office.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kämpfer, P., Falsen, E., Lodders, N. & Schumann, P. (2010). *Sporosarcina contaminans* sp. nov. and *Sporosarcina thermotolerans* sp. nov., two endospore-forming species. *Int J Syst Evol Microbiol* **60**, 1353–1357.
- Krishnamurthi, S., Bhattacharya, A., Mayilraj, S., Saha, P., Schumann, P. & Chakrabarti, T. (2009). Description of *Paenisporosarcina quisquiliarum* gen. nov., sp. nov., and reclassification of *Sporosarcina macmurdoensis* Reddy *et al.* 2003 as *Paenisporosarcina macmurdoensis* comb. nov. *Int J Syst Evol Microbiol* **59**, 1364–1370.
- Krishnamurthi, S., Ruckmani, A., Pukall, R. & Chakrabarti, T. (2010). *Psychrobacillus* gen. nov. and proposal for reclassification of *Bacillus insolitus* Larkin & Stokes, 1967, *B. psychrotolerans* Abd-El Rahman *et al.*, 2002 and *B. psychrodurans* Abd-El Rahman *et al.*, 2002 as *Psychrobacillus insolitus* comb. nov., *Psychrobacillus psychrotolerans* comb. nov. and *Psychrobacillus psychrodurans* comb. nov. *Syst Appl Microbiol* **33**, 367–373.
- Kwon, S. W., Kim, B. Y., Song, J., Weon, H. Y., Schumann, P., Tindall, B. J., Stackebrandt, E. & 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.

- Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M., Murray, B. L., Syme, N., Wynn-Williams, D. D. & De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich archipelago, with the proposal of *Bacillus fumarioli* sp. nov. *Int J Syst Evol Microbiol* **50**, 1741–1753.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Maiden, M. F. J. & Jones, J. G. (1984). A new filamentous gliding bacterium, *Filibacter limicola* gen. nov. sp. nov., from lake sediment. *J Gen Microbiol* **130**, 2943–2959.
- Mesbah, M., Premachandran, U. & 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.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Stackebrandt, E., Ludwig, W., Weizenegger, M., Dorn, S., McGill, T. J., Fox, G. E., Woese, C. R., Schubert, W. & Schleifer, K. H. (1987). Comparative 16S rRNA oligonucleotide analyses and murein types of round-spore-forming bacilli and non-spore-forming relatives. *J Gen Microbiol* **133**, 2523–2529.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- 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.
- Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.
- Tominaga, T., An, S. Y., Oyaizu, H. & Yokota, A. (2009). *Sporosarcina luteola* sp. nov. isolated from soy sauce production equipment in Japan. *J Gen Appl Microbiol* **55**, 217–223.
- 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. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA–DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51**, 1315–1322.
- Wolfgang, W. J., Carpenter, A. N., Cole, J. A., Gronow, S., Habura, A., Jose, S., Nazarian, E. J., Kohlerschmidt, D. J., Limberger, R. & other authors (2011). *Neisseria wadsworthii* sp. nov. and *Neisseria shayegani* sp. nov. isolated from clinical specimens. *Int J Syst Evol Microbiol* **61**, 91–98.
- Yoon, J. H., Lee, K. C., Weiss, N., Kho, Y. H., Kang, K. H. & 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. & Zhang, D. C. (2008). *Sporosarcina antarctica* sp. nov., a psychrophilic bacterium isolated from the Antarctic. *Int J Syst Evol Microbiol* **58**, 2114–2117.
- Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J Comput Biol* **7**, 203–214.