Sporosarcina contaminans sp. nov. and *Sporosarcina thermotolerans* sp. nov., two endospore-forming species

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The taxonomic positions of two Gram-positive, endospore-forming rods, strains CCUG 53915^T and CCUG 53480^T, isolated from an industrial clean-room floor and from a human blood sample, respectively, were studied. 16S rRNA gene sequence similarity studies revealed that both isolates clearly clustered with *Sporosarcina* species. Strain CCUG 53915^T was most closely related to *Sporosarcina koreensis* and *Sporosarcina soli*, showing 99.4 and 99.2% 16S rRNA gene sequence similarities to the type strains of these species, respectively. Strain CCUG 53480^T showed the highest 16S rRNA gene sequence similarities to the type strains of *S. koreensis* (98.7%) and *Sporosarcina saromensis* (98.6%). Strains CCUG 53915^T and CCUG 53480^T had peptidoglycan type A4 α L-Lys–D-Glu. The quinone systems of both strains were composed predominantly of menaquinone MK-7, with small amounts of MK-8. The polar lipid profiles of both strains consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and three unidentified phospholipids. The fatty acid profiles, which comprise anteiso- and iso-branched fatty acids, supported affiliation of the two isolates to the genus *Sporosarcina*. The results of physiological and biochemical tests and DNA–DNA hybridization data allowed a clear phenotypic and genotypic differentiation of both strains from the most closely related *Sporosarcina* species. For this reason, it is proposed that strains CCUG 53915^T (=DSM 22204^T) and CCUG 53480^T (=DSM 22203^T) represent two novel species in the genus *Sporosarcina*, with the names *Sporosarcina contaminans* sp. nov. and *Sporosarcina thermotolerans* sp. nov., respectively.

The genus *Sporosarcina* was described by Kluyver & van Niel (1936) and comprises several Gram-positive, endospore-forming rods that have been isolated from various environmental and also clinical habitats (Claus *et al.*, 1983; Yoon *et al.*, 2001; Reddy *et al.*, 2003; An *et al.*, 2007; Krishnamurthi *et al.*, 2009). The genus is characterized by having L-lysine as the diagnostic amino acid in the peptidoglycan and MK-7 as the characteristic menaquinone. In this study, the phenotypic and genotypic properties of two novel *Sporosarcina* strains isolated from different samples are described.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CCUG 53915^T and CCUG 53480^T are FN298444 and FN298445, respectively.

The cellular fatty acid compositions of the type strains of members of the genus *Sporosarcina* are available with the online version of this paper.

Strain CCUG 53915^T was isolated as a contaminant from an industrial clean-room floor in Göteborg, Sweden, and strain CCUG 53480^T was isolated from a human blood sample of a 54-year-old woman in Göteborg, Sweden. Both strains were isolated on blood agar at 37 °C, but maintained on tryptone soy agar (TSA; Oxoid) at 30 °C.

Gram staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was examined with a Zeiss light microscope at $\times 1000$ magnification using cells that had been grown for 24 h at 30 °C on TSA. Cell morphology data are given in the formal species description.

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). PCR amplification of the 16S rRNA gene was carried out with the primer pair 27F (5'-GAGTTTGATCMTGGCTCAG) and MR 1492R (5'-ACGGYTACCTTGTTACGACTT) (Lane, 1991). For sequen-

cing, standard sequencing primers for the 16S rRNA gene were used. The 16S rRNA gene sequences of strains CCUG 53915^T and CCUG 53480^T were continuous stretches of 1463 and 1420 bp, respectively. Phylogenetic analysis was performed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSUref 95 database (version July 2008; Pruesse *et al.*, 2007). Trees were reconstructed using the maximum-likelihood method with FASTDNAML (Olsen *et al.*, 1994) and 50 % conservatory filter (only alignment columns in which the frequency of the most abundant nucleotide was equal to or above 50 % were included into the calculation). Tree topology was further tested without filters. Tree topologies showed similar groupings regarding CCUG 53915^T, CCUG 53480^T and their nearest relatives. A maximum-likelihood tree is shown in Fig. 1. A tree reconstructed with the neighbour-joining method showed a similar result (data not shown). The 16S rRNA gene sequences of the strains were compared by distance calculations (distance options according to the Kimura-2 model) using the software package MEGA version 4.0 (Tamura *et al.*, 2007).

16S rRNA gene sequence similarity calculations indicated that the closest relatives of strain CCUG 53915^T were *Sporosarcina koreensis* F73^T (GenBank accession no. DQ073393) with 99.4 % similarity and *Sporosarcina soli* 180^T (GenBank accession no. DQ073394) with 99.2 % similarity. The closest relatives of strain CCUG 53480^T were *S. koreensis* F73^T with 98.7 % similarity and *Sporosarcina saromensis* HG645^T (GenBank accession no. AB243859) with 98.6 % similarity.

The peptidoglycan of strains CCUG 53915^T and CCUG 53480^T grown in tryptic soy broth at 28 °C for 1 day was isolated after disruption of the cells by shaking with glass beads and subsequent trypsin digestion according to the method of Schleifer (1985). The amino acids and peptides in the cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using previously described solvent systems (Schleifer, 1985). The

molar ratios of the amino acids were determined by GC (GC 14A; Shimadzu) and GC-MS (320-MS Quadrupole GC/MS; Varian) of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). The hydrolysate (4 M HCl, 100 °C, 16 h) of the purified peptidoglycan of strains CCUG 53915^T and CCUG 53480^T contained the amino acids lysine, alanine and glutamic acid in molar ratios of approximately 1.0:1.7:2.4 and 1.0:1.3:2.0, respectively. The peptides L-Ala-D-Glu and L-Lys-D-Ala were detected in the partial hydrolysate (4 M HCl, 100 °C, 0.75 h) of the peptidoglycan. Dinitrophenylation according to Schleifer (1985) revealed that Glu represents the N terminus of the interpeptide bridge. These data suggest that the peptidoglycan of strains CCUG 53915^T and CCUG 53480^T is of type A4α L-Lys-D-Glu (Schleifer & Kandler, 1972), which is also found in other members of the genus *Sporosarcina* (Reddy *et al.*, 2003; Yoon *et al.*, 2001; Yu *et al.*, 2008; Kwon *et al.*, 2007). Menaquinones and polar lipids were extracted from cells of strains CCUG 53915^T and CCUG 53480^T grown in tryptic soy broth at 28 °C for 1 day. Menaquinones were analysed (Groth *et al.*, 1996) after extraction as described by Collins *et al.* (1977) and polar lipids were determined according to Minnikin *et al.* (1979). Strains CCUG 53915^T and CCUG 53480^T contained the menaquinones MK-7 and MK-8 in the ratios 98:2 (CCUG 53480^T) and 96:4 (CCUG 53915^T) and the phospholipids phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and three unidentified phospholipids.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profiles of both strains were very similar to those of closely related *Sporosarcina* species (see Supplementary Table S1, available in IJSEM Online). A typical fatty acid profile consisting of mainly anteiso- and iso-branched fatty acids was found.

Results of the comparative physiological characterization of all *Sporosarcina* species are given in Table 1. Strains CCUG 53915^T and CCUG 53480^T were also tested with the API

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from GenBank/EMBL (accession numbers are given in parentheses). The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSUref 95 database (version July 2008; Pruesse *et al.*, 2007). Tree building was performed using the maximum-likelihood method with FASTDNAML (Olsen *et al.*, 1994) without conservatory filter. Bar, 0.10 nucleotide substitutions per nucleotide position.

Table 1. Differential phenotypic and physiological characteristics of *Sporosarcina* species

Taxa: 1, CCUG 53915^T; 2, *S. koreensis* F73^T; 3, *S. soli* I80^T; 4, *S. macmurdoensis* (data from Reddy *et al.*, 2003); 5, *S. aquimarina* (Yoon *et al.*, 2001); 6, *S. globispora* (Nakamura, 1984; Rüger, 1983); 7, *S. psychrophila* (Nakamura, 1984); 8, *S. pasteurii* (Yoon *et al.*, 2001); 9, *S. ureae* (Claus & Fahmy, 1986); 10, CCUG 53480^T; 11, *S. saromensis* HG645^T (data from An *et al.*, 2007). Data for taxa 1, 2, 3, 10 and 11 are based on results for the type strain. All of the taxa are positive for catalase activity. +, Positive; –, negative; w, weak; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour*	BE	LO	LO	WH	LO	WH	WH	WH	WH	BE	BE
Cell shape†	R	R	R	R	R	R	R	S	S	R	R
Spore position‡	T	T	C	ST	T	NA	T	T	NA	T	T
Motility	+	+	–	–	+	+	+	+	+	–	+
NaCl tolerance (%)	10	7	5	3	13	5	2	10	3	3	9
Optimum pH	7–8	7	8	7	6.5–7.0	7	7	9	7	7	6.5
Growth at:											
pH 5.7	–	–	–	–	+	–	–	–	–	–	+
5 and 10 °C	–	–	–	+	+	+	+	NA	NA	w	+
30 °C	+	+	+	–	+	+	w	+	+	+	+
37 °C	+	+	+	–	+	–	+	+	w	+	+
40 °C	+	+	–	–	–	–	–	w	+	+	+
Optimum growth temperature (°C)	30	30	30	20	25	25	20	30	25	30–37	27
Anaerobic growth	–	–	–	+	+	+	–	+	–	–	–
Oxidase	+	+	+	–	+	+	+	NA	+	+	+
Urease	–	+	+	–	+	+	+	+	+	–	+
Hydrolysis of:											
Casein	–	–	–	NA	–	–	w	w	–	–	NA
Gelatin	–	+	–	+	+	+	+	+	–	+	+
Starch	–	–	–	+	–	–	w	–	–	–	+
Tween 80	–	–	–	NA	–	–	NA	NA	w	–	NA
Tyrosine	–	–	–	NA	+	NA	–	NA	+	–	NA
Nitrate reduction	NA	–	+	–	+	+	–	+	+	–	–
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%)	NA	46.5	44.5	44	40	40	44.1	39	40.0–41.5§	ND	46

*LO, Light orange; WH, white; BE, beige.

†R, Rod; S, spherical.

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

§Data for 11 strains.

50CH and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). DNA–DNA hybridization experiments were performed with CCUG 53915^T and the type strains of *S. koreensis* and *S. soli*, and with strain CCUG 53480^T and *S. saromensis* HG645^T and the type strain of *S. koreensis* according to the method of Ziemke *et al.* (1998). DNA–DNA similarity values between strains were as follows: CCUG 53915^T and *S. koreensis* DSM 16921^T, 28.0%; CCUG 53915^T and *S. soli* DSM 16920^T, 30.2%; CCUG 53480^T and *S. saromensis* HG645^T, 36.1%; and CCUG 53480^T and *S. koreensis* F73^T, 43.1%.

Peptidoglycan types, quinone systems, and fatty acid and polar lipid profiles, as well as 16S rRNA analyses, show unambiguously that strains CCUG 53915^T and CCUG 53480^T are affiliated with the genus *Sporosarcina*. On the

basis of the observed phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences, two novel *Sporosarcina* species are proposed.

Description of *Sporosarcina contaminans* sp. nov.

Sporosarcina contaminans (con.ta'mi.nans. L. part. adj. *contaminans* contaminating, polluting, isolated as a contaminant of an industrial clean-room floor).

Cells are Gram-positive, strictly aerobic, motile rods (0.8–1.0 × 2.0–3.0 µm). Spherical endospores are formed in a terminal position. Colonies grown on TSA are circular, convex and beige. Optimal temperature for growth is 30 °C; growth occurs at 15–50 °C, but not at 5 or 55 °C. Optimal

pH for growth is 7.0–8.0; growth occurs at pH 6.5–10.5. NaCl is not required for growth, but can be tolerated up to 9 % (w/v). Positive for catalase and oxidase activities. Gelatin and starch are not hydrolysed. H₂S, indole and acetoin are not produced. Nitrate is not reduced. Negative for urease, gelatinase, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and citrate utilization. No acid formation from sugars is observed. Sugar compounds in the API 50CH gallery are not utilized. Some organic acids, such as pyruvate, fumarate and L-malate, are utilized according to the method of Kämpfer *et al.* (1991). Further physiological data are indicated in Table 1. The peptidoglycan is of type A4 α L-Lys-D-Glu and the major menaquinone is MK-7. The polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Major fatty acids are anteiso-C_{15:0} and iso-C_{15:0}; iso-C_{14:0}, iso-C_{17:0} and anteiso-C_{17:0} are also detected.

The type strain is CCUG 53915^T (=DSM 22204^T), isolated as a contaminant from an industrial clean-room floor in Göteborg, Sweden.

Description of *Sporosarcina thermotolerans* sp. nov.

Sporosarcina thermotolerans (ther.mo.to'ler.ans. Gr. n. *thermê* heat; L. part. adj. *tolerans* tolerating; N.L. part. adj. *thermotolerans* heat-tolerating).

Cells are Gram-positive, strictly aerobic, non-motile rods (0.8–1.0 × 2.0–3.0 µm). Spherical endospores are formed in a terminal position. Colonies grown on TSA are circular, convex and beige. Optimal temperature for growth is 30–37 °C; growth occurs at 15–50 °C, but not at 5 or 55 °C. Optimal pH for growth is 7.0–8.0; growth occurs at pH 6.5–8.5. NaCl is not required for growth, but can be tolerated up to 3 % (w/v). Positive for catalase and oxidase activities. Gelatin and starch are hydrolysed. H₂S, indole and acetoin are not produced. Nitrate is not reduced. Positive for gelatinase activity. Negative for urease, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and citrate utilization. Acid is not produced from carbohydrates. No sugars or other compounds in the API 50CH gallery are utilized. The peptidoglycan is of type A4 α L-Lys-D-Glu and the major menaquinone is MK-7. The polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Major fatty acids are anteiso-C_{15:0}, iso-C_{15:0} and iso-C_{14:0}.

The type strain is CCUG 53480^T (=DSM 22203^T), isolated from a human blood sample of a 54-year-old woman in Göteborg, Sweden.

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References

- 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.
- 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.
- Claus, D., Fahmy, F., Rolf, H. J. & Tosunoglu, N. (1983). *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst Appl Microbiol* **4**, 496–506.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- 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.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- Kluyver, A. J. & van Niel, C. B. (1936). Prospects for a natural system of classification of bacteria. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt II* **94**, 369–403.
- 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.
- 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.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester, UK: Wiley.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the N-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.
- Nakamura, L. K. (1984). *Bacillus psychrophilus* sp. nov., nom. rev. *Int J Syst Bacteriol* **34**, 121–123.

- Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994). FASTDNAML: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188–7196.
- Reddy, G. S. N., Matsumoto, G. I. & 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.
- 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.
- 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.
- 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.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.