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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*

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A light-orange-coloured, facultatively anaerobic, rod-shaped bacterium (strain SW28^T), which was isolated from seawater in Korea, was taxonomically studied by a polyphasic approach. This organism formed round terminal endospores in swollen sporangia. The peptidoglycan type is A4α, based on L-Lys-L-Ala-D-Asp. The predominant menaquinone is MK-7 and the major fatty acid is ante-$C_{15:0}$. The G+C content of the DNA is 40 mol%. Phylogenetic analysis based on 16S rDNA sequences showed that strain SW28^T falls within the radiation of a cluster comprising the rRNA group 2 bacilli and non-*Bacillus*-type organisms. Strain SW28^T showed the highest degree of relatedness to the type strain of *Sporosarcina ureae*, sharing 96·8% 16S rDNA similarity. Levels of DNA–DNA relatedness between strain SW28^T and *S. ureae* DSM 2281^T and the type strains of some *Bacillus* species forming a coherent phylogenetic cluster are less than 12·5%. On the basis of phenotypic and chemotaxonomic characteristics, 16S rDNA sequence analysis and DNA–DNA relatedness data, it is proposed that strain SW28^T should be placed in the genus *Sporosarcina* as a new species, *Sporosarcina aquimarina* sp. nov. The type strain is SW28^T (= KCCM 41039^T = JCM 10887^T). From the results of the taxonomic re-evaluation, it is also proposed that *Bacillus globisporus*, *Bacillus psychrophilus* and *Bacillus pasteurii* be transferred to the genus *Sporosarcina* as *Sporosarcina globispora*, *Sporosarcina psychrophila* and *Sporosarcina pasteurii*, respectively.

Keywords: *Sporosarcina aquimarina* sp. nov., marine bacterium, *Bacillus* rRNA group 2, genus *Sporosarcina*, taxonomic reclassification

INTRODUCTION

Traditionally, Gram-positive, aerobic or facultatively anaerobic, spore-forming, rod-shaped bacteria have been assigned to the genus *Bacillus*. This led the single genus *Bacillus* to include phenotypically heterogeneous organisms with a wide range of nutritional requirements, growth conditions and metabolic diversity (Claus & Berkeley, 1986; Slepecky & Hemphill, 1991). Ash *et al.* (1991) revealed the phylogenetic heterogeneity of the genus *Bacillus* by grouping it into five phylogenetically distinct clusters through com-

The GenBank accession numbers for the 16S rDNA sequence of strain SW28^T and for the newly determined 16S rDNA sequence of *Sporosarcina ureae* DSM 2281^T are AF202056 and AF202057, respectively.

parative analysis of 16S rRNA sequences. *Bacillus* species assigned to rRNA group 3 have been reclassified in a new genus, *Paenibacillus* (Ash *et al*., 1993), and *Bacillus* species assigned to rRNA group 4 have been reclassified as members of other new genera, such as *Aneurinibacillus* and *Brevibacillus* (Shida *et al*., 1996). The rRNA group 2 of the genus *Bacillus* comprises round-spore-forming bacilli, which form a radiation of the cluster distinct from members of other rRNA groups (Ash *et al*., 1991; Stackebrandt *et al*., 1987). However, phylogenetic analysis based on 16S rRNA sequences has shown that this group also comprises some non-*Bacillus*-type organisms, such as the genera *Caryophanon*, *Planococcus*, *Filibacter* and *Sporosarcina* (Clausen *et al*., 1985; Farrow *et al*., 1992, 1994; Stackebrandt *et al*., 1987). Members of the *Bacillus* rRNA group 2 are chemotaxonomically characterized by containing L-lysine or D-ornithine at position 3 of the peptide subunit of the peptidoglycan (Rheims *et al*., 1999; Stackebrandt *et al*., 1987). It has been recognized that the L-lysine- and D-ornithine-containing bacilli belonging to this group may be in need of taxonomic re-evaluation. Although the taxonomic status of the rRNA group 2 bacilli has been discussed (Farrow *et al*., 1994; Rheims *et al*., 1999), no proposals have yet been put forward to transfer them into one or several new or existing genera.

In this work, we describe a new, light-orange-coloured, moderately halotolerant, round-spore-forming rod that was isolated from seawater in Korea. To determine its taxonomic status, this organism (strain SW28^T) was characterized phenotypically and genetically. On the basis of the data described below, strain SW28^T is considered as a new species of the genus *Sporosarcina*, for which a new species name, *Sporosarcina aquimarina* sp. nov., is proposed. In this study, we also propose that *Bacillus globisporus*, *Bacillus psychrophilus* and *Bacillus pasteurii* be transferred to the genus *Sporosarcina* as *Sporosarcina globispora*, *Sporosarcina psychrophila* and *Sporosarcina pasteurii*, respectively, on the basis of phylogenetic and chemotaxonomic properties.

METHODS

Bacterial strains and cultural conditions. Strain SW28^T was isolated from seawater in Korea by the dilution plating technique on trypticase soy agar (TSA; BBL) supplemented with (l^{-1}) 24 g NaCl, 7 g $MgSO_4.7H_2O$, 5·3 g $MgCl_2.6H_2O$, 0·7 g KCl and 0·1 g $CaCl_2$ (artificial sea water) (pH 7·5). For investigation of morphological and physiological characteristics, strain SW28^T was cultivated on TSA and in trypticase soy broth (TSB) at 25 °C. Cell mass for analyses of cell wall and menaquinone and for DNA extraction was obtained from TSB cultures. For DNA extraction, some reference strains were cultivated as follows: *B. globisporus* DSM 4^T and *B. psychrophilus* KCTC 3446^T were cultivated at 20 °C in TSB, *B. pasteurii* KCTC 3558^T was cultivated at 30 °C in ATCC medium no. 1376 containing 0·13 M Tris buffer pH 9·0, 20 g yeast extract l^{-1} and 10 g $(NH_4)_2SO_4$ l^{-1}, and *Sporosarcina ureae* DSM 2281^T was cultivated at 30 °C in TSB. All strains were cultivated on a horizontal shaker at 150 r.p.m. and the broth cultures were checked for purity microscopically before they were harvested by centrifugation. For fatty acids methyl ester (FAME) analysis, strain SW28^T, *B. globisporus* DSM 4^T, *B. psychrophilus* KCTC 3446^T and *S. ureae* DSM 2281^T were cultivated at optimal growth temperatures for 3 d on TSA, and *B. pasteurii* KCTC 3558^T was cultivated at 30 °C for 3 d on nutrient agar (Difco) supplemented with urea (2 %, w/v).

Morphological and physiological characterization. Phase-contrast microscopy and transmission electron microscopy (TEM) were used to observe the morphology of cells. Flagellum type was examined by TEM using cells from an exponentially growing culture. The cells were negatively stained with 1 % (w/v) phosphotungstic acid, and after air drying the grids were examined by using a model CM-20 transmission electron microscope (Philips). Hydrolysis of casein, gelatin, starch, hypoxanthine, tyrosine, Tween 80 and xanthine, and production of urease were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were determined as described by Lanyi (1987). Hydrolysis of arbutin was determined according to the method of Kurup & Fink (1975). Catalase activity was determined by bubble production in a 3 % hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1 % *p*-aminodimethylaniline oxalate. Tolerance of sodium chloride was investigated in the presence of 0·5–20 % (w/v) NaCl. Growth at various temperatures was tested in TSB or on TSA at 4, 10, 15, 20, 25, 28, 30, 33, 37, 40, 45 and 50 °C. Acid production from carbohydrates was determined by using the API 50CHB system (bioMérieux). Other physiological tests were performed with the API 20NE system (bioMérieux).

Isolation of DNA. Chromosomal DNAs were isolated and purified according to the method described previously (Yoon *et al*., 1996), with the exception that ribonucelase T1 was used together with ribonuclease A.

Chemotaxonomic characterization. The absence of diaminopimelic acid in the peptidoglycan was established by the method described by Komagata & Suzuki (1987). Preparation of cell walls and determination of peptidoglycan structure were carried out by the methods described by Schleifer & Kandler (1972), with the modification that TLC on cellulose sheets was used instead of paper chromatography. Menaquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass grown on TSA was harvested and fatty acid methyl esters were prepared and identified following the instructions of the Microbial Identification System (MIDI).

Determination of G+C content. This was done by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

DNA–DNA hybridization. This was performed fluorometrically by the method of Ezaki *et al*. (1989) using photobiotin-labelled DNA probes and microdilution wells. *S. ureae* DSM 2281^T, *B. globisporus* DSM 4^T, *B. psychrophilus* KCTC 3446^T and *B. pasteurii* KCTC 3558^T were used as reference strains.

16S rDNA sequencing and phylogenetic analysis. 16S rDNA was amplified by PCR using two universal primers as described previously (Yoon *et al*., 1998). The PCR product was purified by using a QIAquick PCR Purification kit (Qiagen). The purified 16S rDNA was sequenced using an

ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) as recommended by the manufacturer. The purified sequencing reaction mixtures were electrophoresed using an Applied Biosystems model 310 automatic DNA sequencer. The 16S rDNA sequences of strain SW28^T and reference organisms were aligned by using CLUSTAL W software (Thompson *et al.*, 1994) and the resulting alignment was then modified to remove regions containing ambiguous bases and gaps. Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods. The PHYLIP package (Felsenstein, 1993) was used for the neighbour-joining, maximum-likelihood and maximum-parsimony analyses. Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of Jukes & Cantor (1969) and the DNADIST program. The stability of relationships was assessed by a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT and CONSENSE of the PHYLIP package.

Nucleotide sequence accession numbers. GenBank accession numbers for reference 16S rDNA sequences used in this analysis are as follows: L14013 (*Bacillus fusiformis* ATCC 7055^T), X68415 (*B. globisporus* DSM 4^T), X60642 (*Bacillus insolitus* DSM 5^T), X60631 (*B. pasteurii* NCIMB 8841^T), D16277 (*B. psychrophilus* IAM 12468^T), AJ006086 (*Bacillus silvestris* DSM 12223^T), D16280 (*Bacillus sphaericus* IAM 13420^T), X60646 (*Bacillus subtilis* NCDO 1769^T), X70314 (*Caryophanon latum* NCIMB 9533^T), X70315 (*Caryophanon tenue* NCDO 2324^T), X70320 (*Kurthia gibsonii* NCIMB 9758^T), X70321 (*Kurthia zopfii* NCIMB 9878^T), X62172 (*Planococcus citreus* NCIMB 1493^T), X62173 (*Planococcus kocurii* NCIMB 629^T) and AF202057 (*S. ureae* DSM 2281^T).

RESULTS

Morphology

Strain SW28^T was Gram-variable and motile by means of a single polar flagellum (Fig. 1). Cells were rod-shaped and measured approximately 0·9–1·2 μm wide by 2·0–3·5 μm long in 3 d cultures on TSA at 25 °C. Round terminal spores were observed in swollen sporangia. Colonies were smooth, glistening, circular to irregular, flat to raised and light-orange-coloured after 3 d on TSA.

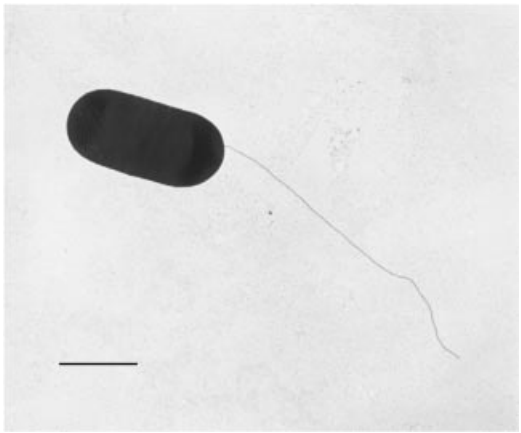

Fig. 1. Transmission electron micrograph of strain SW28^T from an exponentially growing culture. Bar, 1 μm.

Cultural and physiological characteristics

Strain SW28^T was facultatively anaerobic. It grew well in both TSA and TSA supplemented with artificial sea water. Strain SW28^T grew at 4–37 °C but not at 40 °C. The optimal growth temperature was 25 °C and it also grew well at 10–20 °C. The optimal pH for growth was 6·5–7·0 and growth was inhibited at pH values below 5·0. Strain SW28^T grew in the presence of 12 % NaCl and weakly in the presence of 13 % NaCl. Growth did not occur in the presence of more than 14 % NaCl. Strain SW28^T showed catalase, oxidase and urease acitivities. No hydrolysis of aesculin, arbutin, casein, hypoxanthine, starch, Tween 80 or xanthine was observed. Gelatin was hydrolysed and tyrosine was weakly hydrolysed. Nitrate was reduced to nitrite. Arginine was not deaminated and indole was not produced. Acid was produced from *N*-acetylglucosamine, aesculin, fructose, glycerol, 5-ketogluconate, ribose and D-tagatose.

Chemotaxonomic characteristics and DNA base composition

Strain SW28^T did not contain diaminopimelic acid as the diagnostic amino acid in the cell wall peptidoglycan. The cell wall of strain SW28^T contained the amino acids lysine, glutamic acid, aspartic acid and alanine in a molar ratio of about 1 : 1 : 1 : 3. Accordingly, the peptidoglycan type of strain SW28^T is A4α, based on L-Lys-L-Ala-D-Asp, as described by Schleifer & Kandler (1972). The lack of ε- (aminosuccinyl-) lysine in the total hydrolysate and the occurrence of the peptide N^6-Ala-Lys in the partial hydrolysate was helpful to distinguish the rather uncommon type from the more common type A4α, L-Lys-D-Asp. The predominant isoprenoid quinone was an unsaturated menaquinone with seven isoprene units (MK-7). The cellular fatty acid profile of strain SW28^T was compared with those of the type strains of *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii*, which form a coherent cluster with strain SW28^T. The cellular fatty acid profile of strain SW28^T is characterized by having ante-$C_{15:0}$ as the major fatty acid and was similar with those of *S. ureae* DSM 2281^T, *B. globisporus* DSM 4^T, *B. psychrophilus* KCTC 3446^T and *B. pasteurii* KCTC 3558^T (Table 1). The G+C content of strain SW28^T is 40 mol %.

Phylogenetic analysis

The 16S rDNA of strain SW28^T was directly sequenced following PCR amplification and its almost complete nucleotide sequence was determined. The 16S rDNA sequence of strain SW28^T determined was 1508 bp

Table 1. Cellular fatty acid profiles of strain SW28^T and some related organisms

1, *B. globisporus* DSM 4^T; 2, *B. psychrophilus* KCTC 3446^T; 3, *B. pasteurii* KCTC 3558^T; 4, *S. ureae* DSM 2281^T; 5, strain SW28^T.

Fatty acid	1	2	3	4	5
Saturated fatty acids					
$C_{12:0}$				0·2	
$C_{14:0}$	0·6	0·9	1·1	0·8	1·9
$C_{15:0}$	0·4	0·5	3·3	0·7	
$C_{16:0}$	0·9	1·5	4·7	2·1	3·8
$C_{18:0}$		0·2		0·6	0·5
Unsaturated fatty acids					
$C_{16:1}$ $\omega 7c$ alcohol	5·5	2·4	2·8	1·4	0·2
$C_{16:1}$ $\omega 11c$	3·1	1·8	3·2	2·1	0·6
$C_{17:1}$ $\omega 7c$		0·2			
$C_{18:1}$ $\omega 9c$		0·3	0·8	0·5	0·4
Branched fatty acids					
ante-$C_{13:0}$		0·1		0·7	0·3
iso-$C_{14:0}$	3·4	4·1	15·4	2·1	3·6
iso-$C_{15:0}$	4·0	6·4	6·9	7·0	5·4
ante-$C_{15:0}$	61·8	68·4	48·6	68·7	77·3
iso-$C_{16:0}$	1·5	2·1	7·5	1·1	1·9
iso-$C_{17:0}$		0·2		0·5	
ante-$C_{17:0}$	6·9	6·6	4·0	8·8	4·1
iso-$C_{17:1}$ $\omega 10c$	0·3			0·4	
Summed feature 4*	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 which could not be separated by GLC with the MIDI system. Summed feature 4 contained one or more of following fatty acids: iso-$C_{17:1}$ I and/or ante-$C_{17:1}$ B.

long, corresponding to the region between positions 28 and 1524 by comparison with the *Escherichia coli* 16S rRNA sequence. A total of 1289 nucleotides present in all strains between positions 28 and 1431 (*E. coli* numbering) was used to construct phylogenetic trees. In the tree based on the neighbour-joining algorithm, strain SW28^T falls within the radiation of a cluster comprising *Bacillus* species belonging to rRNA group 2 and some non-*Bacillus*-type organisms and forms a coherent cluster with *S. ureae*, *B. globisporus*, *B. psychrophilus*, *B. pasteurii* and *B. insolitus* (Fig. 2). The same topology was also found in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. The 16S rDNA sequence similarity values between strain SW28^T and the type strains of *Bacillus* species and non-*Bacillus* type species belonging to rRNA group 2 whose 16S rRNA gene sequences are known are in the range 92·1–96·8 %. The highest 16S rDNA similarity value was found in the relationship between strain SW28^T and *S. ureae* DSM 2281^T (96·8 %). Levels of 16S rDNA similarity between strain SW28^T and the type strains of some phylogenetically related *Bacillus* species are as follows: *B. globisporus*, 96·2 %; *B. psychrophilus*, 96·2 %; *B. pasteurii*, 96·2 %; and *B. insolitus*, 95·1 %.

DNA–DNA relatedness

DNA–DNA relatedness was determined between strain SW28^T and the type strains of *S. ureae* and some *Bacillus* species of the rRNA group 2 that form a coherent cluster. Strain SW28^T exhibited levels of DNA relatedness of 12·5, 9·7, 10·3 and 8·5 % with *S. ureae* DSM 2281^T, *B. globisporus* DSM 4^T, *B. psychrophilus* KCTC 3446^T and *B. pasteurii* KCTC 3558^T, respectively.

DISCUSSION

The result of 16S rDNA sequence analysis clearly indicates that strain SW28^T falls within the phylogenetic radiation comprising members of the *Bacillus* rRNA group 2. In particular, strain SW28^T exhibited the closest phylogenetic affiliation with *S. ureae* and was also found to form a coherent cluster with *B. globisporus*, *B. psychrophilus*, *B. pasteurii* and *B. insolitus* (Fig. 2). Cells of strain SW28^T and these *Bacillus* species are rods, whereas the genus *Sporosarcina* has spherical cells that are arranged in tetrads or packages (Claus & Fahmy, 1986). However, cellular morphology may not be a meaningful characteristic to decide the taxonomic status of bacterial species at the

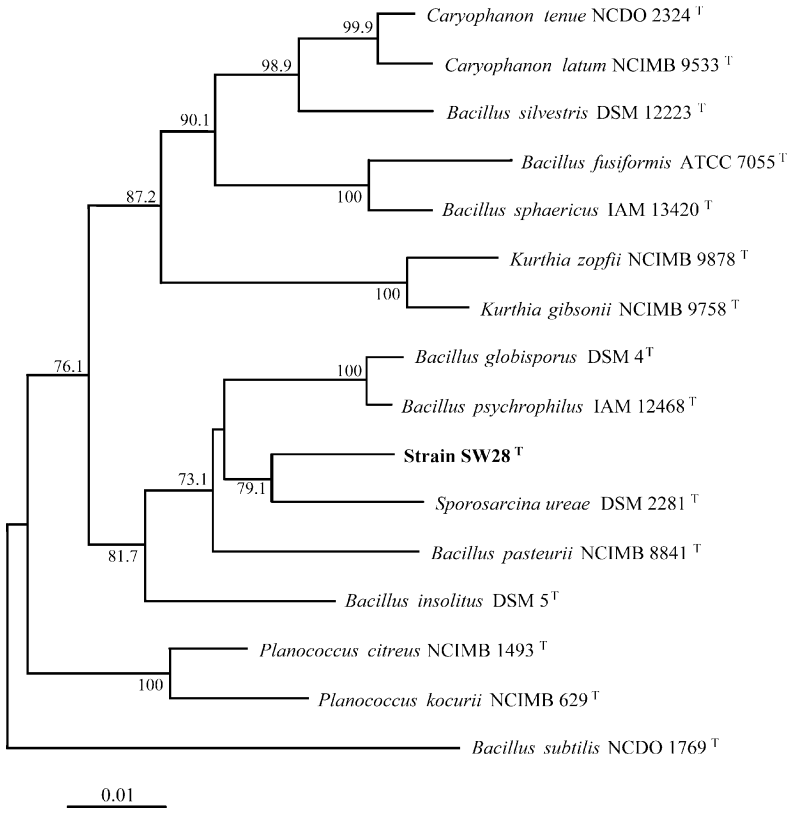

Fig. 2. Neighbour-joining tree based on 16S rDNA sequences showing the positions of strain SW28^T, members of *Bacillus* rRNA group 2 and *Bacillus subtilis*. Scale bar represents 0·01 substitution per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points.

genus level (Koch *et al*., 1994, 1995). Strain SW28^T, *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* have L-lysine as the diagnostic amino acid at position 3 of the peptide subunit of the peptidoglycan, and MK-7 as the predominant menaquinone. The cellular fatty acid profile is similar in strain SW28^T and the type strains of *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* (Table 1), although *B. pasteurii* KCTC 3558^T was cultivated on a different medium. They all contain ante-$C_{15:0}$ as the major fatty acid. It has been expected that *Bacillus* species belonging to rRNA group 2 should be differentiated from members of the *Bacillus* rRNA group 1 by morphology, phylogeny and the cell wall composition (Farrow *et al*., 1994; Rheims *et al*., 1999). Farrow *et al*. (1994) have already shown that *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* cluster together in phylogenetic analyses and form a stable line of descent supported by bootstrapping data, and this stability can be used as evidence for assigning these species to a single, redefined genus, *Sporosarcina*. Nevertheless, no proposal for the taxonomic reclassification of the rRNA group 2 bacilli has been put forward. From the results of chemotaxonomic (Table 2) and phylogenetic analysis, it is likely to be more appropriate that SW28^T be considered as a member of the genus *Sporosarcina* and at least *B. globisporus*, *B. psychrophilus* and *B. pasteurii* be reclassified as members of the genus *Sporosarcina*. Although *B. insolitus* is also phylogenetically related to the redefined phyletic group, it contains D-ornithine instead of L-lysine at position 3 of the peptide subunit of the peptidoglycan. It is therefore better that *B. insolitus* not be included in the redefined genus *Sporosarcina*.

The cell wall peptidoglycan structure of strain SW28^T is important for distinguishing this organism from *S. ureae* and some phylogenetically related *Bacillus* species (Rheims *et al*., 1999). Strain SW28^T has the peptidoglycan structure L-Lys-L-Ala-D-Asp, whereas the type strain of *S. ureae* has the peptidoglycan type based on L-Lys-Gly-D-Glu (Stackebrandt *et al*., 1987). The type strains of *B. globisporus* and *B. psychrophilus* have the peptidoglycan type based on L-Lys-D-Glu (Claus & Fritze, 1989; Stackebrandt *et al*., 1987). The type strain of *B. pasteurii* has the peptidoglycan type based on L-Lys-D-Asp as cited by Schleifer & Kandler (1972). The reanalysis of the cell wall of the type strain of *B. pasteurii* confirmed the previous data. The colony colour and growth on TSA distinguish strain SW28^T from the type strains of *S. ureae* and some *Bacillus* species that form a coherent cluster with strain SW28^T. Strain SW28^T has light-orange-coloured colonies, whereas colonies of the type strains of *B. globisporus*, *B. psychrophilus* and *S. ureae* are cream to yellow. Moreover, *B. pasteurii* KCTC 3558^T showed no growth on TSA. Strain SW28^T also shows a little difference from the type strains of *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* in the composition of fatty acid ante-$C_{15:0}$ (Table 1), al-

Table 2. Chemotaxonomic properties of strain SW28^T and related organisms of the *Bacillus* rRNA group 2

Species	Murein type*	Menaquinone system*	Major fatty acid	G+C content (mol %)*
Strain SW28^T	L-Lys-L-Ala-D-Asp	MK-7	ante-$C_{15:0}$	40
Sporosarcina ureae	L-Lys-Gly D-Glu	MK-7	ante-$C_{15:0}$†	40–42
Bacillus globisporus	L-Lys-D-Glu	MK-7	ante-$C_{15:0}$†	40
Bacillus psychrophilus	L-Lys-D-Glu	MK-7	ante-$C_{15:0}$†	40–41
Bacillus pasteurii	L-Lys-D-Asp‡	MK-7	ante-$C_{15:0}$†	39
Bacillus insolitus	D-Orn-D-Glu	MK-7	NA	36
Bacillus fusiformis	NA	NA	NA	35–36
Bacillus silvestris	L-Lys-D-Glu	MK-7	iso-$C_{15:0}$*	39
Bacillus sphaericus§	L-Lys-D-Asp	MK-7	iso-$C_{15:0}$	37
Caryophanon tenue	L-Lys-D-Glu	MK-6	NA	41–42
Caryophanon latum	L-Lys-D-Glu	MK-6	NA	44–46
Kurthia zopfii	L-Lys-D-Asp	MK-7	NA	36–38
Kurthia gibsonii	L-Lys-D-Asp	MK-7	NA	36–38
Filibacter limicola	L-Lys-D-Glu	MK-7	NA	44
Planococcus citreus	L-Lys-D-Glu	MK-7, MK-8	ante-$C_{15:0}$‖	48–52
Planococccus kocurii	L-Lys-D-Glu	MK-7, MK-8	ante-$C_{15:0}$‖	40–43¶

NA, Data not available.

* Data from Rheims *et al.* (1999).

† Data from this study.

‡ Data from Schleifer & Kandler (1972) and this study.

§ Major fatty acid data from *B. sphaericus* JCM 2502^T (Shida *et al.*, 1997) and other data from *B. sphaericus* NCDO 1767.

‖ Data from Hao & Komagata (1985) and Nakagawa *et al.* (1996).

¶ Data from Hao & Komagata (1985).

though standardized conditions between them were not used. Strain SW28^T exhibits levels of 16S rDNA similarity of 96·8 % with *S. ureae* DSM 2281^T and less than 96·2 % with the type strains of *B. globisporus*, *B. psychrophilus* and *B. pasteurii*. According to the report of Stackebrandt & Goebel (1994), strain SW28^T can be differentiated from *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* only by the results of 16S rDNA sequence analysis. DNA–DNA relatedness between strain SW28^T and *S. ureae* DSM 2281^T and the type strains of some *Bacillus* species which form a phylogenetically coherent cluster is low enough to exclude the possibility of assigning strain SW28^T to one of such species. Levels of DNA relatedness clearly indicate that strain SW28^T is a species different from *S. ureae*, *B. globisporus*, *B. psychrophilus* and *B. pasteurii* (Wayne *et al.*, 1987). Therefore, on the basis of the phenotypic and chemotaxonomic characteristics, the phylogeny and the genomic distinctiveness, strain SW28^T should be placed within the genus *Sporosarcina* as a new species, for which we propose a new name, *Sporosarcina aquimarina* sp. nov. In addition, it is proposed to transfer *B. globisporus* (Larkin and Stokes 1967), *B. psychrophilus* (Nakamura 1984) and *B. pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., respectively.

Description of *Sporosarcina aquimarina* sp. nov.

Sporosarcina aquimarina (a.qui.ma.ri′na. L. n. *aqua* water; L. adj. *marinus* of the sea; M.L. adj. *aquimarina* pertaining to seawater).

Cells are facultatively anaerobic, endospore-forming rods that are approximately 0·9–1·2 μm wide and 2·0–3·5 μm long in 3 d cultures on TSA. Round terminal spores are observed in swollen sporangia. Gram-variable. Motile by means of a single polar flagellum. Colonies are light-orange-coloured, smooth, circular to irregular and raised on TSA. Catalase-, oxidase- and urease-positive. Aesculin, arbutin, casein, hypoxanthine, starch, Tween 80 and xanthine are not hydrolysed. Gelatin is hydrolysed and tyrosine is weakly hydrolysed. Nitrate is reduced to nitrite. Arginine is not deaminated and indole is not produced. Acid is produced from *N*-acetylglucosamine, aesculin, fructose, glycerol, 5-ketogluconate, ribose and D-tagatose. No acid is produced from erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, *β*-methyl-D-xyloside, galactose, glucose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-fucose, L-fucose, D-

arabitol, L-arabitol, gluconate and 2-ketogluconate. Grows in the presence of 13% NaCl. No growth occurs in the presence of more than 14% NaCl. Growth occurs at 4 and 37 °C, but not at 40 °C. The optimal growth temperature is 25 °C. The optimal pH for growth is 6·5–7·0. Growth is inhibited below pH 5·0. The peptidoglycan type is A4α (L-Lys-L-Ala-D-Asp). The predominant menaquinone is MK-7. The major fatty acid is ante-$C_{15:0}$. The G+C content is 40 mol% (determined by HPLC). Isolated from seawater in Korea. The type strain is strain SW28^T, which has been deposited in the Korean Culture Center of Microorganisms as KCCM 41039^T and in the Japan Collection of Microorganisms as JCM 10887^T.

Description of *Sporosarcina globispora* (Larkin and Stokes 1967) comb. nov.

The description is the same as that given by Larkin & Stokes (1967) and Claus & Berkeley (1986).

Description of *Sporosarcina psychrophila* (Nakamura 1984) comb. nov.

The description is the same as that given by Nakamura (1984) and Claus & Berkeley (1986).

Description of *Sporosarcina pasteurii* (Chester 1898) comb. nov.

The description is the same as that given by Chester (1898) and Claus & Berkeley (1986).

Emended description of the genus *Sporosarcina* Kluyver and van Niel 1936

Sporosarcina. (Spo.ro.sar.ci′na. M.L. n. *spora* a spore; M.L. fem. n. *Sarcina* generic name; M.L. fem. n. *Sporosarcina* spore-forming *Sarcina*).

Cells are spherical or rods. Gram-positive or Gram-variable. Endospores are round. Motile (not determined for *Sporosarcina pasteurii*). Facultatively anaerobic or strictly aerobic. Oxidase-positive (not determined for *Sporosarcina pasteurii* but positive for the type strain of *Sporosarcina pasteurii*). Catalase-positive (not determined for *Sporosarcina pasteurii* but positive for the type strain of *Sporosarcina pasteurii*). Gelatin and urea are hydrolysed. Starch is not hydrolysed. Nitrate reduction to nitrite is variable. Growth at 3% NaCl is variable. The predominant menaquinone is MK-7. The major fatty acid is ante-$C_{15:0}$. The diagnostic amino acid at position 3 of the peptide subunit of the peptidoglycan is L-lysine. The G+C content of the genomic DNA is 38–42 mol%. The type species is *Sporosarcina ureae*.

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