Zhongshania aliphaticivorans sp. nov., an aliphatic hydrocarbon-degrading bacterium isolated from marine sediment, and transfer of *Spongiibacter borealis* Jang *et al.* 2011 to the genus *Zhongshania* as *Zhongshania borealis* comb. nov.

Naysim LO, Hyo Jung Kang and Che Ok Jeon

Correspondence
Hyo Jung Kang
hyokang@cau.ac.kr
Che Ok Jeon
cojeon@cau.ac.kr

Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul, 156-756, Republic of Korea

A Gram-staining-negative, facultatively aerobic bacterium, designated SM-2^T, was isolated from a sea-tidal flat of Yellow Sea, South Korea. Cells were catalase- and oxidase-positive motile rods with a single polar flagellum. Growth of strain SM-2^T was observed at 10–37 °C (optimum, 25–30 °C), at pH 5.5–8.5 (optimum, pH 7.0–7.5) and in the presence of 0–11 % (w/v) NaCl (optimum, 2 %). Strain SM-2^T contained ubiquinone-8 (Q-8) as the sole isoprenoid quinone and $C_{17:1}\omega8c$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{17:0}$ and $C_{18:1}\omega7c$ as the major fatty acids. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unidentified lipid were identified as the major cellular polar lipids. The G+C content of the genomic DNA was 52.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SM-2^T formed a tight phyletic lineage with *Zhongshania antarctica* ZS5-23^T, *Zhongshania guokunii* ZS6-22^T and *Spongiibacter borealis* CL-AS9^T, but that *S. borealis* CL-AS9^T was distinct from other species of the genus *Spongiibacter*. Based on 16S rRNA gene sequence similarities, strain SM-2^T was most closely related to *S. borealis* CL-AS9^T, *Z. antarctica* ZS5-23^T and *Z. guokunii* ZS6-22^T, with similarities of 99.5 %, 98.9 % and 98.7 %, respectively, but the DNA–DNA hybridization values among these species were clearly lower than 70 %. On the basis of chemotaxonomic data and molecular properties, we propose strain SM-2^T represents a novel species of the genus *Zhongshania* with the name *Zhongshania aliphaticivorans* sp. nov. (type strain SM-2^T=KACC 18120^T=JCM 30138^T). We also propose the transfer of *Spongiibacter borealis* Jang *et al.* 2011 to the genus *Zhongshania* as *Zhongshania borealis* comb. nov. (type strain CL-AS9^T=KCCM 90094^T=JCM 17304^T).

The genus *Zhongshania* was first proposed by Li *et al.* (2011) as a member of the class *Gammaproteobacteria* and at the time of writing, the genus *Zhongshania* includes only two species, *Zhongshania antarctica* (type species) and *Zhongshania guokunii* (Li *et al.*, 2011), isolated from marine environments. Members of the genus *Zhongshania* are Gram-negative, catalase- and oxidase-positive, aerobic, motile rods that contain $C_{17:1}\omega8c$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{17:0}$ as the major fatty acids (Li *et al.*, 2011). Sea-tidal flats are broad, plain coastal areas that experience exposure and flooding by low and high tides of seawater and are characterized by high nutrient and carbon cycling rates, which may rely upon high microbial abundance and diversity (Stevens *et al.*, 2007). Sea-tidal flats also harbour diverse pollutant-degrading microbial communities, which may play important roles in the remediation of marine environments (Jin *et al.*, 2012). The west coastal areas of the Korean peninsula largely consists of sea-tidal flats. We have made efforts to isolate and characterize pollutant-degrading bacteria from sea-tidal flats (Jin *et al.*, 2012, 2013) and a novel aliphatic hydrocarbon-degrading bacterium, designated strain SM-2^T, belonging to the genus *Zhongshania* was isolated from a sea-tidal flat sample. Here, we describe the taxonomic characteristics of strain SM-2^T using a polyphasic approach. Recently,

Abbreviations: DPG, diphosphatidylglycerol; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SM-2^T is KF982857.

Two supplementary figures and a supplementary table are available with the online version of this paper.

Spongiibacter borealis was described as a member of the genus *Spongiibacter* (Jang *et al.*, 2011). However, phylogenetic analysis based on 16S rRNA gene sequences showed that *S. borealis* formed a tight phylogenetic lineage with members of the genus *Zhongshania* with more than 99.3 % 16S rRNA gene sequence similarities, and was distinctly related to other species of the genus *Spongiibacter* with less than 94.1 % 16S rRNA gene sequence similarities. Therefore, in this study we also propose the transfer of *S. borealis* to the genus *Zhongshania*.

Strain SM-2^T capable of aliphatic hydrocarbon degradation was isolated from a sea-tidal flat using a previously described procedure with some modifications (Jin *et al.*, 2012). For the enrichment and isolation of aliphatic hydrocarbon-degrading bacteria, a tidal flat sediment sample was obtained from the Dangjin coastal area in South Korea (36° 58′ 33.59′′ N 126° 46′ 52.32′′ E). Approximately 0.3 g of a three aliphatic hydrocarbon mixture (C_8 : C_{16} : C_{24},1 : 1 : 1) were added directly into a cotton-plugged 500 ml Erlenmeyer flask containing 10 g tidal flat sediment sample and 100 ml 0.2 μm-filtered seawater. The enrichment culture was incubated at 25 °C and 180 r.p.m. and transferred (1 : 20) into fresh seawater containing the aliphatic hydrocarbon mixture three times every two weeks. To isolate aliphatic hydrocarbon-degrading bacteria from the final enrichment culture, the enrichment culture was diluted and spread onto marine agar 2216 (MA; Becton Dickinson) and incubated at 25 °C for 3 days. The 16S rRNA genes of colonies grown on MA were PCR-amplified using the universal primers F1 and R13 (Jung *et al.*, 2011) and double-digested with a mixture of *Hha*I and *Hae*III. The PCR products with distinct restriction fragment patterns were sequenced and the resulting 16S rRNA gene sequences were analysed using the Nucleotide Similarity Search program (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). From the analysis, a putative novel strain belonging to the genus *Zhongshania*, designated strain SM-2^T, showing aliphatic hydrocarbon-degrading ability was selected for further phenotypic and phylogenetic analyses. Strain SM-2^T was routinely grown aerobically on MA at 25 °C for 3 days, except where indicated, and stored at −80 °C in marine broth 2216 (MB; BD) supplemented with 15 % (v/v) glycerol for preservation. *Z. antarctica* KACC 14066^T, *Z. guokunii* KACC 14532^T, *Spongiibacter marinus* KACC 15453^T and *S. borealis* JCM 17304^T were purchased from their respective culture collection centres and used as reference strains for phenotypic comparisons, fatty acid analysis and DNA–DNA hybridizations.

The 16S rRNA gene amplicon of strain SM-2^T was ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer's instructions and was sequenced with the M13 reverse and T7 primers of the TOPO cloning kit. The resulting 16S rRNA gene sequence (1403 nt) was checked manually for the evaluation of quality and gaps. Sequence similarities between strain SM-2^T and related taxa were evaluated using the Nucleotide Similarity Search program and aligned using the fast secondary-structure aware Infernal aligner available in Ribosomal Database Project (RDP) (Nawrocki & Eddy, 2007). Phylogenetic trees using the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms were reconstructed by the PHYLIP software (version 3.68, Felsenstein, 2002) and the resulting tree topologies were evaluated using bootstrap analyses referred to 1000 resampled datasets within the PHYLIP package. Maximum-likelihood (ML) analysis with bootstrap values was performed by RAxML-HPC BlackBox (version 7.2.8) of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org; Stamatakis *et al.*, 2005) at the San Diego Supercomputer Center.

Comparative analysis based on the 16S rRNA gene sequences showed that strain SM-2^T was most closely related to *S. borealis* CL-AS9^T (99.5 % 16S rRNA gene sequence similarity), *Z. antarctica* ZS5-23^T (98.9 %) and *Z. guokunii* ZS6-22^T (98.7 %). Phylogenetic analysis using the NJ algorithm showed that strain SM-2^T formed a tight phylogenetic lineage with the members of the genus *Zhongshania* with 100 % bootstrap value (Fig. 1). *S. borealis* CL-AS9^T, showing the highest 16S rRNA gene sequence similarity (99.5 %) with strain SM-2^T, also formed a tight phylogenetic lineage with strain SM-2^T as well as members of the genus *Zhongshania*, but *S. borealis* CL-AS9^T was clearly distinct from other species of the genus *Spongiibacter*. Phylogenetic trees reconstructed using the ML and MP algorithms also showed that strain SM-2^T and *S. borealis* CL-AS9^T formed a tight phyletic lineage with members of the genus *Zhongshania* but that *S. borealis* CL-AS9^T was distinct from other species of the genus *Spongiibacter*.

Genomic DNA from strain SM-2^T and the type strains of *S borealis*, *Z. antarctica* and *Z. guokunii* was extracted according to the procedure of Sambrook & Russell (2001). Pairwise DNA–DNA hybridization analyses were reciprocally performed in triplicate to evaluate DNA–DNA relatedness among the four closely related strains using the DIG High Prime DNA Labelling kit (Roche Applied Science), as described previously (Lee *et al.*, 2011). Hybridization signals were scanned and analysed using Adobe Photoshop CS6 (version 13.0). Signals produced by the hybridization of the probes to the identical target DNA were taken to be 100 %, and the signal intensities from self-hybridizations of serial dilutions were used for the calculation of the DNA–DNA relatedness among the four strains. The DNA–DNA relatedness values among the four closely related strains were clearly lower than 70 %, the threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001) (Table 1).

Growth of strain SM-2^T was tested at 25 °C on R2A agar (BD), laboratory-prepared Luria–Bertani (LB) agar, nutrient agar (NA; BD), tryptic soy agar (TSA; BD) and MA, which were additionally supplemented with approximately 2 % (w/v) NaCl (final concentration). Growth of strain

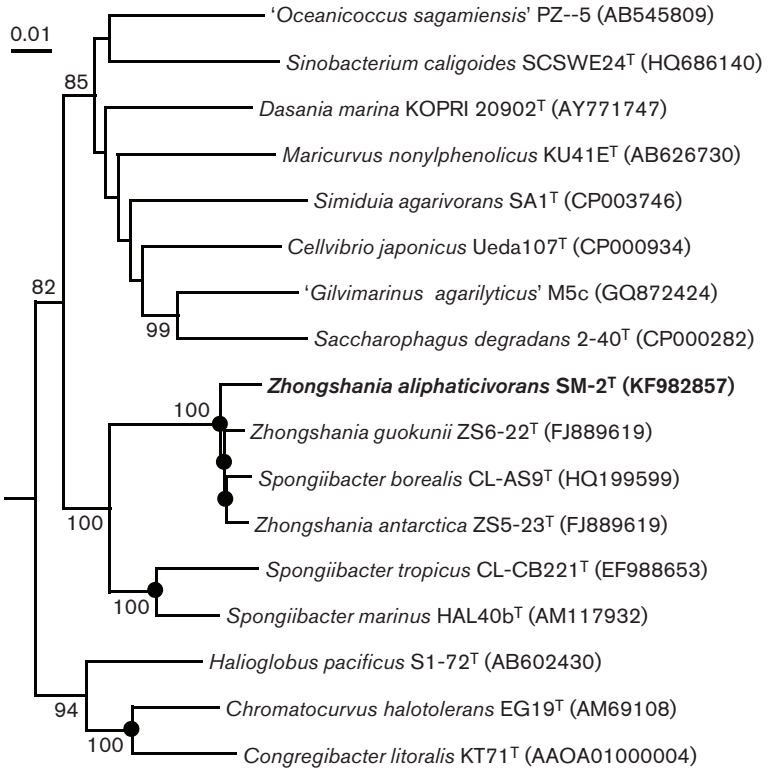

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain SM-2^T, *S. borealis* CL-AS9^T and related taxa. Bootstrap values are shown on nodes in percentages of 1000 replicates; only values >70 % are given. *Thermotoga maritima* MSB8^T (GenBank accession no. M21774) was used as an outgroup (not shown). Filled circles indicate that the corresponding nodes were also recovered in trees generated by the MP and ML algorithms. Bar, 0.01 changes per nucleotide position.

SM-2^T was assessed in MB at different temperatures (5, 10, 15, 20, 25, 30, 35, 37 and 40 °C) and pH values (5.0–11.0 at 0.5 pH unit intervals). MB with pH <8.0 and pH 8.0–11.0 was prepared using the Na_2HPO_4–NaH_2PO_4 and Na_2CO_3–$NaHCO_3$ buffers, respectively (Gomori, 1955). After sterilization (121 °C, 15 min), the pH values were adjusted again if necessary. Growth at different NaCl concentrations (0–10 % at 1 % intervals) was investigated using MB prepared in the laboratory according to the BD formula. Gram staining was tested using the bioMérieux Gram stain kit according to the manufacturer's instructions. Anaerobic growth was assessed on MA and on MA under anaerobic conditions (with 4–10 % CO_2) using the GasPak Plus system (BBL) at 25 °C for 20 days. Cell morphology of strain SM-2^T and the presence of flagella were investigated using phase-contract microscopy and transmission electron microscopy (JEM-1010; JEOL) with cells from an exponentially grown culture in MB at 25 °C. Oxidase activity was evaluated by the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was tested by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994). Nitrate reduction was assessed according to the method of Lányí (1987). The following properties of strain SM-2^T and reference strains were evaluated in parallel under the same conditions at 25 °C. Hydrolysis of Tweens 20 and 80, casein, starch and tyrosine was tested on MA according to the methods described previously (Lányí, 1987; Smibert & Krieg, 1994). Acid production from D-galactose, lactose, D-mannose, D-sorbitol and sucrose was assessed as described by Leifson (1963). Substrate utilization as a single carbon and energy source was determined in a marine minimal medium containing 1 % (w/v) of each substrate (L-arabinose, D-fructose, D-glucose, glycerol, pyruvate and xylose) as described by Yurkov *et al.* (1994). Additional enzymic activities, biochemical features

Table 1. Pairwise DNA–DNA hybridization among strain SM-2^T and type strains of *S. borealis* and species of the genus *Zhongshania*

Strain	Hybridization (% ±SD) with labelled DNA from:			
	1	2	3	4
1. SM-2^T	100	44.2±6.4	32.8±4.8	36.9±5.3
2. *S. borealis* JCM 17304^T	52.6±4.3	100	33.6±5.5	41.1±3.4
3. *Z. antarctica* KACC 14066^T	44.3±5.2	39.9±6.2	100	31.3±6.7
4. *Z. guokunii* KACC 14532^T	42.6±2.7	42.9±5.7	39.0±6.6	100

and utilization of carbon sources were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog) according to the instructions of the manufacturers except that inocula were prepared by resuspending cells in artificial seawater. Antibiotic susceptibility was tested as described previously (Jeong *et al.*, 2013).

Strain SM-2^T grew well on R2A agar supplemented with 2 % NaCl and on MA, but did not grow on NA and TSA supplemented with 2 % NaCl. Cells were Gram-staining-negative, facultatively anaerobic, motile rods with a single polar flagellum (0.4–0.6 μm in width and 1.0–2.0 μm in length) (Fig. S1, available in the online Supplementary Material). Strain SM-2^T was resistant to ampicillin, carbenicillin, gentamicin and kanamycin, but sensitive to lincomycin, oleandomycin, neomycin, novobiocin and tetracycline. Other phenotypic characteristics of strain SM-2^T are presented in Table 2, the species description and Table S1. Most properties such as motility with a single polar flagellum, oxidase- and catalase-positive reactions and nitrate reduction ability, are in accordance with those of species of the genus *Zhongshania*, whereas some other properties such as growth temperature range, starch hydrolysis and glucose utilization allow the differentiation of strain SM-2^T from other species of the genus *Zhongshania* (Table 2 and Table S1).

Isoprenoid quinones of strain SM-2^T were analysed using a HPLC (model LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 × 4.6 mm, Kromasil; Akzo Nobel) as described previously (Komagata & Suzuki, 1987). Methanol/2-propanol (2 : 1, v/v) was used as an eluent at a flow rate of 1 ml min^{-1}. For the analysis of cellular fatty acids, strain SM-2^T and the four reference strains were cultivated in MB at 25 °C and microbial cells were harvested at the same growth phase (exponential phase, OD_{600}=0.8). The cellular fatty acids were saponified, methylated and extracted using the

Table 2. Phenotypic characteristics of strain SM-2^T and type strains of related species of the genera *Zhongshania* and *Spongiibacter*

Strains: 1, SM-2^T (data from this study); 2, *S. borealis* JCM 17304^T (Jang *et al.*, 2011); 3, *Z. antarctica* KACC 14066^T (Li *et al.*, 2011); 4, *Z. guokunii* KACC 14532^T (Li *et al.*, 2011); 5, *S. marinus* KACC 15453^T (Graeber *et al.*, 2008). All strains are positive for the following characteristics: hydrolysis of Tweens 20 and 80 and aesculin; alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase activities; and assimilation of D-mannitol. All strains are negative for Gram staining, indole production, hydrolysis of casein, tyrosine, gelatin and urea; acid production from D-galactose, D-mannose and sucrose; utilization of L-arabinose; trypsin, α-chymotrypsin, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase and 4-nitrophenyl-β-D-galactopyranoside activities; and assimilation of potassium gluconate, D-mannose, *N*-acetylglucosamine, capric acid and adipic acid. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	10−37	4−30	4−35	4−35	10−40
NaCl range for growth (%, w/v)	0−11.0	1.0−8.0	0−9.0	0−7.5	1.0−7.0
pH range for growth	5.5−8.5	5.8−9.3	5.5−9.0	5.5−9.0	6.5−9.5
Nitrate reduction*	+	+	+	+	−
Hydrolysis of starch*	+	−	−	−	−
Acid production from:*					
Lactose	−	+	−	−	−
D-Sorbitol	−	+	−	+	−
Substrate utilization of:*					
D-Fructose	+	−	+	+	−
D-Glucose	+	+	−	−	−
Glycerol	−	+	−	+	−
Pyruvate	+	W	−	−	−
Xylose	−	+	−	−	−
Enzyme activities (API ZYM)*					
Lipase (C14), valine arylamidase,	−	−	W	−	−
Naphthol-AS-BI-phosphohydrolase	+	W	W	+	+
Assimilation of: (API 20NE)*					
D-Glucose	+	+	−	−	−
Maltose	−	W	+	−	−
Malic acid	+	W	W	−	−
Trisodium citrate	−	−	−	−	+
Phenylacetic acid	−	W	−	−	W
DNA G+C content (mol%)	52.2	53.6	51.5	51.8	69.1

*These analyses were performed using the same conditions in this study.

standard MIDI protocol. The fatty acid methyl esters were analysed by GC (Hewlett Packard 6890) and identified by using the TSBA6 database of the Microbial Identification System (Sherlock version 6.0B; Sasser, 1990). The DNA G+C content of strain SM-2^T was determined by the fluorimetric method (Gonzalez & Saiz-Jimenez, 2002) using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). The polar lipids of strain SM-2^T and *Z. antarctica* KACC 14066^T were analysed by TLC using cells harvested during the exponential growth phase as described by Minnikin *et al.* (1977). The following reagents were used to detect different polar lipids: 10 % ethanolic molybdatophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), the Dittmer-Lester reagent (for phospholipids) and α-naphthol/sulfuric acid (for glycolipids) and the polar lipids were confirmed using authentic standard polar lipids (Sigma).

The only respiratory lipoquinone detected in strain SM-2^T was ubiquinone-8 (Q-8). The major cellular fatty acids of strain SM-2^T were $C_{17:1}\omega8c$ (31.0 %), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 23.3 %), $C_{17:0}$ (9.4 %) and $C_{18:1}\omega7c$ (8.1 %). The overall fatty acid profile of strain SM-2^T was similar to those of the reference strains of the genus *Zhongshania*; there were some differences in the respective proportions of some components (Table 3). The major cellular polar lipids of strain SM-2^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and one unidentified lipid (L), while *Z. antarctica* KACC 14066^T contained PE, PG, DPG and phosphatidylinositol (PI) (Fig. S2), which might allow differentiation of the two strains. The DNA G+C content of strain SM-2^T was 52.2 mol%, which was in the range of those of the previously reported species of the genus *Zhongshania* (Li *et al.*, 2011).

In conclusion, the physiological, biochemical and phylogenetic properties of strain SM-2^T support its description as a novel species of the genus *Zhongshania*, for which the name *Zhongshania aliphaticivorans* sp. nov. is proposed. The major lipoquinone, major fatty acids, DNA G+C content and nitrate reduction of *Spongiibacter borealis* were also in accordance with those of members of the genus *Zhongshania* and strain SM-2^T (Li *et al.*, 2011). The DNA G+C content of *S. borealis* (53.6 mol%) is quite different from that of the type species of the genus *Spongiibacter* (*S. marinus*, 69.1 mol%) and *S. borealis* reduces nitrate unlike *S. marinus* (Table 2), which can differentiate *S. borealis* from the genus *Spongiibacter*. Therefore, we propose the transfer of *Spongiibacter borealis* to the genus *Zhongshania* as *Zhongshania borealis* comb. nov.

Table 3. Cellular fatty acid composition of strain SM-2^T and type strains of related species of the genera *Zhongshania* and *Spongiibacter*

Strains: 1, SM-2^T; 2, *S. borealis* JCM 17304^T; 3, *Z. antarctica* KACC 14066^T; 4, *Z. guokunii* KACC 14532^T; 5, *S. marinus* KACC 15453^T. All data are from this study. Data are expressed as percentages of the total fatty acids; fatty acids amounting to <0.5 % in all strains are not shown. Major components (>5.0 %) are highlighted in bold. TR, Trace amount (<0.5 %); −, not detected.

Fatty acid	1	2	3	4	5
Saturated					
$C_{9:0}$	−	−	−	−	0.9
$C_{10:0}$	0.6	0.6	TR	TR	TR
$C_{11:0}$	1.2	TR	TR	TR	0.6
$C_{12:0}$	0.9	TR	1.0	1.0	TR
$C_{14:0}$	1.2	1.2	2.3	2.0	1.0
$C_{15:0}$	−	2.0	4.0	4.9	**8.0**
$C_{16:0}$	**5.7**	**11.7**	**8.3**	**9.3**	3.5
$C_{17:0}$	**9.4**	**5.7**	**5.7**	**6.4**	**5.0**
$C_{18:0}$	0.8	1.3	0.7	0.9	0.7
Hydroxy					
$C_{10:0}$ 3-OH	1.9	**5.0**	1.9	2.6	1.5
iso-$C_{11:0}$ 3-OH	TR	0.7	TR	TR	2.5
$C_{11:0}$ 3-OH	3.0	1.5	1.2	1.4	3.8
$C_{12:0}$ 3-OH	TR	0.7	0.6	0.7	TR
$C_{12:1}$ 3-OH	−	−	−	TR	0.6
Unsaturated					
$C_{15:1}\omega8c$	0.6	−	TR	TR	0.9
$C_{15:1}\omega6c$	1.1	−	0.5	TR	TR
iso-$C_{17:1}\omega9c$	−	TR	0.7	TR	0.6
$C_{17:1}\omega8c$	**31.0**	**13.0**	**18.0**	**16.6**	**40.8**
$C_{17:1}\omega6c$	1.0	TR	0.6	0.6	0.7
$C_{18:1}\omega7c$	**8.1**	**12.1**	**12.3**	**10.6**	**9.1**
Branched					
iso-$C_{13:0}$	−	TR	TR	−	0.9
iso-$C_{15:0}$	−	1.2	1.0	TR	1.7
anteiso-$C_{15:0}$	**5.5**	2.8	TR	−	−
iso-$C_{16:0}$	TR	0.6	TR	TR	−
iso-$C_{17:0}$	−	1.7	0.7	TR	0.8
anteiso-$C_{17:0}$	−	1.4	TR	−	−
Unknown					
11.799	2.2	**6.3**	4.6	4.7	TR
Summed feature 3*	**23.3**	**26.8**	**31.8**	**33.1**	**11.3**

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contains $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

Description of *Zhongshania aliphaticivorans* sp. nov.

Zhongshania aliphaticivorans (a.li.pha.ti.ci.vo′rans. N.L. part. adj. *aliphaticivorans* aliphatic; L. part. adj. *vorans* consuming; N.L. adj. *aliphaticivorans* aliphatic hydrocarbon-consuming).

Cells are Gram-staining-negative, facultatively anaerobic, motile rods with a single flagellum (0.4–0.6 μm wide and 1.0–2.0 μm long). Colonies on MA are ivory, circular, convex and smooth. Growth occurs at 10–37 °C (optimum, 25–30 °C), pH 5.5–8.5 (optimum, pH 7.0–7.5) and 0–11 % (w/v) NaCl (optimum, 2 %). Oxidase- and catalase-positive. Hydrolyses Tweens 20 and 80 and starch, but not casein or tyrosine. Nitrate is reduced to nitrite, but nitrogen gas is not produced. Acid is not produced from any of the carbohydrates tested. Utilizes D-glucose, D-fructose and pyruvate,

but not L-arabinose, glycerol or xylose. In the API ZYM strip, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-glucosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-galactosidase, β-glucosidase, α-mannosidase and α-fucosidase activities are negative. In the API 20NE strip, positive results for hydrolysis of aesculin and assimilation of malic acid, glucose and D-mannitol, but negative results for hydrolysis of urea and gelatin, indole production, arginine dihydrolase and β-galactosidase activity and assimilation of D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. In Biolog GN2 MicroPlates, utilizes Tween 40, Tween 80, *N*-acetyl-D-galactosamine, D-serine, putrescine, 2-aminoethanol, *i*-erythitol, gentiobiose, α-D-glucose, D-psicose, succinic acid monomethyl ester, acetic acid, α-ketobutyric acid, DL-lactic acid, L-aspartic acid, glycyl L-aspartic acid, L-glutamic acid, thymidine, D-mannitol, D-saccharic acid, bromosuccinic acid and DL-α-glycerol phosphate; other organic substrates included in the Biolog GN2 MicroPlates are not utilized. Contains PE, PG, DPG and one unidentified lipid as polar lipids. The major cellular fatty acids are $C_{17:1}\omega8c$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{17:0}$ and $C_{18:1}\omega7c$. The only isoprenoid quinone is Q-8.

The type strain is SM-2^T (=KACC 18120^T=JCM 30138^T), isolated from a sea-tidal flat of Dangjin bay in South Korea. The DNA G+C content of the type strain is 52.2 mol%.

Description of *Zhongshania borealis* (Jang *et al.* 2011) comb. nov.

Zhongshania borealis (bo.re.a′lis. L. fem. adj. *borealis* related to the north, boreal).

Basonym: *Spongiibacter borealis* Jang *et al.* 2011.

The description is as given for *Spongiibacter borealis* by Jang *et al.* (2011).

The type strain is CL-AS9^T (=KCCM 90094^T=JCM 17304^T).

Acknowledgements

This work was supported by grants from the National Institute of Biological Resources (NIBR), the Ministry of Environment (MOE) of the Republic of Korea (NIBR nos. 2014-02-066 and 014-02-001).

References

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Graeber, I., Kaesler, I., Borchert, M. S., Dieckmann, R., Pape, T., Lurz, R., Nielsen, P., von Döhren, H., Michaelis, W. & Szewzyk, U. (2008). *Spongiibacter marinus* gen. nov., sp. nov., a halophilic marine bacterium isolated from the boreal sponge *Haliclona* sp. 1. *Int J Syst Evol Microbiol* **58**, 585–590.

Jang, G. I., Hwang, C. Y., Choi, H.-G., Kang, S.-H. & Cho, B. C. (2011). Description of *Spongiibacter borealis* sp. nov., isolated from Arctic seawater, and reclassification of *Melitea salexigens* Urios *et al.* 2008 as a later heterotypic synonym of *Spongiibacter marinus* Graeber *et al.* 2008 with emended descriptions of the genus *Spongiibacter* and *Spongiibacter marinus*. *Int J Syst Evol Microbiol* **61**, 2895–2900.

Jeong, S. H., Park, M. S., Jin, H. M., Lee, K., Park, W. & Jeon, C. O. (2013). *Aestuariibaculum suncheonense* gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from a tidal flat and emended descriptions of the genera *Gaetbulibacter* and *Tamlana*. *Int J Syst Evol Microbiol* **63**, 332–338.

Jin, H. M., Kim, J. M., Lee, H. J., Madsen, E. L. & Jeon, C. O. (2012). *Alteromonas* as a key agent of polycyclic aromatic hydrocarbon biodegradation in crude oil-contaminated coastal sediment. *Environ Sci Technol* **46**, 7731–7740.

Jin, H. M., Choi, E. J. & Jeon, C. O. (2013). Isolation of a BTEX-degrading bacterium, *Janibacter* sp. SB2, from a sea-tidal flat and optimization of biodegradation conditions. *Bioresour Technol* **145**, 57–64.

Jung, J. Y., Kim, J. M., Jin, H. M., Kim, S. Y., Park, W. & Jeon, C. O. (2011). *Litorimonas taeanensis* gen. nov., sp. nov., isolated from a sandy beach. *Int J Syst Evol Microbiol* **61**, 1534–1538.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lee, S. H., Shim, J. K., Kim, J. M., Choi, H.-K. & Jeon, C. O. (2011). *Henriciella litoralis* sp. nov., isolated from a tidal flat, transfer of *Maribaculum marinum* Lai *et al.* 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and emended description of the genus *Henriciella*. *Int J Syst Evol Microbiol* **61**, 722–727.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Li, H. J., Zhang, X. Y., Chen, C. X., Zhang, Y. J., Gao, Z. M., Yu, Y., Chen, X. L., Chen, B. & Zhang, Y. Z. (2011). *Zhongshania antarctica* gen. nov., sp. nov. and *Zhongshania guokunii* sp. nov., *gammaproteobacteria* respectively isolated from coastal attached (fast) ice and surface seawater of the Antarctic. *Int J Syst Evol Microbiol* **61**, 2052–2057.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.

Nawrocki, E. P. & Eddy, S. R. (2007). Query-dependent banding (QDB) for faster RNA similarity searches. *PLOS Comput Biol* **3**, e56.

Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, D.C.: American Society for Microbiology.

Stamatakis, A., Ott, M. & Ludwig, T. (2005). RAxML-OMP: An efficient program for phylogenetic inference on SMPs. In *Proceedings of 8th International Conference on Parallel Computing Technologies (PaCT2005), Lecture Notes in Computer Science* 3506, 288–302, Springer Verlag.

Stevens, H., Brinkhoff, T., Rink, B., Vollmers, J. & Simon, M. (2007). Diversity and abundance of Gram positive bacteria in a tidal flat ecosystem. *Environ Microbiol* **9**, 1810–1822.

Yurkov, V., Stackebrandt, E., Holmes, A., Fuerst, J. A., Hugenholtz, P., Golecki, J., Gad'on, N., Gorlenko, V. M., Kompantseva, E. I. & Drews, G. (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.