# 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*

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A Gram-negative, rod-shaped and motile strain, designated CL-AS9$^T$, was isolated from polar seawater of the Arctic. Analysis of the 16S rRNA gene sequence of the strain showed an affiliation with the genus *Spongiibacter*, sharing 93.9 % and 93.7 % sequence similarities with the type strains of *Spongiibacter tropicus* CL-CB221$^T$ and *Spongiibacter marinus* HAL40b$^T$, respectively. Phylogenetic analyses revealed that strain CL-AS9$^T$ formed a separate branch that was distinct from a clade comprising *Spongiibacter marinus* HAL40b$^T$, *Spongiibacter tropicus* CL-CB221$^T$ and *Melitea salexigens* 5IX/A01/131$^T$. Cells of the strain grew optimally at 20–25 °C and pH 6.6–8.0 in the presence of 3–4 % (w/v) sea salts. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified aminophospholipid. The major quinone was ubiquinone 8. The major cellular fatty acids were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (23.1 %), $C_{17:1}\omega8c$ (22.1 %) and $C_{18:1}\omega7c$ (15.6 %). The genomic DNA G+C content was 53.6 mol%. Based on the phylogenetic, chemotaxonomic and phenotypic data presented, we propose the name *Spongiibacter borealis* sp. nov. with the type strain CL-AS9$^T$ (=KCCM 90094$^T$ =JCM 17304$^T$) and the reclassification of *Melitea salexigens* as a later heterotypic synonym of *Spongiibacter marinus*. We also provide emended descriptions of the genus *Spongiibacter* and *Spongiibacter marinus*.

The genus *Spongiibacter* in the class *Gammaproteobacteria* was established by Graeber *et al.* (2008) with *Spongiibacter marinus* as the type species. At the time of writing, two species of the genus *Spongiibacter* have been reported from marine environments: *Spongiibacter marinus* was isolated from a boreal sponge (Graeber *et al.*, 2008) and *Spongiibacter tropicus* from a marine *Synechococcus* culture (Hwang & Cho, 2009). Both species required NaCl for growth (1–7 % and 1–9 % for *Spongiibacter marinus* and *Spongiibacter tropicus*, respectively) and had the same temperature range of 10 to 40 °C for growth (Graeber *et al.*, 2008; Hwang & Cho, 2009). The major fatty acids of the two species were $C_{17:1}\omega8c$ and $C_{18:1}\omega7c$ (Hwang & Cho, 2009).

Eight months after the proposal of the genus *Spongiibacter*, the genus *Melitea* was proposed by Urios *et al.* (2008) with *Melitea salexigens*, a novel gammaproteobacterium isolated from sea surface layer. However, the close taxonomic relationship between the genera *Spongiibacter* and *Melitea* was not noticed until recently. During the present study, we recognized that *Spongiibacter marinus* HAL40b$^T$ and *Melitea salexigens* 5IX/A01/131$^T$ shared 99.2 % 16S rRNA

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-AS9$^T$ is HQ199599.

A supplementary table and figure are available with the online version of this paper.

gene sequence similarity. This similarity value is higher than the threshold of 97 % (Rosselló-Mora & Amann, 2001) or 98.7–99 % (Stackebrandt & Ebers, 2006) above which DNA–DNA relatedness experiments should be mandatory for confirming separate species.

In this study, a marine bacterium was isolated and subjected to a polyphasic taxonomic analysis. In addition, *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$ were compared using a polyphasic taxonomic approach to clarify the taxonomic positions of the two species.

Coastal seawater was collected near the Korean Arctic Dasan station at Svalbard in April 2010. An aliquot (100 µl) of seawater was spread on various media including marine agar 2216 (MA, Difco) and incubated under aerobic conditions at 4–20 °C for a month. Among 56 colonies isolated and identified by 16S rRNA gene sequencing (data not shown), only one strain (designated CL-AS9$^T$) was affiliated with the genus *Spongiibacter*. Strain CL-AS9$^T$ has been subsequently purified on fresh MA. Strain CL-AS9$^T$ was maintained both on MA at 20 °C and in marine broth 2216 (MB, Difco) supplemented with 30 % (v/v) glycerol at −80 °C.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by a boiling method (Englen & Kelley, 2000). The crude extract served as the DNA template for PCR, which included *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using the *AccuPrep* PCR purification kit (Bioneer) and direct sequence determination of the purified 16S rRNA gene was performed using sequencing primers (27F, 518F, 800R and 1492R; Lane, 1991; Anzai *et al.*, 1997) with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen, Seoul, Korea. The almost complete 16S rRNA gene sequence of strain CL-AS9$^T$ (1429 bp) was obtained and compared with available 16S rRNA gene sequences in GenBank using BLASTN searches (Altschul *et al.*, 1990). The 16S rRNA gene sequence of strain CL-AS9$^T$ was aligned by the jPHYDIT program (Jeon *et al.*, 2005) with the sequences of the type strains of other phylogenetically related species obtained from GenBank and Ribosomal Database Project II (Cole *et al.*, 2007). Accurate multiple alignment was made manually according to the 16S rRNA secondary structure information implemented in the jPHYDIT program. Phylogenetic trees were obtained by use of the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods and 100 replications for the maximum-likelihood method. Phylogenetic analyses were carried out using MEGA 4 (Tamura *et al.*, 2007) and PAUP 4.0 (Swofford, 1998). Likelihood parameters were estimated by using the hierarchical ratio test in MODELTEST, version 3.04 (Posada & Crandall, 1998).

Morphological and physiological tests were performed as follows. Gram-staining was performed as described by Smibert & Krieg (1994). Unless otherwise specified, all biochemical characteristics of strain CL-AS9$^T$ and *Spongiibacter tropicus* CL-CB221$^T$ were based on cultures grown on MA at 25 °C. Motility of the cells was assessed by the hanging drop method (Skerman, 1967) with cells grown in MB for 5 days. Cellular morphology and the presence of flagella were observed using transmission electron microscopy (EX2; JEOL). Anaerobic growth was checked on MA by using the GasPak anaerobic system (BBL) at 25 °C for 3 weeks. The temperature range for growth was examined on the basis of colony formation on MA incubated at 4 °C and temperatures ranging from 5 to 45 °C, using increments of 5 °C. The pH range (pH 4.1–9.9, using increments of approximately 1 pH unit) for growth in MB was determined by assessing changes in optical density at a wavelength of 600 nm ($OD_{600}$) over an incubation period of 10 days at 25 °C; prior to autoclaving the medium, its pH was adjusted using 1 M NaOH and 1 M HCl solutions. Salt tolerance of strain CL-AS9$^T$ was determined by assessing changes in $OD_{600}$ in synthetic ZoBell broth (Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate 0.1 g; distilled water, 1 l) with varying concentrations (0–10 % in increments of 1 %, and 15 %, w/v) of NaCl and sea salts (Sigma) at 25 °C.

Oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Hydrolysis of DNA, gelatin, starch and Tweens 40 and 80, ornithine and lysine deaminase activities, and Voges–Proskauer and methyl red tests were determined according to Hansen & Sørheim (1991). $H_2S$ production was tested as described by Bruns *et al.* (2001). In addition, other enzyme activities were assayed in duplicate using the API ZYM and API 20NE kits (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater (NaCl, 24 g; $MgCl_2 . 6H_2O$, 10.9 g; $Na_2SO_4$, 4 g; $CaCl_2 . 2H_2O$, 1.5 g; KCl, 0.7 g; $NaHCO_3$, 0.2 g; KBr, 0.1 g; $H_3BO_3$, 0.027 g; $SrCl_2 . 6H_2O$, 0.03 g; NaF, 0.003 g; distilled water, 1 l; Lyman & Fleming, 1940). Carbon utilization was tested using the basal broth medium supplemented with yeast extract (NaCl, 23.6 g; KCl, 0.64 g; $MgCl_2 . 6H_2O$, 4.53 g; $MgSO_4 . 7H_2O$, 5.94 g; $CaCl_2 . 2H_2O$, 1.3 g; $NaNO_3$, 0.2 g; $NH_4Cl$, 0.2 g; yeast extract, 0.05 g; distilled water, 1 l; Bruns *et al.*, 2001) containing 0.4 % carbon source. Growth was monitored by measuring $OD_{600}$ using a spectrophotometer (Ultraspec 2000; Pharmacia Biotech) twice a week for approximately one month. Carbon utilization was scored as negative when growth rate was equal to or less than that in the negative control with no carbon source.

Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional

TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). The quinone system was determined according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985) using *Salinisphaera dokdonensis* CL-ES53$^T$ (Bae *et al.*, 2010) as a reference strain. The fatty acid methyl esters in whole cells of strain CL-AS9$^T$ grown on MA at 30 °C for 3 days were analysed by using gas chromatography according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms (KCCM) in Seoul, Korea. The genomic DNA G+C content of strain CL-AS9$^T$ was analysed by HPLC (HP 100; Hewlett Packard) analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), after DNA was extracted by the method of Marmur (1961). Lambda phage DNA was used for calibration and the genomic DNA of *Spongiibacter tropicus* CL-CB221$^T$ as a reference.

To compare characteristics of *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$, the following experiments were performed for both strains at the same time. Unless otherwise specified, all characteristics of *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$ were based on cultures grown on MA at 30 °C for 3 days, and the same methods were employed as described above. Genomic DNA–DNA relatedness was determined by dot-blot hybridization (Kim *et al.*, 2007). Pre-hybridization, hybridization (temperature of 45.8 °C) and detection were performed by using a DIG labelling and detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. The DNA–DNA hybridization experiment was repeated on two different days. Quinone system and polar lipids were determined as described above. Fatty acid methyl esters were analysed for cells of *M. salexigens* 5IX/A01/131$^T$ grown on MA at 30 °C for 3 days, which were identical to conditions employed for fatty acid analysis of other species of the genus *Spongiibacter* (Hwang & Cho, 2009; this study). Physiological tests (i.e. temperature, pH and NaCl ranges for growth) and all of the above biochemical tests were performed for *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$.

Sequence analysis of the 16S rRNA gene showed that strain CL-AS9$^T$ belonged to the class *Gammaproteobacteria* (Fig. 1). Strain CL-AS9$^T$ was most closely related to *Spongiibacter tropicus* CL-CB221$^T$ (93.9 % similarity) and next to *Spongiibacter marinus* HAL40b$^T$ (93.7 % similarity) and *Melitea salexigens* 5IX/A01/131$^T$ (93.7 % similarity); no other species in the class *Gammaproteobacteria* shared more than 92 % sequence similarity with strain CL-AS9$^T$.

Tree topologies inferred from three tree-making algorithms showed that strain CL-AS9$^T$ formed a separate branch that was distinct from a clade (hereafter referred to as the *Spongiibacter* clade) comprising *Spongiibacter tropicus*, *Spongiibacter marinus* and *M. salexigens* (Fig. 1). Thus, the low sequence similarities (93.7–93.9 %) between strain CL-AS9$^T$ and species with validly published names, and the phylogenetic position of strain CL-AS9$^T$, indicated that our strain represents a novel species in the genus *Spongiibacter*.

The morphological, physiological and biochemical characteristics of strain CL-AS9$^T$ are given in the species description and Table 1. The major fatty acids of strain CL-AS9$^T$ were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 23.1 %), $C_{17:1}\omega8c$ (22.1 %) and $C_{18:1}\omega7c$ (15.6 %). The presence of a high amount of summed feature 3 and similar amounts of $C_{17:1}\omega8c$ and $C_{18:1}\omega7c$ could clearly differentiate strain CL-AS9$^T$ from other species of the genus *Spongiibacter* and *M. salexigens* (Table 1). Other fatty acids of strain CL-AS9$^T$ are shown in Supplementary Table S1 (available in IJSEM Online). The major polar lipids of strain CL-AS9$^T$ were diphosphatidylglycerol, phosphatidylglycerol and an unidentified aminophospholipid (Supplementary Figure S1). The absence of two unidentified aminophospholipids (APL2 and APL4) could differentiate strain CL-AS9$^T$ from *Spongiibacter marinus* and *M. salexigens* (Supplementary Figure S1). The genomic DNA G+C content of strain CL-AS9$^T$ was 53.6 mol%, which

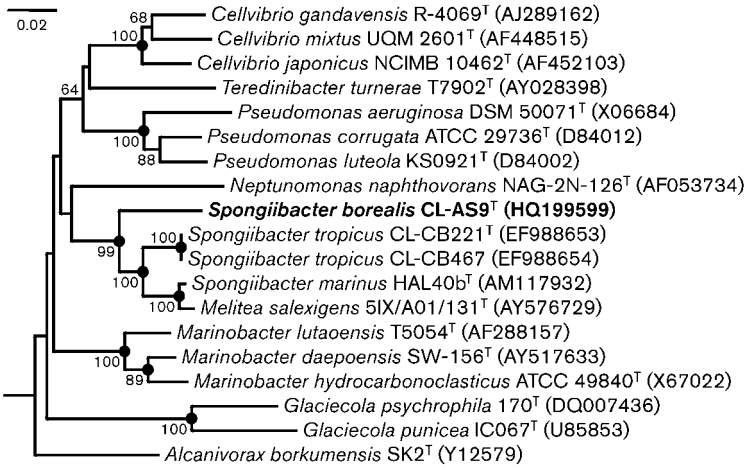


**Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of strain CL-AS9$^T$ and related members of the class *Gammaproteobacteria* on the basis of 16S rRNA gene sequence. Only bootstrap values above 60 % are shown (1000 resamplings) at branching points. Solid circles indicate that the corresponding nodes were also obtained in the maximum-likelihood and maximum-parsimony trees. *Thermotoga maritima* MSB8$^T$ (M21774) was used as an outgroup. Bar, 0.02 nucleotide substitutions per site.

is lower (>3.6 mol%) than those of members of the *Spongiibacter* clade (Table 1).

Furthermore, strain CL-AS9$^T$ can be differentiated from other species in the *Spongiibacter* clade by the ability to grow at 4–5 °C, inability to grow at 35 °C or above and inability to grow in the presence of NaCl as sole salt (Table 1). Strain CL-AS9$^T$ can be distinguished from *Spongiibacter tropicus* by the ability to hydrolyse Tween 40. Strain CL-AS9$^T$ can be distinguished from *Spongiibacter marinus* and *M. salexigens* by the inability to produce lipase (C14) and leucine arylamidase, and the ability to utilize pyruvate as sole carbon source (Table 1).

To determine the taxonomic positions of *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$, a polyphasic approach was employed. Phylogenetic analyses of 16S rRNA gene sequences of both type strains revealed that they formed a robust clade within the genus *Spongiibacter* (Fig. 1) with a similarity of 99.2 %. This grouping was supported by high bootstrap values (neighbour-joining, 100 %; maximum-likelihood, 100 %; and maximum-parsimony, 99 %). The level of DNA–DNA relatedness between *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$ was 83±5 % (reciprocal 85±0.4 %), indicating that they belonged to the same species (Rosselló-Mora & Amann, 2001). Fatty acid patterns were similar between *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$ (Supplementary Table S1). The major quinone was ubiquinone 8 (Q-8). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and unidentified aminophospholipid APL1 for both species (Supplementary Figure S1). Phenotypic characteristics were mostly identical (32 of 33 enzyme activities and 17 of 20 sole carbon source utilization) between *Spongiibacter marinus* HAL40b$^T$ and *M. salexigens* 5IX/A01/131$^T$.

Overall, the phylogenetic, chemotaxonomic and phenotypic data obtained in this study indicate that strain CL-AS9$^T$ should be assigned to a novel species in the genus *Spongiibacter*, for which the name *Spongiibacter borealis* sp. nov. is proposed. In addition, it is proposed to unite the species *Melitea salexigens* Urios *et al.* 2008 and *Spongiibacter marinus* Graeber *et al.* 2008. According to Rules 38, 42 and 24b(2) of the Bacteriological Code (Lapage *et al.*, 1992), the name *Spongiibacter marinus* has

**Table 1.** Selected characteristics that differentiate strain CL-AS9$^T$ from other species in the *Spongiibacter* clade

Strains: 1, CL-AS9$^T$ (this study); 2, *Spongiibacter tropicus* CL-CB221$^T$ (Hwang & Cho, 2009); 3, *Spongiibacter marinus* HAL40b$^T$ (Graeber *et al.*, 2008); 4, *Melitea salexigens* 5IX/A01/131$^T$ (Urios *et al.*, 2008). Fatty acids were analysed from cells grown on marine agar at 30 °C for 3 days (data for *M. salexigens* were obtained from this study). +, Positive; –, negative; w, weakly positive.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Isolation source | Arctic seawater | *Synechococcus* culture | Marine sponge | Seawater |
| Temperature range for growth (optimum) (°C) | 4–30 (20–25) | 10–40 (30–35) | 10–40 (20–30) | 15–37 (30) |
| pH range for growth (optimum) | 5.8–9.3 (6.6–8.0) | 6–10 (7–8) | 6.5–9.5 (7–9) | 6–10 (8) |
| Growth in the presence of NaCl as sole salt* | – | + | + | + |
| Hydrolysis of Tween 40* | + | – | + | + |
| API ZYM test* | | | | |
| *N*-Acetyl-β-glucosaminidase | – | – | + | – (w)† |
| Esterase (C4) | + | w | + | + (–)† |
| Leucine arylamidase | – | – | + | + |
| Lipase (C14) | – | – | + | + |
| Utilization of:* | | | | |
| Arabinose | – | – | + | – |
| D-Glucose | – | – | + | – (+)† |
| D-Mannose | w | – | + | – (+)† |
| Pyruvate | + | + | – | – |
| Major fatty acids (%) | | | | |
| $C_{16:0}$ | 7.4 | 12.9–14.4 | 3.2‡ | 3.9 |
| $C_{17:0}$ | 7.2 | 4.4–4.6 | 11.2‡ | 17.3 |
| $C_{17:1}\omega8c$ | 22.1 | 14.7–15.3 | 44.1‡ | 35.8 |
| $C_{18:1}\omega7c$ | 15.6 | 31.2–31.5 | 6.8‡ | 5.9 |
| $C_{11:0}$ 3-OH | 2.1 | 2.1–2.5 | 6.3‡ | 6.5 |
| Summed feature 3 ($C_{16:1}\omega7c$ / iso-$C_{15:0}$ 2-OH) | 23.1 | 11.4–12.5 | 6.3‡ | 4.8 |
| DNA G+C content (mol%) | 53.6 | 57.7 | 69.1 (60.6)‡ | 57.2 |

*Data for *Spongiibacter marinus* HAL40b$^T$ and *Melitea salexigens* 5IX/A01/131$^T$ were obtained from this study.
†Data in parentheses are from Urios *et al.* (2008).
‡Data from Hwang & Cho (2009).

priority and hence should be used for the unified taxon, with *Melitea salexigens* as a later heterotypic synonym.

### Emended description of *Spongiibacter* Graeber *et al.* 2008

The characteristics of the genus are as described by Graeber *et al.* (2008), with the following amendment. Motility is variable. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified aminophospholipid. The major quinone is Q-8.

### Emended description of *Spongiibacter marinus* Graeber *et al.* 2008

The characteristics of the species are as described by Graeber *et al.* (2008) with the above modifications and the following amendment. Negative for hydrolysis of starch, DNA and gelatin. Negative for ornithine and lysine deaminase activities, Voges–Proskauer and methyl red tests. $H_2S$ is not produced. According to the API ZYM test, *N*-acetyl-$\beta$-glucosaminidase activity is strain dependent. According to the API 20NE test, positive for aesculin hydrolysis, but negative for arginine dihydrolase, $\beta$-galactosidase (PNPG) and gelatinase, glucose fermentation, indole production, nitrate reductase and urease. Utilization of arabinose, D-glucose and D-mannose as sole carbon source is strain dependent, but acetate, L-arginine, cellobiose, citrate, D-fructose, D-galactose, glycerol, *myo*-inositol, lactose, mannitol, L-ornithine, pyruvate, L-rhamnose, D-salicin, sorbitol, succinate and xylose are not utilized. The minor polar lipids are three unidentified aminophospholipids. The G+C content of DNA is 57.2–60.6 mol%.

### Description of *Spongiibacter borealis* sp. nov.

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

Gram-negative, strictly aerobic, motile (by single polar flagellum), rods approximately 0.6–0.7 μm wide and 1.3–2.5 μm long. After 5 days on marine agar plates at 25 °C, colonies are creamy, circular and convex, and approximately 1 mm in diameter. Grows at 4–30 °C (optimum 20–25 °C) and pH 5.8–9.3 (optimum pH 6.6–8.0). No growth occurs in the presence of NaCl as sole salt. Growth occurs with 1–8 % (w/v) sea salts (optimum 3–4 %). Positive for oxidase and catalase. Tweens 40 and 80 are hydrolysed, but DNA, gelatin and starch are not. Negative for ornithine and lysine deaminase activities, Voges–Proskauer and methyl red tests. $H_2S$ is not produced. According to the API ZYM test, positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase, but negative for *N*-acetyl-$\beta$-glucosaminidase, $\alpha$-chymotrypsin, cystine arylamidase, $\alpha$-fucosidase, $\alpha$- and $\beta$-galactosidases, $\alpha$- and $\beta$-glucosidases, $\beta$-glucuronidase, leucine arylamidase, lipase (C14), $\alpha$-mannosidase, trypsin and valine arylamidase. According to the API 20NE test, positive for aesculin hydrolysis, but negative for arginine dihydrolase, $\beta$-galactosidase (PNPG) and gelatinase, glucose fermentation, indole production, nitrate reductase and urease. Pyruvate and D-mannose (weakly positive) are utilized as sole carbon source, but acetate, arabinose, L-arginine, cellobiose, citrate, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, mannitol, L-ornithine, L-rhamnose, D-salicin, sorbitol, succinate and xylose are not utilized. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified aminophospholipid. The minor polar lipids are two unidentified aminophospholipids. The major quinone is ubiquinone 8. The major cellular fatty acids are $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{17:1}\omega8c$ and $C_{18:1}\omega7c$. The DNA G+C content of the type strain is 53.6 mol%.

The type strain, CL-AS9$^T$ (=KCCM 90094$^T$ =JCM 17304$^T$), was isolated from Arctic seawater.

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## References

**Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990).** Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

**Anzai, Y., Kudo, Y. & Oyaizu, H. (1997).** The phylogeny of the genera *Chryseomonas*, *Flavimonas*, and *Pseudomonas* supports synonymy of these three genera. *Int J Syst Bacteriol* **47**, 249–251.

**Bae, G. D., Hwang, C. Y., Kim, H. M. & Cho, B. C. (2010).** *Salinisphaera dokdonensis* sp. nov., isolated from surface seawater. *Int J Syst Evol Microbiol* **60**, 680–685.

**Bruns, A., Rohde, M. & Berthe-Corti, L. (2001).** *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.

**Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. (2007).** The ribosomal database project (RDP-II): introducing *myRDP* space and quality controlled public data. *Nucleic Acids Res* **35** (Database issue), D169–D172.

**Collins, M. D. (1985).** Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

**Englen, M. D. & Kelley, L. C. (2000).** A rapid DNA isolation procedure for the identification of *Campylobacter jejuni* by the polymerase chain reaction. *Lett Appl Microbiol* **31**, 421–426.

**Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

**Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

**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.

**Hansen, G. H. & Sørheim, R. (1991).** Improved method for phenotypical characterization of marine bacteria. *J Microbiol Methods* **13**, 231–241.

**Hwang, C. Y. & Cho, B. C. (2009).** *Spongiibacter tropicus* sp. nov., isolated from a *Synechococcus* culture. *Int J Syst Evol Microbiol* **59**, 2176–2179.

**Jeon, Y.-S., Chung, H., Park, S., Hur, I., Lee, J.-H. & Chun, J. (2005).** jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.

**Jukes, T. H. & Cantor, C. R. (1969).** Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

**Kim, Y.-G., Choi, D. H., Hyun, S. & Cho, B. C. (2007).** *Oceanobacillus profundus* sp. nov., isolated from a deep-sea sediment core. *Int J Syst Evol Microbiol* **57**, 409–413.

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

**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: Wiley.

**Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992).** *International Code of Nomenclature of Bacteria (1990 Revision). Bacteriological Code.* Washington, DC: American Society for Microbiology.

**Lyman, J. & Fleming, R. H. (1940).** Composition of sea water. *J Mar Res* **3**, 134–146.

**Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.

**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.

**Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

**Posada, D. & Crandall, K. A. (1998).** MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

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

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

**Skerman, V. B. D. (1967).** *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.

**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, DC: American Society for Microbiology.

**Stackebrandt, E. & Ebers, J. (2006).** Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.

**Swofford, D. L. (1998).** Phylogenetic analysis using parsimony (PAUP), version 4. Sunderland, MA: Sinauer Associates.

**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.

**Urios, L., Agogué, H., Intertaglia, L., Lesongeur, F. & Lebaron, P. (2008).** *Melitea salexigens* gen. nov., sp. nov., a gammaproteobacterium from the Mediterranean Sea. *Int J Syst Evol Microbiol* **58**, 2479–2483.