Salinisphaera dokdonensis sp. nov., isolated from surface seawater

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A Gram-negative, strictly aerobic bacterium, designated CL-ES53^T, was isolated from surface water of the East Sea in Korea. Cells of strain CL-ES53^T were short rods and motile by means of monopolar flagella. Strain CL-ES53^T grew with 4–21 % NaCl (optimum 10 %) and at 5–40 °C (optimum 25 °C) and pH 5.2–8.8 (optimum pH 6.3–7.2). The major isoprenoid quinone was Q-8. The major fatty acids were $C_{18:1}\omega7c$ (42.0 %), $C_{18:1}\omega9c$ (14.8 %) and $C_{14:0}$ (9.4 %). The genomic DNA G+C content was 64.9 mol%. Analysis of the 16S rRNA gene sequence of strain CL-ES53^T revealed that it was a member of the genus *Salinisphaera* and most closely related to *Salinisphaera shabanensis E1L3A*^{T} (96.9 % sequence similarity) and *Salinisphaera hydrothermalis* EPR70^T (93.8 %). Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain CL-ES53^T formed a robust cluster with *S. shabanensis E1L3A*^{T}. Although the 16S rRNA gene sequence similarity between strain CL-ES53^T and *S. shabanensis E1L3A*^{T} was rather high (96.9 %), DNA–DNA relatedness between these strains was 12 %, suggesting that they represent genomically distinct species. Strain CL-ES53^T was differentiated from *S. shabanensis E1L3A*^{T} and *S. hydrothermalis* EPR70^T on the basis of optimum temperature for growth and certain phenotypic characteristics. The phylogenetic analysis and physiological and chemotaxonomic data show that strain CL-ES53^T should be classified in the genus *Salinisphaera* within a novel species, for which the name *Salinisphaera dokdonensis* sp. nov. is proposed. The type strain is CL-ES53^T (=KCCM 90064^T =DSM 19549^T).

The genus *Salinisphaera* in the class *Gammaproteobacteria* was established by Antunes *et al.* (2003) with *Salinisphaera shabanensis* as the type species. Until now, two *Salinisphaera* strains have been isolated from extreme environments: *S. shabanensis E1L3A*^{T} was isolated from the brine–seawater interface (1331 m depth, 21–23 % salinity) of the Shaban Deep in the Red Sea (Antunes *et al.*, 2003) and *Salinisphaera hydrothermalis* EPR70^T was isolated from the deep-sea hydrothermal vents (about 2500 m depth) on the East Pacific Rise (Crespo-Medina *et al.*, 2009). Cells of *S. shabanensis E1L3A*^{T} are monotrichous cocci that show a remarkable physiological flexibility, as seen by quite broad growth ranges for oxygen (anaerobic and aerobic), temperature (5–42 °C, optimum 30–37 °C) and NaCl (1–28 %, optimum 10 %) (Antunes *et al.*, 2003). Cells of *S. hydrothermalis* EPR70^T are short rods that are motile by means of one or more flagella, strictly aerobic, mesophilic (30–35 °C, optimum 20–40 °C), halotolerant (1–25 % NaCl, optimum 2.5 % NaCl) and facultatively chemolithoautotrophic (Crespo-Medina *et al.*, 2009). This study describes a strictly aerobic, moderately halophilic, short-rod-shaped bacterium, strain CL-ES53^T, which was isolated from the surface of offshore seawater.

Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-ES53^T is EF988634.

Seawater was collected from the surface in the vicinity of Dokdo, an island in the East Sea, Korea. An aliquot (100 µl) of seawater was spread on MY solid medium, which was used to isolate polysaccharide-producing bacteria by Bouchotroch *et al.* (2001), and incubated under aerobic conditions at 30 °C for 1 week. Strain CL-ES53^T was isolated and subsequently purified on marine agar (MA; Difco) at 30 °C, because strain CL-ES53^T grew well on both MA and MY plates, by picking a single colony and streaking it onto fresh MA four times. The strain was maintained on MA at 30 °C and preserved in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at −80 °C.

For 16S rRNA gene sequence amplification by PCR, DNA was extracted from a single colony by the boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for PCR, which used *Taq* DNA polymerase (Promega) and primers 27F and 1492R (Lane, 1991). PCR amplification products were purified by using the AccuPrep PCR purification kit (Bioneer) and direct sequence determination of the purified amplification products was performed using sequencing primers (27F,

518F, 800R and 1492R; Lane, 1991; Anzai *et al.*, 1997) and an Applied Biosystems automated sequencer (ABI 3730XL) at Macrogen (Seoul, Korea). The almost-complete 16S rRNA gene sequence of strain CL-ES53^T (1403 bp) was obtained and compared with 16S rRNA gene sequences available in GenBank using the BLASTN algorithm (Altschul *et al.*, 1990). The sequence of strain CL-ES53^T was aligned by the jPHYDIT program (Jeon *et al.*, 2005) with those from related taxa in the class *Gammaproteobacteria* 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 using 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 version 4 (Tamura *et al.*, 2007) and PAUP version 4.0 (Swofford, 1998). Likelihood parameters were estimated using the hierarchical ratio test in MODELTEST version 3.04 (Posada & Crandall, 1998). Genomic DNA–DNA relatedness was determined by dot-blot hybridization (Kim *et al.*, 2007a). Pre-hybridization, hybridization and detection were performed using a DIG labelling and detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. The experiment was repeated on different days.

In the original paper of Antunes *et al.* (2003), morphological and physiological characteristics of *S. shabanensis E1L3A*^{T} were described for cultures grown in SD0 medium or on SD0 medium agar. However, we found that strain CL-ES53^T and *S. shabanensis* JCM 11575^T grew better on MA supplemented with 7.5 % NaCl (hereafter referred to as mMA) than on SD0 medium agar (not shown). Thus, unless otherwise specified, all characteristics of strain CL-ES53^T and *S. shabanensis* JCM 11575^T were based on cultures grown on mMA at 25 °C. Gram-staining was performed as described by Smibert & Krieg (1994). Motility of the cells was observed by the hanging-drop method (Skerman, 1967). Cell morphology and the presence of flagellum were examined using transmission electron microscopy (EX2; JEOL). Anaerobic growth was checked on MA, mMA and ZOF medium (Lemos *et al.*, 1985) supplemented with agar (1.5 %) incubated in the GasPak anaerobic system (BBL) at 25 °C for 20 days. The presence of poly-β-hydroxybutyrate (PHB) granules was assessed using epifluorescence microscopy (BX60; Olympus) after Nile blue A staining (Ostle & Holt, 1982). The presence of endospores was assessed with malachite green staining (Smibert & Krieg, 1994). The temperature range for growth was determined on the basis of colony formation on mMA incubated at 5–50 °C in increments of 5 °C. The pH range for growth was determined by assessing changes in OD_{600} over the incubation period (up to 10 days) in MB supplemented with 7.5 % (w/v) NaCl at 25 °C. Prior to autoclaving, the pH was adjusted to pH 4–10 in increments of 1 pH unit using 1 M NaOH and 1 M HCl. After autoclaving and cooling, the pH of the media was determined to be pH 4.2, 5.2, 6.3, 7.2, 8.0, 8.8 and 9.8. The tolerance of strain CL-ES53^T and *S. shabanensis* JCM 11575^T to NaCl was determined by assessing changes in OD_{600} in synthetic ZoBell broth (l^{-1} distilled water: 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate) supplemented with 0, 0.5 and 1–25 % (in increments of 1 %) NaCl (w/v) at 25 °C.

Catalase, oxidase, H_2S production and hydrolysis of aesculin and casein were assayed according to the protocols described by Smibert & Krieg (1994). Hydrolysis of starch, gelatin and DNA and degradation of Tweens 40 and 80 were determined according to Hansen & Sørheim (1991). Other enzyme activities were assayed 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 (l^{-1} distilled water: 24 g NaCl, 10.9 g $MgCl_2.6H_2O$, 4 g Na_2SO_4, 1.5 g $CaCl_2.2H_2O$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.027 g H_3BO_3, 0.03 g $SrCl_2.6H_2O$, 0.003 g NaF; Lyman & Fleming, 1940) supplemented with 7.6 % (w/v) NaCl. Acid production was examined for up to 2 weeks with the API 50CH kit (bioMérieux), using the API 50 CHB/E medium (bioMérieux) supplemented with 10 % (w/v) NaCl. For the API ZYM, API 20NE and API 50CH tests, *Alteromonas marina* KCCM 41638^T and *Terribacillus goriensis* KCCM 42329^T, grown on MA under their optimal growth conditions (Kim *et al.*, 2007b; Yoon *et al.*, 2003), served as reference strains in addition to *S. shabanensis* JCM 11575^T and *S. hydrothermalis* JCM 155140^T. *T. goriensis* KCCM 42329^T and *S. shabanensis* JCM 11575^T were used as a positive and a negative control, respectively, for hydrolysis tests for starch, casein, gelatin, DNA and Tweens 40 and 80; these tests were performed for strain CL-ES53^T and the two type strains of *Salinisphaera* species on mMA and for *T. goriensis* CL-GR16^T on MA. To examine carbon utilization, a modified basal broth medium supplemented with yeast extract (l^{-1} distilled water: 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2.6H_2O$, 5.94 g $MgSO_4.7H_2O$, 1.3 g $CaCl_2.2H_2O$, 0.2 g $NaNO_3$, 0.2 g NH_4Cl, 0.05 g yeast extract; Bruns *et al.*, 2001), 7.64 % NaCl (w/v) and 0.4 % carbon source was used (Suzuki *et al.*, 2001). Growth was monitored by OD_{600} using a spectrophotometer (Ultraspec 2000; Pharmacia Biotech). OD_{600} was measured for 14 days. Carbon utilization was scored as negative when growth rate was equal to or less than that in the negative control with no carbon source. Growth rate was measured by assessing changes in OD_{600}. Resistance to antibiotics was determined on mMA incubated at 30 °C for 2 weeks by the disc diffusion plate method (Bauer *et al.*, 1966).

The fatty acid methyl esters in whole cells of strain CL-ES53^T, *S. shabanensis* JCM 11575^T and *S. hydrothermalis* JCM 15514^T were analysed by gas chromatography according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). The three strains were grown on tryptic soy agar (TSA; Difco) supplemented with 9.5 % (w/v) NaCl at 25 °C for 6 days. The G+C content of genomic DNA extracted by the method of Marmur (1961) was determined by HPLC analysis (Hewlett Packard HP 100) of deoxyribonucleosides as described by Mesbah *et al.* (1989). Lambda phage DNA was used as the standard and DNA of *S. shabanensis* JCM 11575^T as the reference. Isoprenoid quinone composition was determined according to Minnikin *et al.* (1984) and analysed by HPLC at the KCCM as described by Collins (1985).

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain CL-ES53^T belonged to the genus *Salinisphaera* in the class *Gammaproteobacteria* (Fig. 1). Strain CL-ES53^T was most closely related to *S. shabanensis E1L3A*^{T} (96.9 % sequence similarity) and *S. hydrothermalis* EPR70^T (93.8 %). The tree topologies inferred from three tree-making algorithms showed that strain CL-ES53^T formed a robust cluster with *S. shabanensis E1L3A*^{T} (Fig. 1). DNA–DNA relatedness between strain CL-ES53^T and *S. shabanensis E1L3A*^{T} was 12 %, which is much lower than the level accepted to delineate species (Vandamme *et al.*, 1996). Thus, the phylogenetic and genomic evidence indicated that our isolate represents a novel species in the genus *Salinisphaera*.

The phenotypic characteristics of strain CL-ES53^T are given in the species description and in Table 1, alongside some phenotypic characteristics of *S. shabanensis* JCM 11575^T and *S. hydrothermalis* JCM 15514^T that were determined in this study. The major fatty acids of strain CL-ES53^T were $C_{18:1}\omega7c$ (42.0 %), $C_{18:1}\omega9c$ (14.8 %) and $C_{14:0}$ (9.4 %); the detailed fatty acid composition is shown in Table 2. The major isoprenoid quinone in both strain CL-ES53^T and *S. shabanensis E1L3A*^{T} was ubiquinone-8 (Q-8). The genomic DNA G+C content of strain CL-ES53^T was 64.9 mol%.

There were some phenotypic and chemotaxonomic features that differentiated strain CL-ES53^T from the other *Salinisphaera* species (Table 1). The temperature for optimal growth of strain CL-ES53^T (25 °C) was lower than those of *S. shabanensis* and *S. hydrothermalis* (30–37 and 30–35 °C, respectively). Strain CL-ES53^T could be differentiated from its closest phylogenetic neighbour, *S. shabanensis E1L3A*^{T}, on the basis of its abilities to hydrolyse starch and Tween 80, its weak abilities to produce lipase (C14) and valine arylamidase and its inability to produce alkaline phosphatase. In addition, different proportions of major fatty acids ($C_{18:1}\omega7c$ and $C_{18:1}\omega9c$) could differentiate strain CL-ES53^T from *S. shabanensis E1L3A*^{T} (Table 2). Strain CL-ES53^T could be distinguished from *S. hydrothermalis* EPR70^T on the basis of the optimum NaCl concentration for growth, its ability to produce oxidase and its inabilities to produce α-chymotrypsin, reduce nitrate, hydrolyse aesculin, utilize D-fructose and galactose as sole carbon sources and produce acid from D- and L-arabitol, aesculin and D- and L-fucose (Table 1).

Overall, on the basis of the polyphasic data presented in this study, strain CL-ES53^T should be placed in the genus *Salinisphaera* within a novel species, for which the name *Salinisphaera dokdonensis* sp. nov. is proposed.

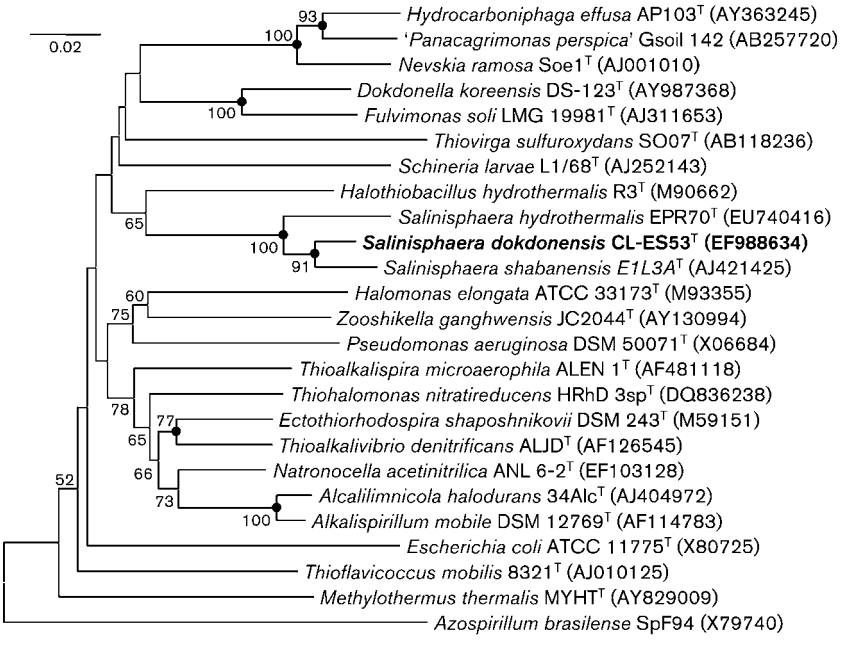

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of *Salinisphaera dokdonensis* sp. nov. CL-ES53^T and related members of the class *Gammaproteobacteria* on the basis of 16S rRNA gene sequences. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Solid circles indicate that the corresponding nodes were also obtained in the maximum-likelihood and the maximum-parsimony trees. The sequence of *Azospirillum brasilense* SpF94 was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential characteristics of strain CL-ES53^T and the type strains of other *Salinisphaera* species

Strains: 1, *Salinisphaera dokdonensis* sp. nov. CL-ES53^T (data from this study); 2, *S. shabanensis E1L3A*^{T} (unless indicated, data from Antunes *et al.*, 2003); 3, *S. hydrothermalis* EPR70^T (Crespo-Medina *et al.*, 2009). +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3*
Cell characteristics			
Shape	Short rod	Coccus†	Short rod
Size (μm)	0.3–0.6 × 0.5–0.9	0.7–1.2	0.3–0.5 × 0.8–1.0
Growth requirements			
Oxygen	Strictly aerobic	Facultatively anaerobic	Strictly aerobic
NaCl range (optimum) (%, w/v)	4–21 (10)	1–28 (10)	1–25 (2.5)
Temperature range (optimum) (°C)	5–40 (25)	5–42 (30–37)	20–40 (30–35)
Oxidase	+	+	−
Hydrolysis of:			
Starch	+	−	+‡
Tween 80	+	−	+‡
API ZYM test results			
Acid phosphatase	w	w‡	+‡
Alkaline phosphatase	−	+‡	−‡
α-Chymotrypsin	−	−‡	w‡
Lipase (C14)	w	−‡	w‡
Valine arylamidase	w	−‡	w‡
API 20NE test results			
Aesculin hydrolysis	−	−‡	+‡
Nitrate reductase	−	−‡	+‡
Acid production from (API 50CH):			
Aesculin	−	−‡	+‡
Amygdalin	−	w‡	−‡
D-Arabitol	−	−‡	w‡
L-Arabitol	−	−‡	w‡
D-Fucose	−	−‡	w‡
L-Fucose	−	−‡	w‡
Utilization as sole carbon source			
D-Fructose	−	−	+
Galactose	−	−	+
D-Glucose	+	−/w‡	+
Glycerol	w	−/w‡	+
DNA G+C content (mol%)	64.9	61.8	64.0

*Hydrolysis of DNA, casein, Tween 40 and gelatin was negative (this study).
†Short rods after incubation in marine broth supplemented with 7.5 % (w/v) NaCl at 25 °C for 6 days (this study).
‡Data from this study: all strains were grown on MA supplemented with 7.5 % NaCl at 25 °C for 6 days prior to each test.

Description of *Salinisphaera dokdonensis* sp. nov.

Salinisphaera dokdonensis (dok.do.nen′sis. N.L. fem. adj. *dokdonensis* of Dokdo, a Korean island, from where the type strain was isolated).

Gram-negative, strictly aerobic and motile by a single polar flagellum. Non-spore-forming and short-rod-shaped cells, approximately 0.3–0.6 μm wide and 0.5–0.9 μm long. Oxidase- and catalase-positive. Cells contain PHB granules. Colonies are circular, convex and creamy and about 1 mm in diameter after incubation on MA supplemented with 7.5 % (w/v) NaCl at 25 °C for 6 days. Growth occurs at 5–40 °C (optimum 25 °C), at pH 5.2–8.8 (optimum pH 6.3–7.2) and with 4–21 % NaCl (optimum 10 % NaCl). Tween 80 and starch are hydrolysed, but gelatin, aesculin, casein, DNA and Tween 40 are not. Negative for H_2S production. According to API ZYM tests, positive for esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, weakly positive for acid phosphatase, lipase (C14) and valine arylamidase and negative for *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, α-mannosidase and trypsin. According to API 20NE tests, negative for aesculin hydrolysis, glucose fermentation,

Table 2. Cellular fatty acid content of strain CL-ES53^T and the type strains of other *Salinisphaera* species

Strains: 1, *Salinisphaera dokdonensis* sp. nov. CL-ES53^T; 2, *S. shabanensis* JCM 11575^T; 3, *S. hydrothermalis* JCM 15514^T. Data were obtained in this study from cells grown for 6 days; *S. hydrothermalis* JCM 15514^T was also examined after 4 days. The analysis was repeated on different days; mean values are shown. Values are percentages of total fatty acids. ND, Not detected; tr, trace amount (<0.5 %).

Fatty acid	1	2	3	
			6 days	4 days
Straight-chain				
$C_{10:0}$	tr	ND	ND	ND
$C_{12:0}$	3.6	tr	tr	1.1
$C_{14:0}$	9.4	6.7	2.5	5.5
$C_{16:0}$	3.8	2.0	4.8	15.9
$C_{17:0}$	tr	ND	ND	1.2
$C_{18:0}$	8.1	11.6	9.3	13.6
$C_{20:0}$	ND	tr	ND	ND
Unsaturated				
$C_{14:1}\omega5c$	0.6	ND	ND	ND
$C_{16:1}\omega5c$	1.9	2.1	3.4	2.4
$C_{16:1}\omega9c$	tr	ND	tr	ND
$C_{17:1}\omega6c$	ND	tr	ND	ND
$C_{18:1}\omega7c$	42.0	70.2	12.0	20.4
$C_{18:1}\omega9c$	14.8	0.9	tr	5.4
$C_{18:3}\omega6c$(6,9,12)	ND	tr	1.4	0.9
$C_{20:1}\omega7c$	ND	tr	ND	ND
$C_{20:1}\omega9c$	tr	ND	ND	ND
$C_{20:2}\omega6c$(6,9)	ND	ND	1.3	2.5
Hydroxy				
$C_{12:0}$ 2-OH	ND	tr	1.2	1.5
$C_{12:0}$ 3-OH	ND	0.6	2.2	2.9
$C_{14:0}$ 2-OH	ND	ND	1.1	2.4
$C_{16:0}$ 3-OH	ND	ND	ND	0.6
$C_{18:0}$ 3-OH	ND	ND	ND	0.6
Branched				
$C_{13:0}$ anteiso	ND	ND	ND	0.7
$C_{16:0}$ iso	tr	ND	ND	ND
$C_{17:0}$ cyclo	1.0	ND	0.9	ND
$C_{19:0}\omega8c$ cyclo	2.3	2.0	55.6	13.7
10-Methyl $C_{17:0}$	ND	tr	ND	ND
11-Methyl $C_{18:1}\omega7c$	ND	tr	2.0	4.5
Summed features*				
2	2.5	tr	0.7	0.6
3	6.5	1.1	1.0	1.3
5	ND	ND	ND	0.5
7	3.2	ND	ND	ND

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of one or more of unknown ECL 10.928, $C_{14:0}$ 3-OH and iso-$C_{16:1}$. Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 5 consisted of $C_{18:2}\omega6,9c$ and/or anteiso-$C_{18:0}$. Summed feature 7 consisted of one or more of $C_{19:1}\omega6c$, ECL 18.846 and $C_{19:0}\omega10c$ cyclo.

nitrate reduction, indole production, arginine dihydrolase, β-galactosidase, gelatinase and urease. D-Glucose and glycerol (weak growth) are utilized as sole carbon sources, but acetate, *N*-acetyl-D-glucosamine, betaine, citrate, ethanol, D-fructose, D-galactose, lactose, D-mannose, raffinose, succinate, trehalose, urea and xylose are not utilized. Acid is not produced from *N*-acetylglucosamine, D-adonitol, starch, amygdalin, arbutin, D- and L-arabitol, cellobiose, dulcitol, erythritol, aesculin, D-fructose, D- and L-fucose, D-galactose, gentiobiose, D-glucose, glycerol, glycogen, inositol, inulin, lactose (bovine origin), D-lyxose, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, L-rhamnose, D-ribose, sucrose, salicin, D-sorbitol, L-sorbose, D-tagatose, trehalose, turanose, xylitol and D- and L-xylose. Sensitive to (μg per disc) ampicillin (6), cephalexin (20), chloramphenicol (20), erythromycin (10), nalidixic acid (20), penicillin G (6), polymyxin B (25) and tetracycline (20), but resistant to gentamicin (6), kanamycin (20) and streptomycin (6). The isoprenoid quinone is Q-8. The major fatty acids are $C_{18:1}\omega7c$, $C_{18:1}\omega9c$ and $C_{14:0}$. The genomic DNA G+C content of the type strain is 64.9 mol%.

The type strain is CL-ES53^T (=KCCM 90064^T =DSM 19549^T), isolated from surface seawater in the vicinity of Dokdo, an island in the East Sea, Korea.

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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.

Antunes, A., Eder, W., Fareleira, P., Santos, H. & Huber, R. (2003). *Salinisphaera shabanensis* gen. nov., sp. nov., a novel, moderately halophilic bacterium from the brine-seawater interface of the Shaban Deep, Red Sea. *Extremophiles* **7**, 29–34.

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.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.

Bouchotroch, S., Quesada, E., Del Moral, A., Llamas, I. & Béjar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **51**, 1625–1632.

Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appen-

daged 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. & other authors (2007). The Ribosomal Database Project (RDP-II): introducing *myRDP* space and quality controlled public data. *Nucleic Acids Res* **35**, D169–D172.

Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.

Crespo-Medina, M., Chatziefthimiou, A., Cruz-Matos, R., Pérez-Rodríguez, I., Barkay, T., Lutz, R. A., Starovoytov, V. & Vetriani, C. (2009). *Salinisphaera hydrothermalis* sp. nov, a mesophilic, halotolerant, facultative autotrophic, thiosulfate-oxidizing gammaproteobacterium from deep-sea hydrothermal vents, and emended description of the genus *Salinisphaera*. *Int J Syst Evol Microbiol* **59**, 1497–1503.

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.

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

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. (2007a). *Oceanobacillus profundus* sp. nov., isolated from a deep-sea sediment core. *Int J Syst Evol Microbiol* **57**, 409–413.

Kim, Y.-G., Hwang, C. Y., Yoo, K. W., Moon, H. T. & Cho, B. C. (2007b). *Pelagibacillus goriensis* gen. nov., sp. nov., a moderately halotolerant bacterium isolated from coastal water off the east coast of Korea. *Int J Syst Evol Microbiol* **57**, 1554–1560.

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.

Lemos, M. L., Toranzo, A. E. & Barja, J. L. (1985). Modified medium for the oxidation-fermentation test in the identification of marine bacteria. *Appl Environ Microbiol* **49**, 1541–1543.

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 microorganisms. *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.

Ostle, A. G. & Holt, J. G. (1982). Nile blue A as a fluorescent stain for poly-β-hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.

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

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.

Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001). Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.

Swofford, D. L. (1998). PAUP* – Phylogenetic analysis using parsimony, 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.

Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* **60**, 407–438.

Yoon, J.-H., Kim, I.-G., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2003). *Alteromonas marina* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1625–1630.