Stakelama pacifica gen. nov., sp. nov., a new member of the family Sphingomonadaceae isolated from the Pacific Ocean

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A Gram reaction-negative, weakly motile, non-spore-forming, rod-shaped, aerobic bacterium designated strain JLT832′ was isolated from surface water of the central Pacific Ocean and formed yellow colonies on rich organic (RO) medium. The strain was oxidase-negative and catalase-positive. Acid was produced from mannitol, glucose, sucrose, lactose, sorbitol, maltose, (+)-trehalose and β-fructose. No acid was produced from β- (+)-xylose. The major cellular fatty acids of strain JLT832′ were C18:1ω7c, C14:02-OH and C16:0. The major polar lipids were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Ubiquinone-10 and spermidine were present as the major quinone and polyamine, respectively. The genomic DNA G+C content of strain JLT832′ was 66.0 ± 0.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the new isolate formed a tight branch within the family Sphingomonadaceae but was clearly separate from established genera in this family. The sequence similarities between the new isolate and type strains of established genera ranged from 90.5 to 94.9%. Based on these data, strain JLT832′ constitutes a novel genus and species, for which the name Stakelama pacifica gen. nov., sp. nov. is proposed. The type strain of Stakelama pacifica is JLT832′ (=CGMCC 1.7294′ =LMG 24686′).

The family Sphingomonadaceae was established by Kosako et al. (2000) and was further divided into families Sphingomonadaceae and Erythrobacteraceae by Lee et al. (2005). The genus Sphingomonas was first described by Yabuuchi et al. (1990) and was later divided into four genera, Sphingomonas, Sphingobium, Novosphingobium and Sphingopyxis (Takeuchi et al., 2001), based on both phylogenetic analysis of 16S rRNA gene sequences and chemotaxonomic and phenotypic differences. Many novel genera of this clade have been described, such as Sphingomonas, Sphingobium, Novosphingobium, Sphingopyxis, Zymomonas, Sandaracinobacter, Sphingosinicella, Sandarakinorhabdus and Blastomonas.

Strain JLT832′ was isolated from the surface of the Pacific Ocean at 174° 21.70′ E 20° 34.44′ N and formed a deep branch within the family Sphingomonadaceae. In this study, we characterized strain JLT832′ by using a polyphasic approach.

Strain JLT832′ was initially isolated with seawater medium (SW) containing (per litre seawater filtered by 0.2 μm film) 1 g peptone, 1 g yeast extract, 20 μg vitamin B12 and 1 ml of a trace element solution (ES) as described by Drews (1983). The strain was identified by growing on modified rich organic (RO) medium (Yurkov et al., 1999) containing (per litre distilled water) 1 g peptone, 1 g yeast extract, 20 g NaCl, 1 g sodium acetate, 0.3 g KCl, 0.5 g MgSO4.7H2O, 0.03 g CaCl2.7H2O, 0.3 g NH4Cl, 0.3 g K2HPO4, 20 μg vitamin B12 and 1.0 ml ES, at pH 8.0 and 28 °C.

Cell morphology was examined using light microscopy (BX61; Olympus) and TEM (H600; Hitachi). The Gram

Abbreviations: UPLC-Q-TOF-MS, ultra-performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry; MR, methyl red; VP, Voges–Proskauer.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JLT832′ is EU581829.

A transmission electron micrograph of negatively stained cells and polar lipid profiles of strain JLT832′ are available as supplementary material with the online version of this paper.
reaction was tested according to standard procedures (Gerhardt et al., 1994). Catalase activity was determined by adding drops of 3% H$_2$O$_2$ to an overnight colony and assessed by the formation of bubbles. The pH and temperature ranges for growth were determined by adjusting the RO medium to a final pH of 5.5–9.5 (at intervals of 0.5 pH units) with HCl and NaOH, and by incubation at 0, 5, 10, 15, 25, 28, 30, 37 and 40 °C. Growth in NaCl 1–6% (w/v) (increments of 0.5%) was investigated in RO medium. Oxidase and urease activity, hydrolysis of casein, starch, gelatin and Tween 80 and reduction of nitrate and nitrite were determined as described by Dong & Cai (2001) on RO medium. Denitrification was tested by growing the strain anaerobically in the presence of nitrate (Zumft, 1992). H$_2$S production was tested as described by Bruns et al. (2001). Indole production, methyl red (MR) test and Voges–Proskauer (VP) reaction were determined as described by Denner et al. (2001).

The ability of strain JLT832$^T$ to utilize substrates as sole carbon and energy sources was examined on RO medium without organic compounds at 28 °C for 7–14 days (Williams et al., 1983). Acid production from carbohydrates was assessed using the procedures outlined by Cappuccino & Sherman (2002). Antibiotic susceptibility was tested by using the disc-diffusion plate (Kirby–Bauer) method according to Fraser & Jorgensen (1997) and Andrews (2008).

Cellular fatty acid composition was determined as described previously (Hu et al., 2004) by growing cells of strain JLT832$^T$ on marine agar 2216e (MA; Difco) for 2 days at 28 °C. Polyamines were extracted and derivatized as described by Busse & Auling (1988) and analysed by HPLC using the equipment previously described by Stolz et al. (2007). Polar lipids were identified by two-dimensional TLC and detection with sulfuric acid/ethanol (1:2, v/v) followed by heating at 150 °C for 3 min, according to the procedures described by Minnikin et al. (1984). Isoprenoid quinones were analysed as described by Hiraishi et al. (1998) using ultra-performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry (UPLC-Q-TOF-MS) in an electrospray ionization method (Romano et al., 2006).

Genomic DNA was extracted following the method of Marmur (1961) from cells that were grown in RO medium for 2 days at 28 °C, washed and then resuspended in the buffer. Purity was assessed by measuring absorbance at $A_{280}/A_{260}$ and $A_{230}/A_{260}$ ratios (Johnson, 1994). The 16S rRNA gene was amplified and sequenced as described by Rainey et al. (1996) and sequences were aligned using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1990) to determine the approximate phylogenetic affiliation. Phylogenetic trees were constructed using neighbour-joining and maximum-parsimony algorithms in MEGA 4.0.
software (Kumar et al., 2004). DNA G+C contents were
determined by thermal denaturation (Marmur & Doty,
1962) using DNA of Escherichia coli DH5α as a standard for
calibration of the Tm.

Strain JLT832T cells were strictly aerobic, heterotrophic,
Gram reaction-negative, short rods. Flagella were observed
(Supplementary Fig. S1, available in IJSEM Online).
Colonies were gold–yellow, glossy, opaque, circular and
about 1 mm in diameter. JLT832T grew in the presence of
5–10% (w/v) NaCl (optimum 2%), at 5–37 °C (optimum
28 °C) and pH 6–9 (optimum pH 8). Strain JLT832T was
positive for catalase, aerobic nitrate reduction, H₂S
production and VP reaction and negative for oxidase,
urease, aerobic nitrite reduction, anaerobic nitrite reduc-
tion, indole production, MR test and hydrolysis of gelatin,
starch, Tween 80 and casein. Acid was produced from
mannitol, glucose, sucrose, lactose, sorbitol, maltose,
(+)-trehalose and D-fructose. No acid was produced from
(+)-D-xylose.

JLT832T exhibited a polyamine pattern with the following
compounds (per g dry weight): spermidine (17.6 μmol),
putrescine (0.2 μmol), cadaverine (0.6 μmol) and traces
(<0.1 μmol) of 1,3-diaminopropane and spermine. This
polyamine pattern was in agreement with the traits
reported for the genera Blastomonas, Sphingobium,
Novosphingobium and Sphingopyxis, while the presence of
homospermidine was restricted in species of Zymomonas,
Sphingosincola and Sphingomonas (Busse et al., 1999;
Takeuchi et al., 2001; Hamana et al., 2003). The major
polar lipids were sphingoglycolipid, diphostatidylgly-
cerol, phosphatidylglycerol and phosphatidylethanolamine
(Supplementary Fig. S2). Q-10 was the sole respiratory

Table 1. Differentiation of physiological and biochemical characteristics between strain JLT832T and closely related species within
the family Sphingomonadaceae

<table>
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<th>Characteristic</th>
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<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>66.0±0.5</td>
<td>63.6–63.7</td>
<td>65</td>
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<td>ND</td>
<td>ND</td>
<td>66±1.0</td>
<td>48.5±1.0</td>
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quinone. Cellular fatty acids were C_{18:1\alpha\delta\text{C}} (72.88%), C_{14:0} 2-OH (11.93%), C_{16:0} (11.53%), C_{18:1\alpha\delta\text{C}} (2.02%) and C_{16:1\omega7\text{C}} (1.64%) (Supplementary Table S1).

The DNA G+C content of JLT832T was 66 ± 0.5 mol%. The complete 16S rRNA gene sequence (1412 bp) of strain JLT832T was determined. Phylogenetic trees based on 16S rRNA gene sequences (1374 bp compared) showed that strain JLT832T forms a distinct phylogenetic lineage within the family Sphingomonadaceae (Fig. 1) and exhibits the highest sequence similarity to Novosphingobium subarcticum SMCC B0478T (94.9%), followed by Novosphingobium mathurensense SM 117T (94.3%) and Sphingosinicella microcystinovorans ICM 13185T (94.3%).

No currently recognized species in the genera Novosphingobium, Sphingosinicella and Zymomonas utilizes sorbitol, mannitol, lactose, (+)-trehalose, cellobiose, lactic acid, sodium d-gluconate, inositol, glycerol, mannitol, acetic acid, ethanol and malonic acid are utilized. Sucrose, glucose, (+)-d-galactose, (+)-l-malic acid, l-proline and l-threonine are used weakly. (+)-l-Sorbose, d-fructose, dextrin, trisodium citrate, oxalic acid, l-alanine, l-lysine, glycine and l-arginine are not utilized. The type strain was sensitive (μg per disc) to ampicillin (10), penicillin (10), tetracycline (30), chloramphenicol (30), erythromycin (15), cefoperazone (75), cefotaxime (30), ciprofloxacin (5), clarithromycin (15), gentamicin (120) and streptomycin (300); resistant to clindamycin (2), sulfamethoxazole (300), vancomycin (30) and cefalothin (30).

The type strain, JLT832T (=CGMCC 1.7294T =LMG 24686T), was isolated from surface water at 174° 21.70’ E 20° 34.44’ N in the Pacific Ocean.

Acknowledgements

The authors would like to thank Dr Hans-Jürgen Busse, Institut für Bakteriologie, Mykologie und Hygiene, University of Veterinary Medicine Vienna, for the analysis of the polyamine pattern. This work was supported by the MOST 973 project (2007CB815904), the NSFC projects (40632013 and 40821063), the SOA project (200805068) and the COMRA project (DYXM-115-02-4-3).

References


Fraser, S. L. & Jorgensen, J. H. (1997). reappraisal of the antimicrobial susceptibilities of Chryseobacterium and Flavobacterium species and