Microbulbifer gwangyangensis sp. nov. and Microbulbifer pacificus sp. nov., isolated from marine environments

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Two novel Gram-stain-negative, chemoheterotrophic and strictly aerobic bacteria, strains GY2T and SPO729T, were isolated from a tidal flat at Gwangyang Bay in Korea and a marine sponge sample from the Pacific Ocean, respectively. The two strains were halotolerant, catalase- and oxidase-positive, and non-motile rods. Optimum temperature and pH for growth of both strains were observed to be 35 °C and pH 7.0–7.5, but optimum salinity for strain SPO729T (2–3% (w/v)) was slightly higher than that for strain GY2T (1–2%). The major cellular fatty acids of both strains were C16:0, iso-C15:0, iso-C17:0, iso-C17:1ω9c, C18:1ω7c, iso-C17:0 and iso-C11:0 3-OH. The genomic DNA G+C contents of strains GY2T and SPO729T were 55.1 and 57.9 mol%, respectively, and ubiquinone 8 (Q-8) was detected as the sole respiratory quinone from the two strains. Phylogenetic analysis based on 16S rRNA gene sequences showed that strains GY2T and SPO729T formed tight phyletic lineages with members of the genus Microbulbifer. Strain GY2T was closely related to Microbulbifer okinawensis ABABA23T (98.2%), strain SPO729T (98.0%) and Microbulbifer donghaiensis CN85T (97.0%); strain SPO729T was closely related to M. okinawensis ABABA23T (98.3%) and M. donghaiensis CN85T (98.2%). The DNA–DNA relatedness values of strain GY2T with M. okinawensis ABABA23T, strain SPO729T and M. donghaiensis CN85T were 40.0 ± 2.1%, 13.1 ± 3.9% and 16.2 ± 5.8%, respectively, whereas those of strain SPO729T with M. okinawensis ABABA23T and M. donghaiensis CN85T were 48.0 ± 4.0% and 34.6 ± 9.3%, respectively. On the basis of phenotypic and molecular features, it is concluded that the two strains GY2T and SPO729T represent two novel species of the genus Microbulbifer, for which the names Microbulbifer gwangyangensis sp. nov. and Microbulbifer pacificus are proposed; the type strains are GY2T (=KACC 16188T=JCM 17800T) and SPO729T (=KCCM 42667T=JCM 14507T), respectively.

The genus Microbulbifer, of the family Alteromonadaceae of the Gammaproteobacteria, was first proposed by González et al. (1997) to accommodate Gram-negative, strictly aerobic bacteria, mainly isolated from marine sediments. At the time of writing, the genus Microbulbifer contains 14 species, Microbulbifer hydrolyticus (González et al., 1997), Microbulbifer salipaludis (Yoon et al., 2003a), Microbulbifer elongatus (Yoon et al., 2003b), Microbulbifer maritimus (Yoon et al., 2004), Microbulbifer celer (Yoon et al., 2007), Microbulbifer agarlyticus and Microbulbifer thermotolerans (Miyazaki et al., 2008), Microbulbifer halophilus (Tang et al., 2008), Microbulbifer donghaiensis (Wang et al., 2009), Microbulbifer epialgicus and Microbulbifer variabilis (Nishijima et al., 2009), Microbulbifer chitinilyticus and Microbulbifer okinawensis (Baba et al., 2011), Microbulbifer marinus and Microbulbifer yueqingensis (Zhang et al., 2012) and Microbulbifer taiwanensis (Kämpfer et al., 2012). Tidal flats are broad, plain marshes with low-gradient depth, or muddy coastal areas that experience exposure and flooding by seawater between low and high tides. In addition, the varying influences of fresh river water and saline marine waters cause physical conditions, principally temperature, salinity and acidity, to vary more widely than those in any

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GY2T and SPO729T are JF751045 and DQ993341, respectively.

A supplementary figure is available with the online version of this paper.
other marine environments. Therefore, sea-tidal flats contain a wealth of valuable biological resources such as micro-organisms and marine animals. In the course of studies investigating microbial communities inhabiting tidal flats, several bacterial community members have been characterized (Jin et al., 2011a, 2011b; Jung et al., 2011; Lee et al., 2011; Park et al., 2011). Here, we describe the taxonomic characterization of a novel Microbulbifer species isolated from a tidal flat of the South Sea in Korea with the taxonomic characterization of another novel Microbulbifer species isolated from a marine sponge in the Pacific Ocean.

Strain GY2T was isolated from a surface tidal flat (less than 5 cm depth) of Gwangyang Bay (34°56′00.49″N, 127°36′18.71″E) of the South Sea in February, 2011, using a previously described procedure with some modifications (Jung et al., 2011). Briefly, a tidal flat sample was serially diluted with artificial seawater (ASW; 20 g NaCl, 2.9 g MgSO4, 4.53 g MgCl2·6H2O, 0.64 g KCl, 1.75 g CaCl2·2H2O, 1.83 g Na2SO4·10H2O, 0.03 g Na2HPO4·12H2O, 0.005 g K2SO4·H2O, 0.005 g Na2CO3·10H2O, pH 7.8) spread on marine agar (Marine agar 2216, MA; BD) and incubated at 25 °C for 3 days. Colonies were randomly selected and crude genomic DNA was prepared as described previously (Lu et al., 2006). PCR amplification of 16S rRNA genes was performed using universal primers, F1 and R13 (Jin et al., 2011b) and the amplicons were double-digested with HaeIII and HhaI. Based on their restriction fragment patterns, PCR products with unique fragment patterns were selected and sequenced using the F1 primer. The resulting 16S rRNA gene sequences were analysed using the BLAST program (Altschul et al., 1997) in GenBank, and the search results were used as a guide to classify the strains. From the analysis, a novel strain belonging to the genus Microbulbifer, designated strain GY2T, was selected for further phenotypic and phylogenetic analysis. In this study, additionally we characterized the taxonomic properties of another member of the genus Microbulbifer, strain SPO729T, which was isolated from a marine sponge found at 20 m depth in a tropical area, Weno Island, Chuuk State, Federated State of Micronesia (FSM) in the Pacific Ocean. Strains GY2T and SPO729T were routinely grown aerobically on MA at 35 °C for 2 days, except where indicated otherwise. The two novel strains were stored at −80 °C in marine broth (MB; BD) supplemented with 10% (v/v) glycerol. Microbulbifer hydrolyticus DSM 11525T and Microbulbifer donghaiensis JCM 15145T, purchased from DSMZ (Germany) and JCM (Japan), respectively, and Microbulbifer okinawensis ABAA23T (a gift from Dr Y. Nogi, Extremobiosphere Research Program, JAMSTEC, Japan) were used as reference strains for phenotypic characterization and DNA–DNA hybridizations.

The 16S rRNA gene amplitcons of strains GY2T and SPO729T were each ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer’s instructions for almost full sequencing. The inserted 16S rRNA genes were sequenced with the M13 reverse and T7 primers of the TOPO cloning kit. The resulting almost complete 16S rRNA gene sequences of strains GY2T (1497 nt) and SPO729T (1495 nt) were checked manually for the evaluation of quality. Sequence similarity values of strains GY2T and SPO729T were evaluated using the Nucleotide Similarity Search program at EzTaxon (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) and aligned with closely related taxa using the greengenes alignment program (http://greengenes.lbl.gov; DeSantis et al., 2006). Phylogenetic trees using the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms were constructed by the PHYLIP software version 3.6 (Felsenstein, 2002). The resulting tree topologies were evaluated using bootstrap analyses based on 1000 resampled datasets within the PHYLIP package. Maximum-likelihood (ML) analysis with bootstrap values was performed using 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 of the 16S rRNA gene sequences showed that strain GY2T was closely related to M. okinawensis ABABA23T and M. donghaiensis CN85T with similarities of 98.2 and 97.0%, respectively, whereas strain SPO729T was closely related to M. okinawensis ABABA23T and M. donghaiensis CN85T with similarities of 98.3 and 98.2%, respectively, which suggested that strains GY2T and SPO729T were very closely related to each other. The 16S rRNA gene sequence similarity value between strains GY2T and SPO729T was 98.0%. Phylogenetic analysis based on 16S rRNA gene sequences using the NJ, MP and ML algorithms showed that strains GY2T and SPO729T formed tight phylogenetic lineages with members of the genus Microbulbifer (Fig. 1).

DNA–DNA hybridization was carried out to evaluate the levels of DNA relatedness among strains GY2T and SPO729T and other closely related reference strains, M. okinawensis ABABA23T and M. donghaiensis JCM 15145T, as described previously (Lee et al., 2011). Briefly, different concentrations of the extracted genomic DNA for slot hybridization were blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech) in three replicates after denaturation using NaOH solution and heating (80 °C). Each DNA sample (4 μg) fragmented by a HaeIII digestion was used individually as a labelled DNA probe for cross-hybridization. Random primed DNA labelling with digoxigenin (DIG)-dUTP and detection of hybrids by enzyme immunoassay on nylon membrane were performed using DIG High Prime DNA Labelling kit (Roche Applied Science) according to the manufacturer’s instructions and standard procedures (Sambrook & Russell, 2001). The hybridization signals were captured and analysed with Quantity One (ver. 4.62; Bio-Rad). The signal produced by hybridization of the probe to the homologous target DNA was taken to be 100% and signal intensities by the self-hybridization of the series of dilutions were used for the calculation of the levels of DNA relatedness among strains GY2T and SPO729T and reference strains, M. donghaiensis JCM 15145T and M.
Transmission electron microscopy (JEM-1010, JEOL) with morphology and the presence of flagella were studied using a commercial kit according to the instructions of the manufacturer. Cell size and shape were determined by electron microscopy. The ionic requirement for Mg²⁺ in the laboratory according to the formula of the medium.

Growth at various NaCl concentrations ranging from 0 to 2 M in marine broths with different NaCl concentrations prepared in seawater. The pH values were verified after sterilization for pH 4.5–10.0 (at 0.5-pH-unit intervals) prior to sterilization at 0–45 °C (121 °C, 15 min) and adjusted again when necessary. Growth was assessed on MA and MA supplemented with potassium nitrate (0.1 %, w/v) under anaerobic conditions using the GasPak Plus system (BBL) at 35 °C for 20 days. Hydrolysis of casein, Tween 80, Tween 20, tyrosine, starch and xylan was investigated on MA and MA supplemented with potassium nitrate (0.1 %, w/v) under anaerobic conditions using the GasPak Plus system (BBL) at 35 °C for 20 days.

The phenotypic analysis of strains GY2T and SPO729T was performed as described below. Growth was assessed on MA at 0–45 °C (at 5 °C intervals) and in MB adjusted to pH 4.5–10.0 (at 0.5-pH-unit intervals) prior to sterilization using Na₂HPO₄–NaH₂PO₄ and Na₂CO₃–NaHCO₃ buffers for pH <8.0 and pH 8.0–10.0, respectively (Gomori, 1955). The pH values were verified after sterilization at 121 °C (15 min) and adjusted again when necessary. Growth at various NaCl concentrations ranging from 0 to 10 % (w/v, at 1 % intervals) was investigated using marine broths with different NaCl concentrations prepared in the laboratory according to the formula of the medium. The ionic requirement for Mg²⁺ or Ca²⁺ was determined as described previously (Nishijima et al., 2009). Gram staining was performed using the bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology and the presence of flagella were studied using transmission electron microscopy (JEM-1010, JEOL) with: 

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Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strains GY2T and SPO729T and related taxa. Bootstrap values are shown as percentages of 1000 replicates, when greater than 70 %. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Escherichia coli ATCC 11775T (X80725) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Two novel Microbulbifer species

okinawensis ABABA23T. The hybridization experiments were always confirmed by cross-hybridizations. The DNA–DNA relatedness value between strains GY2T and SPO729T was 13.1 ± 3.9 %, which suggested that the two isolates could be classified into different novel species within the genus Microbulbifer. The DNA–DNA relatedness values of strain GY2T with M. okinawensis ABABA23T and M. donghaiensis JCM 15145T were 40.0 ± 2.1 % and 16.2 ± 5.8 %, respectively, whereas those of SPO729T with M. okinawensis ABABA23T and M. donghaiensis JCM 15145T were 48.0 ± 4.0 % and 34.6 ± 9.3 %, respectively, which are clearly below the 70 % threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001).

The phenotypic analysis of strains GY2T and SPO729T was performed as described below. Growth was assessed on MA at 0–45 °C (at 5 °C intervals) and in MB adjusted to pH 4.5–10.0 (at 0.5-pH-unit intervals) prior to sterilization using Na₂HPO₄–NaH₂PO₄ and Na₂CO₃–NaHCO₃ buffers for pH <8.0 and pH 8.0–10.0, respectively (Gomori, 1955). The pH values were verified after sterilization at 121 °C (15 min) and adjusted again when necessary. Growth at various NaCl concentrations ranging from 0 to 10 % (w/v, at 1 % intervals) was investigated using marine broths with different NaCl concentrations prepared in the laboratory according to the formula of the medium. The ionic requirement for Mg²⁺ or Ca²⁺ was determined as described previously (Nishijima et al., 2009). Gram staining was performed using the bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology and the presence of flagella were studied using transmission electron microscopy (JEM-1010, JEOL) with...
The only respiratory lipoquinone detected in both strains

Staphylococcus aureus (Acar, 1980). Top marine agar (0.5 %)

held at about 45 °C was inoculated with marine broth
culture (approximately 10^8 cells ml^-1) of the isolates and
poured over the MA plates forming a homogeneous top
layer. Filter-paper discs (6 mm, Whatman) containing
antibiotics were placed onto the top layer of the agar plates.
The agar plates were incubated for 3 days at 35 °C and
their growth inhibitions were evaluated. Antibiotics were
added at the following concentrations (µg per disc unless
stated otherwise): ampicillin (10), polymyxin B (100 U),
streptomycin (50), penicillin G (20 U), chloramphenicol
(100), tetracycline (30), kanamycin (30), lincomycin (15),
oleandomycin (15), carbencillin (100), neomycin (30) and
novobiocin (5).

Both strains $GY2^T$ and $SPO729^T$ were Gram-stain-negative,
obligately aerobic and straight non-motile rods. Cell size of
strain $GY2^T$ was 0.3–0.4 µm wide and 2.0–4.5 µm long
(Fig. S1, available at IJSEM online), whereas that of strain
$SPO729^T$ was 0.3–0.4 µm wide and 4.0–6.0 µm long (data
not shown). The two novel strains and the reference species
of the genus Microbulbifer grew at similar temperature
ranges and optimum temperatures. However, a compar-
ison of physiological and biochemical characteristics
showed differences in results for colony pigmentation,
NaCl concentration for growth, hydrolysis of gelatin and
tyrosine, some enzyme activities, acid production and
sensitivities to antibiotics (Table 1). More physiological
and biochemical characteristics of strains $GY2^T$ and
$SPO729^T$ are presented in Table 1 and in the species
descriptions.

Cell mass for the analysis of isoprenoid quinone and fatty
acids was obtained from cultures in the exponential growth
phase. In particular, for the analysis of cellular fatty acids,
strains $GY2^T$ and $SPO729^T$ and reference strains were
cultivated in MB at 35 °C and their cells were harvested at
the same growth phase (OD600 0.8). Analysis of fatty acid
methyl esters was performed by gas chromatography
(Agilent 6890N) according to the instructions of the
Sherlock Microbial Identification System (MIDI; Microbial
ID) based on TSBA 40 database. Isoprenoid quinones of
strains $GY2^T$ and $SPO729^T$ 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). Total
genomic DNA from strains $GY2^T$ and $SPO729^T$ for the
DNA G+C determination was extracted using a Wizard
Genomic DNA Purification kit (Promega) according to the
manufacturer’s instructions and their DNA G+C contents
were determined by the fluorometric method (Gonzalez &
Saiz-Jimenez, 2002) using SYBR Green I and a real-time
PCR thermocycler (Bio-Rad).

The only respiratory lipoquinone detected in both strains
$GY2^T$ and $SPO729^T$ was ubiquinone-8 (Q-8), in line with
all other members of the genus Microbulbifer. Both strains
$GY2^T$ and $SPO729^T$ contained iso-C17:0, iso-C17:1ω9c,
iso-C18:0, iso-C18:1ω9c, iso-C15:0, C16:0, C18:1ω07c,
iso-C17:0 3-OH, iso-C16:0 and iso-C15:0 as the major cellular fatty acids, which were in common with
those of the reference strains of the genus Microbulbifer
grown under the same conditions; there were only limited
differences in the respective proportions of some com-
ponents (Table 2). The genomic DNA G+C contents of
strains $GY2^T$ and $SPO729^T$ were 55.1 and 57.9 mol%,
respectively, which were similar to those of other species of
the genus Microbulbifer (Baba et al., 2011; Zhang et al.,
2012). Therefore, the phenotypic and DNA features of
strains $GY2^T$ and $SPO729^T$ as well as phylogenetic
inference support their descriptions as two novel species
within the genus Microbulbifer, for which the names
Microbulbifer gwangyangensis sp. nov. Microbulbifer pacifi-
cus sp. nov. are proposed.

Description of Microbulbifer gwangyangensis
sp. nov.

Microbulbifer gwangyangensis (gwang.yang.en’sis. N.L.
masc. adj. gwangyangensis of or belonging to Gwangyang
Bay where strain $GY2^T$ was isolated).

Cells are Gram-stain-negative, chemoheterotrophic, obli-
gately aerobic, non-spor-forming and non-motile rods, 0.3–0.4 µm wide and 2.0–4.5 µm long. Resting coccoid
cells 0.7–0.9 µm in diameter are observed. Colonies on MA
are cream-coloured, circular and convex with slightly
irregular edges. Growth occurs at 10–40 °C (optimum,
35 °C), at pH 5.5–9.0 (optimum, pH 7.0–7.5) and in the
presence of 0–9.0 % (w/v) NaCl (optimum, 1.0–2.0 %).
Anaerobic growth is not observed after 20 days at 35 °C
on MA or MA supplemented with nitrate. Oxidase and
catalase are positive. Mg^{2+} and Ca^{2+} are not required
for growth. Tween 20, Tween 80, casein and starch are
hydrolysed, while DNA, tyrosine and xylan are not. H_{2}S is
not produced. Voges–Proskauer reaction is positive. Nitrate is
reduced to nitrite, but nitrogen gas is not produced. In
API 20E tests, gelatinase activity is positive, but indole
production, citrate utilization and ß-nitrophenyl-ß-D-galac-
topyranosidase, arginine dihydrolase, lysine and ornithine
decarboxylase, tryptophan deaminase and urease activities
are negative. In API ZYM tests, alkaline phosphatase,
esterase (C4), esterase lipase (C8), lipase (C14), valine
arilamidase, leucine arilamidase, cystine arilamidase, ß-
chymotrypsin, acid phosphatase, naphthol-AS-BI-phospho-
hydrolase and N-acetyl-ß-glucosaminidase activities are
positive; ß-glucosidase activity is weak; trypsin, ß-galactosi-
dase, ß-galactosidase, ß-glucuronidase, ß-glucosidase, ß-
mannosidase and ß-fucosidase activities are negative. D-
glucose and maltose are utilized as a sole carbon and energy
sources, but D-fructose, D-galactose, sucrose, lactose, D-
mannose, DL-malic acid, melibiose, raffinose, D-sorbitol and
D-mannitol are not utilized. Acid is produced from D-
glucose, L-rhamnose, cellulbiose and maltose, but not from
D-fructose, lactose, D-galactose, D-mannose, L-arabinose,
sucrose, melibiose, D-xyllose, trehalose, D-sorbitol, D-
mannitol and raffinose. Strain $GY2^T$ is resistant to polymyxin B,
streptomycin, novobiocin, lincomycin, oleandomycin and

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carbenicillin, but sensitive to penicillin G, chloramphenicol, ampicillin, kanamycin, neomycin and tetracycline. The major respiratory quinone is Q-8. The major cellular fatty acids (>5% of the total fatty acids) are iso-C_{15:0}, C_{16:0}, C_{18:1}ω7c, iso-C_{17:1}ω9c, iso-C_{11:0} 3-OH, iso-C_{17:0} and iso-C_{11:0}.

The type strain is GY2^T (=KACC 16189^T=JCM 17800^T), which was isolated from sediment of a tidal flat of the Gwangyang Bay in South Korea. The DNA G+C content of the type strain is 55.1 mol%.

### Description of Microbulbifer pacificus sp. nov.

*Microbulbifer pacificus* (pa.ci’fi.cus. L. masc. adj. *pacificus* peaceful, pertaining to the Pacific Ocean from which the type strain was isolated).

Cells are Gram-stain-negative, chemoheterotrophic, obligately aerobic, non-spore-forming and non-motile rods 0.3–0.4 μm wide and 4.0–6.0 μm long. Resting coccolid cells, 0.8–1.1 μm in diameter, are observed. Colonies on MA are ivory-coloured, circular and convex with slightly irregular edges. Growth occurs at 15–45 °C (optimum, 35–37 °C).

### Table 1. Differential phenotypic characteristics of strains GY2^T and SPO729^T and phylogenetic neighbours of the genus *Microbulbifer*

<table>
<thead>
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<th>Characteristic</th>
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<tbody>
<tr>
<td>Colony colour</td>
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<td>Ivory</td>
<td>Cream</td>
<td>Light yellow</td>
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<td>Growth temperature (°C)</td>
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<td>Range</td>
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<td>Optimum</td>
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<td>37*</td>
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<td>Growth at 10% NaCl</td>
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<td>Nitrate reduction</td>
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<td>Voges–Proskauer reaction</td>
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<td>Hydrolysis of:</td>
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<td>Enzyme activities (API ZYM)</td>
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<td>Susceptibility to:</td>
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<tr>
<td>Polymyxin B</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>55.1</td>
<td>57.9</td>
<td>60.2*</td>
<td>57.8†</td>
<td>57.5‡</td>
</tr>
</tbody>
</table>

*Data from Baba *et al.* (2011).
†Data from Wang *et al.* (2009).
‡Data from Miyazaki *et al.* (2008).
Table 2. Whole cellular fatty acid compositions (%) of strains GY2T and SPO729T and related type strains of members of the genus Microbulbifer

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Saturated</td>
<td></td>
<td></td>
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<tr>
<td>C10:0</td>
<td>1.9</td>
<td>1.0</td>
<td>2.1</td>
<td>1.4</td>
<td>3.4</td>
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<tr>
<td>C14:0</td>
<td>1.6</td>
<td>0.7</td>
<td>0.7</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>C15:0</td>
<td>2.7</td>
<td>1.2</td>
<td>1.5</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>13.6</td>
<td>9.3</td>
<td>11.6</td>
<td>11.5</td>
<td>12.7</td>
</tr>
<tr>
<td>C17:0</td>
<td>3.4</td>
<td>1.7</td>
<td>2.9</td>
<td>0.8</td>
<td>2.2</td>
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<tr>
<td>C18:0</td>
<td>0.9</td>
<td>1.2</td>
<td>1.5</td>
<td>1.1</td>
<td>2.2</td>
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<tr>
<td>Branched</td>
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<tr>
<td>iso-C11:0</td>
<td>6.3</td>
<td>6.0</td>
<td>7.1</td>
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<tr>
<td>iso-C15:0</td>
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<td>27.1</td>
<td>24.7</td>
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<tr>
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<td>9.3</td>
<td>7.1</td>
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<tr>
<td>iso-C17:1ω9c</td>
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<td>13.0</td>
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<tr>
<td>anteiso-C17:0</td>
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<td>0.6</td>
<td>TR</td>
<td>–</td>
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<td>Unsaturated</td>
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<tr>
<td>C17:1ω8c</td>
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<td>1.2</td>
<td>TR</td>
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<td>C18:1ω7c</td>
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<tr>
<td>11-Methyl-C18:1ω7c</td>
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<td>TR</td>
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<td>C10:0 3-OH</td>
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<td>2.0</td>
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<td>3.1</td>
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<tr>
<td>iso-C11:0 3-OH</td>
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<td>6.5</td>
<td>8.5</td>
<td>15.5</td>
<td>8.2</td>
</tr>
<tr>
<td>Cyclo</td>
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<td>6.0</td>
</tr>
<tr>
<td>Cyclo-C19:1ω8c</td>
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<td>Summed features*</td>
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<td>3.7</td>
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<td>7</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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</tr>
</tbody>
</table>

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 comprises C16:1ω7c and/or C16:1ω6c and/or iso-C15:0 2-OH. Summed feature 7 comprises unknown (equivalent chain-length) 18.846 and/or C19:1ω6c.

35 °C), at pH 6.0–10.0 (optimum, pH 7.0–7.5) and in the presence of 1.0–7.0 % (w/v) NaCl (optimum, 2.0–3.0 %). Anaerobic growth is not observed after 20 days at 35 °C on MA or MA supplemented with nitrite. Oxidase and catalase are positive. Mg2+ and Ca2+ are not required for growth. Tween 20, Tween 80, casein, DNA and starch are hydrolysed, while tyrosine and xylan are not. H2S is not produced. Voges–Proskauer reaction is negative. Nitrate is reduced to nitrite, but nitrogen gas is not produced. In API 20E tests, indole production, citrate utilization and o-nitrophenyl-β-D-galactopyranosidase, arginine dihydrolase, lysine and ornithine decarboxylase, tryptophan deaminase, gelatinase and urease activities are negative. In API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, leucine arylamidase, β-xymothripsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive; cystine arylamidase activity is weak; trypsin, γ-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, β-mannosidase and x-fucosidase activities are negative. D-fructose, D-glucose, maltose and D-sorbitol are utilized as sole carbon and energy sources, but D-galactose, sucrose, lactose, D-mannose, D-malic acid, melibiose, raffinose and D-mannitol are not utilized. Acid is produced from D-galactose, D-glucose and maltose, but not from L-arabinose, cellobirose, lactose, D-fructose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose and D-xylene. Strain SPO729T is resistant to ampicillin, lincomycin, novobiocin, oleandomycin, streptomycin and tetracycline, but sensitive to carbencillin, chloramphenicol, kanamycin, neomycin, penicillin G and polymyxin B. The major respiratory quinone is Q-8. The major cellular fatty acids (>5% of the total fatty acids) are iso-C15:0, C16:0, C18:1ω7c, iso-C17:1ω9c, iso-C11:0 3-OH, iso-C17:0 and iso-C11:0.

The type strain is SPO729T (=KCCM 42667T=JCM 14507T), which was isolated from a marine sponge found at 20 m depth in a tropical area, Weno Island, Chuuk State, Federated State of Micronesia (FSM), located in the Pacific Ocean. The DNA G+C content of the type strain is 57.9 mol%.

Acknowledgements

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Two novel Microbulbifer species


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