Microbulbifer taiwanensis sp. nov., isolated from coastal soil

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A Gram-negative, non-spore-forming rod (CC-LN1-12T) was isolated from coastal soil samples of Lutao Island (Green Island), Taiwan, and its taxonomic position was studied. 16S rRNA gene sequence analysis showed that isolate CC-LN1-12T was grouped into the Microbulbifer cluster, with the highest similarities to Microbulbifer okinawensis ABABA23T (97.9 %), Microbulbifer maritimus TF-17T (97.7 %) and Microbulbifer donghaiensis CN85T (97.7 %), similarities to all other species of the genus Microbulbifer were lower than 96.8 %. The polyamine pattern contained the major compounds spermidine and cadaverine. The fatty acid profile, comprising the major fatty acids iso-C₁₅ : 0, iso-C₁₇ : 1ω₉c, C₁₈ : 1ω₇c and iso-C₁₁ : 0 3-OH as the major hydroxylated fatty acid, supported the affiliation of strain CC-LN1-12T to the genus Microbulbifer. DNA–DNA hybridizations between strain CC-LN1-12T and Microbulbifer okinawensis ABABA23T, M. donghaiensis CN85T and M. maritimus JCM 12187T resulted in relatedness values of 21.5 % (14.3 %, reciprocal analysis), 35.9 % (48.5 %, reciprocal analysis) and 48.1 % (52.1 %, reciprocal analysis), respectively. From these data, as well as from physiological and biochemical tests, strain CC-LN1-12T could be clearly differentiated from the most closely related species of the genus Microbulbifer. It is concluded that strain CC-LN1-12T represents a novel species, for which the name Microbulbifer taiwanensis sp. nov. is proposed. The type strain is CC-LN1-12T (=LMG 26125T=CCM 7856T).

In 1997, the genus Microbulbifer was proposed by González et al. (1997) for a novel rod-shaped and strictly aerobic marine bacterium allocated to the class Gammaproteobacteria with only one species Microbulbifer hydrolyticus. A second species, Microbulbifer salipaludis, was added to the genus by Yoon et al. (2003a), and subsequently, several other species, Microbulbifer maritimus (Yoon et al., 2004), Microbulbifer agarilyticus and Microbulbifer thermotolerans (Miyazaki et al., 2008), Microbulbifer variabilis and Microbulbifer epialgicus (Nishijima et al., 2009), Microbulbifer celer (Yoon et al., 2007), Microbulbifer elongatus (Yoon et al., 2003b), Microbulbifer halophilus (Tang et al., 2008), Microbulbifer donghaiensis (Wang et al., 2009), Microbulbifer chitinilyticus and Microbulbifer okinawensis (Baba et al., 2011), have been added to this genus. The majority of these species have been isolated from marine habitats.

Strain CC-LN1-12T was isolated from coastal soil samples from Lutao Island (Green Island; 22° 39’ N 121° 29’ E), Taiwan, on Marine Agar 2216 (MA; Difco), incubated at 28°C. The strain was maintained on this agar at 28°C.

Morphological properties, Gram-staining and cell morphology were observed by phase-contrast microscopy as...
described by Kämpfer & Kroppenstedt (2004). Gram-behaviour was also examined by the KOH test (Moaledj, 1986).

The 16S rRNA gene sequence was analysed as described previously (Kämpfer et al., 2003). Multiple sequence alignment and analysis of the data were performed using the MEGA (molecular evolutionary genetics analysis) version 4 software package (Tamura et al., 2007). Genetic distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining (Fig. 1) and maximum-parsimony methods (results not shown) were performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain CC-LN1-12T was a continuous stretch of 1487 bp. Sequence similarity calculation analysis indicated that the closest relatives of strain CC-LN1-12T were Microbulbifer okinawensis ABABA23T (97.9 %), Microbulbifer maritimus JCM 12187T (97.7 %) and Microbulbifer donghaiensis CN85T (97.7 %). The 16S rRNA gene sequence similarity to all other species of the genus Microbulbifer was lower than 96.7 % (96.2 % to the type strain of the type species Microbulbifer hydrolyticus IRE-31T).

Fatty acid analysis was performed according to the method described by Kämpfer & Kroppenstedt (1996) and generated using a GC (HP6890; Hewlett Packard) with Sherlock MIDI software version 2.11 and TSBA peak naming table version 4.1.

The fatty acid profile of strain CC-LN1-12T was similar to those of the other closely related species of the genus Microbulbifer. Strain CC-LN1-12T showed some minor quantitative differences in comparison to other species (Table 1). The typical major fatty acids iso-C15:0, iso-C17:0 3-OH and C18:1 ω7c as well as the major hydroxylated fatty acid iso-C11:0 3-OH could be found in strain CC-LN1-12T. For quinone and polar lipid analysis, cells were grown on MA. Quinone analysis was performed according to the method described by Busse & Auling (1988) and HPLC analysis was conducted as described by Stolz et al. (2007). The polyamine pattern of strain CC-LN1-12T is composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, three aminolipids, one unknown lipid and one glycolipid. The same polar lipid pattern was found in Microbulbifer donghaiensis CN85T, but the glycolipid was missing. In Microbulbifer maritimus JCM 12187T, one aminolipid (AL2) was not detectable. The analysis revealed that diphosphatidylglycerol could be absent in this strain, but a weak lipid spot was detectable (lipid Lx) exhibiting the chromatographic behaviour of diphosphatidylglycerol. This spot was not detectable after molybdenum blue staining, which is a prerequisite for its identification as diphosphatidylglycerol. However, the non-staining ability of lipid Lx with molybdenum blue might have been due to the very low amount of lipid Lx in the extract (Fig. 2). Hence, it is possible that diphosphatidylglycerol is present in Microbulbifer but that the amounts are too low to be detected with molybdenum blue.

For production of biomass for polyamine extraction, cells were grown at 37 °C on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) supplemented with 3.1 % sea salts (Sigma-Aldrich) and harvested at the late exponential growth phase. Extraction was carried out as described by Busse & Auling (1988) and HPLC analysis was conducted as described by Stolz et al. (2007). The polyamine pattern of strain CC-LN1-12T contained spermidine [5.1 μmol (g dry weight)−1], cadaverine [3.4 μmol (g dry weight)−1], putrescine [0.3 μmol (g dry weight)−1] and traces of spermine and 1,3-diaminopropane [both <0.1 μmol (g dry weight)−1]. This polyamine pattern, especially the presence of major amounts of cadaverine, is quite rare among bacteria but similar characteristics have also been reported to be present in two other species of the genus Microbulbifer, namely Microbulbifer salipaludis and Microbulbifer hydrolyticus (Hamana et al., 2006). The results of the comparative...
physiological characterization using identical test conditions (Kämpfer et al., 1991) for strain CC-LN1-12ᵀ and closely related species of the genus Microbulbifer are presented in Table 2 and the species description.

DNA–DNA hybridizations between strain CC-LN1-12ᵀ and M. okinawensis ABABA23ᵀ, M. donghaiensis CA85ᵀ and M. maritimus JCM 12187ᵀ were performed according to Ziemke et al. (1998) and resulted in values of 21.5 % (14.3 %, reciprocal analysis), 35.9 % (48.5 %, reciprocal analysis), and 48.1 % (52.1 %, reciprocal analysis), respectively.

These data clearly demonstrate that strain CC-LN1-12ᵀ exhibits traits that are typical characteristics of the genus Microbulbifer, such as a quinone system with ubiquinone Q-8 and a fatty acid profile consisting of iso-C₁₅:₀, iso-C₁₇:₁₀₉c, C₁₈:₁₀₇c and the major hydroxylated fatty acid iso-C₁₁:₀ 3-OH.

The novel strain could be distinguished from the most closely related species of the genus Microbulbifer on the basis of physiological traits. The results from DNA–DNA hybridization experiments, in addition to the differences in the 16S rRNA gene sequences, suggest that strain CC-LN1-12ᵀ is a representative of a novel species of the genus Microbulbifer for which the name Microbulbifer taiwanensis sp. nov. is proposed.

### Description of Microbulbifer taiwanensis sp. nov.

Microbulbifer taiwanensis (tai.wan.en’sis. N.L. masc. adj. taiwanensis pertaining to Taiwan from where the strain was originally isolated).

Rod-shaped, sometimes coccoid cells, about 2–3.5 µm in diameter. No resting stages observed. Not motile. Gram-negative, oxidase- and catalase-positive and showing an oxidative metabolism. Unable to grow at 15 °C or 50 °C. Cells grow in the presence of 7.0 % NaCl (w/v) (3–4 % optimum). Good growth occurs after 24 h on MA (Difco) at 28 °C, only very weak growth occurs on tryptone soy agar, nutrient agar and R2A agar (all Oxoid). No growth occurs on MacConkey agar (Oxoid) at 28 °C. Colonies on MA convert from yellowish to brown and are circular, translucent and shiny with entire edges. The quinone system is ubiquinone Q-8. The phospholipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, three aminolipids, one unknown lipid and one glycolipid. Major fatty acids are iso-C₁₅:₀, iso-C₁₇:₁₀₉c and C₁₈:₁₀₇c and the major hydroxylated fatty acid is iso-C₁₁:₀ 3-OH. The polyamine pattern contains spermidine and cadaverine as major compounds. The results of physiological tests (including differentiating characteristics determined under identical conditions) are indicated in Table 2. Produces acid from D-glucose (weakly), sucrose, maltose and D-mannitol. No acid is produced from adonitol, L-arabinose, D-arabitol, dulcitol, erythritol, i-inositol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol or D-xyllose. Tests for urease activity, indole production, hydrolysis of casein and aesculin, gelatin, starch, DNA and tyrosine give a negative result. Tests for hydrogen sulphide production and for the activity of arginine dihydrolase, tyrosine give a negative result. Tests for gelatin, hydrogen sulphide and aesculin are negative. The following compounds are not utilized as a sole source of carbon in a mineral-salt medium (Kämpfer et al., 1991): N-acetyl-D-glucosamine, D-glucose, maltose, D-mannose, D-fructose, trehalose, maltose, pyruvate, acetate, propionate, N-acetylgalactosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, glucosanate, glycerol, D-mannitol, maltitol, z-melibiose, D-mannitol, melibiose, L-arabinose, D-galactose, D-glucose, maltose, sucrose, L-arabinose, and D-mannitol.

### Table 1. Cellular fatty acid composition (%) of strain CC-LN1-12ᵀ and the type strains of the most closely related Microbulbifer species

<table>
<thead>
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<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td><strong>Straight chain</strong></td>
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<tr>
<td>C₁₀:₀</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
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<td>C₁₂:₀</td>
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<td>1.0</td>
<td>1.3</td>
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<td>C₁₄:₀</td>
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<td><strong>Branched</strong></td>
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<td>iso-C₁₁:₀</td>
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<td>33.0</td>
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<td>3.3</td>
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<td>anteiso-C₁₅:₀</td>
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<td>8.4</td>
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<td>iso-C₁₇:₁₀₉c</td>
<td>25.2</td>
<td>19.3</td>
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<td>C₁₅:₁₀₈c</td>
<td>1.4</td>
<td>0.6</td>
<td>0.5</td>
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<td>C₁₇:₁₀₈c</td>
<td>2.5</td>
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<td>iso-C₁₁:₀ 3-ΟΗ</td>
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<td>3.1</td>
<td>1.0</td>
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<tr>
<td><strong>Summed features</strong></td>
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<tr>
<td>3</td>
<td>8.0</td>
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<td>4</td>
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</table>

*The double-bond position indicated by a capital letter is unknown.
†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained one or more of the following fatty acids: 2-ΟΗ and/or C₁₆:₁₀₇c. Summed feature 4 contained one or more of the following fatty acids: anteiso-C₁₇:₁ B and/or iso-C₁₇:₁ I.

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Fig. 2. Total polar lipid profile of strains CC-LN1-12T (a), *M. donghaiensis* CN85T (b) and *M. maritimus* JCM 12187T (c) after staining with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; AL1, AL2, AL3, unidentified aminolipids; PL1, unidentified phospholipid; GL1, unidentified glycolipid; L1, L2, unidentified lipid; Lipid Lx exhibited the chromatographic behaviour of DPG, but after detection with molybdenum blue it did not become visible as a blue spot, probably due to the very low amount in the extract.

Table 2. Differential phenotypic characteristics of strain CC-LN1-12T and the type strains of the most closely related *Microbulbifer* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3†</th>
<th>4†</th>
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<th>7</th>
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<th>9</th>
<th>10</th>
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<tr>
<td>Cell shape</td>
<td>Cocci and rods</td>
<td>Cocci and rods</td>
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<td>Cocci and rods</td>
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<td>Cocci and rods</td>
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<td>Cocci and rods</td>
<td>Rods</td>
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<tr>
<td>Colony colour‡</td>
<td>B</td>
<td>C</td>
<td>LY</td>
<td>YB</td>
<td>B</td>
<td>C</td>
<td>GY</td>
<td>YB</td>
<td>GY</td>
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<td>Growth at/with:</td>
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<td>10 % NaCl</td>
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<td>–</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<td>pH 5.0</td>
<td>–</td>
<td>( + )</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>( + )</td>
<td>ND</td>
<td>+</td>
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<td>45 °C</td>
<td>( + )</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<td>ONPG (β-galactosidase)</td>
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<td>–</td>
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<td>Aesculin</td>
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<td>Acid production from:</td>
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<td>L-Arabinose</td>
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<td>–</td>
<td>–</td>
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<td>Cellobiose</td>
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<td>+</td>
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<td>D-Glucose</td>
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<td>+</td>
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<td>L-Rhamnose</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Sucrose</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Data exactly in accordance with those of Baba *et al.* (2011).
†Data exactly in accordance with those of Wang *et al.* (2009).
‡B, brown; C, cream; GY, greyish yellow; LY, light yellow; YB, yellowish brown.
§Data from Yoon *et al.* (2004).
L-rhamnose, D-ribose, sucrose, salicin, D-xylene, adonitol, i-
inositol, D-sorbitol, putrescine, cis-aconitate, trans-aconitate, 4-
aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-
hydroxybutyrate, itaconate, 2-oxoglutarate, suberate, citrate, 
mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylala-
nine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-
tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and 
phenylacetate. The chromogenic substrates (Kämpfer et al., 
2003) 2-deoxyxymidine-2′-p-nitrophenyl-phosphate and L-
alanine-p-nitroanilide are hydrolysed. The substrates 
p-nitrophenyl-α-D-glucopyranoside, p-nitrophenyl-β-D-
galactopyranoside, p-nitrophenyl-β-D-glucopyranoside, 
p-nitrophenyl-β-D-xylopyranoside, p-nitrophenyl-β-D-
gluconuride, bis-p-nitrophenyl-phosphate, bis-p-nitrophenyl-
phenyl-phosphonate, bis-p-nitrophenyl-phosphoryl-choline, 
γ-L-glutamate-p-nitroanilide and L-proline-p-nitroanilide are 
not hydrolysed.

The type strain, CC-LN1-12T (=LMG 26125T=CCM 7856T), was isolated from coastal soil samples from Lutao 
Island (Green Island), Taiwan.

Acknowledgements

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strain of M. okinawensis prior to publication of the species 
description.

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Proteobacteria: distributions of homospermidine within the class 
Alphaproteobacteria, hydroxyputrescine within the class Betapro-
teobacteria, norspermidine within the class Gammaproteobacte-
ria, and spermidine within the classes Deltaproteobacteria and 

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