**Sphingomicrobium lutaoense** gen. nov., sp. nov., isolated from a coastal hot spring

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A yellowish pigmented, Gram-negative, rod-shaped, non-spore-forming bacterium (strain CC-TBT-3T), was isolated on marine agar 2216 from a coastal hot spring of Green Island (Lutao), located off Taitung, Taiwan. 16S rRNA gene sequence analysis of strain CC-TBT-3T showed a relatively low similarity (<95.5%) to representatives of the genera *Novosphingobium*, *Sphingosinicella* and *Sphingomonas* of the *Sphingomonadaceae*, with the most related strain being the type strain of *Novosphingobium soli*. In addition to the relatively low 16S rRNA gene sequence similarity to members of established species, the isolate also showed some unique chemotaxonomic features, including the presence of some glycolipids with unusual chromatographic behaviour. The major components of the polar lipid profile were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid and three unidentified glycolipids. The major respiratory quinone was ubiquinone Q-10. The polyamine pattern was characterized by the triamine sym-homospermidine as a major component. Although the predominant fatty acids were C18:1ω7c and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), the isolate did not show the typical hydroxyl fatty acids, such as C14:0 2-OH, C15:0 2-OH and C16:0 2-OH, found in members of the genera *Novosphingobium*, *Sphingomonas* and *Sphingosinicella*, but showed instead high amounts of C18:1ω9c (12.0%). The DNA G+C content of strain CC-TBT-3T was 63.4 mol%. 16S rRNA gene sequence, chemotaxonomic and physiological analyses revealed that strain CC-TBT-3T represents a novel species in a new genus in the family *Sphingomonadaceae* for which the name *Sphingomicrobium lutaoense* gen. nov., sp. nov. is proposed; the type strain is of the type species *S. lutaoense*, CC-TBT-3T (=DSM 24194T = CCM 7794T).

**Abbreviations:** pNA, para-nitroanilide; pNP, para-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-TBT-3T is EU564841.

Three supplementary figures are available with the online version of this paper.

On the basis of 16S rRNA gene sequence and chemotaxonomic analyses, Takeuchi et al. (2001) proposed a dissection of the former genus *Sphingomonas* (Yabuuchi et al., 1990, 1999), and established the genera *Sphingobium*, *Novosphingobium* and *Sphingopyxis*. Maruyama et al. (2006) added an additional genus, *Sphingosinicella*, to this group of
taxa sharing a number of phenotypic features, including the presence of sphingolipids, yellow-pigmented colonies, the presence of ubiquinone Q-10 as the predominant quinone and, as shown by Geueke et al. (2007), a polyamine pattern with the triamine \textit{sym}-homospermidine as the major component.

Strain CC-TBT-3\textsuperscript{T} was isolated from water samples collected from a coastal hot spring (water temperature 55 °C; pH 7.3; salinity 32 %) located on a volcanic island (Green Island) in the Pacific Ocean, off the east coast of Taiwan (22° 39’ N 121° 29’ E). The strain was isolated and maintained on marine agar 2216 (MA; Difco) after incubation at 45 °C for 48 h. Subcultivation was done on MA at 28 °C for 2 days. The strain was preserved at −80 °C in marine broth 2216 (MB; Difco) with 20 % (v/v) glycerol or by lyophilization.

The strain grew as small yellowish colonies (<0.5 mm) with a glossy surface after 48 h at 28 °C on tryptic soy agar (TSA). Morphology of cells grown on TSA at 28 °C was investigated by phase-contrast microscopy. During exponential growth, CC-TBT-3\textsuperscript{T} cultures grew as single rod-shaped cells, 1.5 ± 0.5 mm long and 1.0 ± 0.1 mm wide and motile in the early exponential phase. Cells stained Gram-negative and were positive for cytochrome \textit{c} oxidase in a slightly delayed reaction as determined by using an oxidase test (Merck). Endospores could not be detected by phase-contrast microscopy. CC-TBT-3\textsuperscript{T} grew well on MA and moderately on R2A agar (Oxoid) at 28 °C. No growth was observed on nutrient agar (NA) or CASO agar (both Oxoid). The strain was able to grow at 20–36 °C. No growth was observed at <15 °C or at >45 °C. Growth was tested at various NaCl concentrations in R2A broth; strain CC-TBT-3\textsuperscript{T} grew in 1–7 % (w/v) NaCl. The pH range for growth (also tested in R2A broth) was 6.5–10.5; no growth was observed at pH 5.5 or 11.5.

For 16S rRNA gene sequencing, chromosomal DNA was extracted as described by Pitcher et al. (1989) and a 1462 bp fragment of the 16S rRNA gene was amplified using primers targeting conserved regions of the 16S rRNA gene, i.e. 8F (8–27, \textit{E. coli} numbering; Brosius et al., 1981) and 1492R (1492–1510) (Lane, 1991). Sequencing of the DNA fragment was done with primers 8F, 1492R, and two additional reverse primers, 907R (907–926) and 1100R (1100–1115) (Lane, 1991), using the Big Dye terminator cycle sequencing reaction kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems). DNA sequences were analysed with the software package \textit{MEGA} (Kumar et al., 2004) and phylogenetic reconstructions were performed with the \textit{ARB} software package (Ludwig et al., 2004). Similarities were calculated on the basis of a pair-wise comparison (\textit{ARB} software) and phylogenetic trees were obtained by the neighbour-joining and maximum-likelihood methods; bootstrap values based on 1000 replicates were determined for the neighbour-joining analysis. 16S rRNA gene sequence analysis revealed that strain CC-TBT-3\textsuperscript{T} had highest similarity to the type strain of \textit{Novosphingobium soli} (accession no. FJ425737; 95.1 % similarity) and lower sequence similarities (<97.4 %) to all other members of the genera \textit{Novosphingobium}, \textit{Sphingomonas} and \textit{Sphingosinibacter} (Fig. 1). Additional phylogenetic trees (Figs S1, S2 and S3) are available in ISJEM Online.

The DNA G+C content, determined as described previously (Urdiain et al., 2008), was 63.4 mol% (standard deviation of ±0.6).

Fatty acids were analysed from biomass grown on MA as described by Kämpfer & Kroppenstedt (1996) using an HP 6890 GC with Sherlock MIDI software version 2.11 and TSBA peak naming table version 4.1.

The dominant fatty acids of CC-TBT-3\textsuperscript{T} were C\textsubscript{18:1}ω7\textsubscript{c} (42.4 %), summed feature 3 (C\textsubscript{16:1}ω7\textsubscript{c} and/or iso-C\textsubscript{15:0} 2-\textsubscript{OH}; 16.3 %) and C\textsubscript{18:1} 2-\textsubscript{OH} (12.0 %). In addition, the following fatty acids were detected: C\textsubscript{16:1}ω5\textsubscript{c} (2.6 %), C\textsubscript{16:0} (5.8 %), C\textsubscript{17:1}ω8\textsubscript{c} (1.7 %), C\textsubscript{17:1}ω6\textsubscript{c} (9.2 %), C\textsubscript{17:0} (1.4 %), C\textsubscript{18:1}ω9\textsubscript{c} (1.8 %), C\textsubscript{18:0} (0.7 %) and 11-methyl C\textsubscript{18:1}ω7\textsubscript{c} (6.2 %). It is interesting to note that the hydroxylated fatty acids C\textsubscript{14:1} 2-\textsubscript{OH}, C\textsubscript{15:0} 2-\textsubscript{OH} and C\textsubscript{16:0} 2-\textsubscript{OH} were not detected in strain CC-TBT-3\textsuperscript{T}. Takeuchi et al. (2001) distinguished members of the genus \textit{Novosphingobium} from those of the genera \textit{Sphingomonas sensu stricto}, \textit{Sphingopyxis} and \textit{Sphingobium} by the absence of other major 2-\textsubscript{OH} fatty acids in addition to C\textsubscript{14:1}. However, strain CC-TBT-3\textsuperscript{T} contained a high amount of C\textsubscript{18:1} 2-\textsubscript{OH}, which was not found in representatives of the genus \textit{Novosphingobium}, \textit{Sphingomonas sensu stricto}, \textit{Sphingopyxis}, \textit{Sphingobium} and \textit{Sphingosinibacter} in such high amounts.

Quinones and polar lipids were extracted from biomass grown at 28 °C in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) supplemented with 3 % sea salts (Sigma) following the integrated procedure described by Tindall (1990a, b) and Altenburger et al. (1996). Polyamines were extracted from biomass grown on PYE (supplemented with 3 % sea salts), harvested at the late exponential growth phase and extracted as described previously (Busse & Auling, 1988). HPLC analyses were carried out using the apparatus described by Stolz et al. (2007). The quinone system was composed of ubiquinone Q-10 (89 %) and Q-9 (11 %). The major polar lipids were diposphatidyglycerol, phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid and three unidentified glycolipids (GL2, GL3 and GL4; Fig. 2). Furthermore, minor amounts of another unidentified glycolipid and three unidentified polar lipids that were not stained with any of the specific staining reagents used (ninhydrin, molybdenum blue or \textit{a}-naphthol) were detected. To the best of our knowledge, no species within the family \textit{Sphingomonadaceae} has so far been reported to contain a glycolipid that shows a chromatographic behaviour similar to glycolipid GL2. Furthermore, the absence of phosphatidylinomethylthanolamine and phosphatidylmethylethanolamine has only rarely been reported among species of the genus \textit{Sphingomonas} including \textit{Sphingomonas haloraomaticans} (Wittich et al., 2007) and \textit{Sphingomonas glacialis
The polyamine pattern consisted of \([\text{mmol (g dry weight)}^{-1}]\) sym-homospermidine (18.5), spermidine (0.2), putrescine (0.2), cadaverine (0.1) and traces of spermine (0.1). This polyamine pattern is similar to those found in members of the genera *Sphingomonas* and *Sphingosinicella*, but differs from those of members of other genera of the family *Sphingomonadaceae* (Busse et al., 1999; Takeuchi et al., 2001; Geueke et al., 2007).

Strain CC-TBT-3T did not hydrolyse the \(\beta\)-peptide H-hVal-hAla-hLeu-OH in MB that was supplemented with 5 mM H-hVal-hAla-hLeu-OH and incubated at 28 °C for 12 days. A \(\beta\)-peptidyl aminopeptidase gene \((\betaapA)\) could not be amplified by PCR with different pairs of conserved primers. These results distinguish strain CC-TBT-3T from *Sphingosinicella* species that are capable of utilizing \(\beta\)-peptides as sole sources of carbon and nitrogen (Geueke et al., 2007).

Further characterization of strain CC-TBT-3T was performed using a substrate assimilation panel and enzyme tests with chromogenic substrates \([\text{para-nitrophenyl- (pNP) and para-nitroanilide (pNA)-linked substrates]}\) (Kämpfer et al., 1991). Only a few positive results could be recorded. CC-TBT-3T was able to utilize fumarate, DL-3 hydroxybutyrate and pyruvate. No growth was observed with the majority of other carbon sources tested. Details are given in the species description.

A combination of the observed chemotaxonomic and physiological differences (presence of a unique glycolipid, production of \(C_{18:1}\) 2-OH instead of other hydroxylated fatty acids and several physiological features) warrant the proposal of a novel species in a separate genus, *Sphingomicrobium lutaoense* gen. nov. sp. nov., to accommodate strain CC-TBT-3T.

**Description of *Sphingomicrobium* gen. nov.**

*Sphingomicrobium* [Sphin.go.mic.ro'bi.um. N.L. n. *sphingosinum* (from Gr. gen. n. *sphingos* of sphinx, and suff. -ine) sphingosine; N.L. pref. *sphingo* pertaining to sphingosine; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Sphingomicrobium* a sphingosine-containing microbe].

Cells are Gram-negative, non-motile, aerobic, non-sporulating, irregular rods. Growth is visible on MA and (slowly) on R2A agar, but not on NA or TSA. Optimum growth occurs between 20 and 36 °C. The fatty acid profile is characterized by a large amount of unsaturated \((C_{18:1}(\Delta 7c))\) fatty acids. In addition, the hydroxylated fatty acid \(C_{18:1}\) 2-OH is detected in high amounts. The
polyamine pattern is characterized by the predominant triamine sym-homospermidine. The predominant polar lipids are diphasphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid and three unidentified glycolipids (GL2, GL3 and GL4). The respiratory lipoquinone is ubiquinone Q-10. Not able to hydrolyse the β-peptide H-βhVal-βhAla-βhLeu-OH. As shown by 16S rRNA gene sequence analysis, the genus belongs to the family Sphingomonadaceae. The type species is Sphingomicrobium lutaoense.

**Description of Sphingomicrobium lutaoense sp. nov.**

*Sphingomicrobium lutaoense* (lu.ta.o.en’se. N.L. neut. adj. *lutaoense* of or belonging to Lutao, an island of Taiwan).

The species shares all characteristics of the genus. Cells are rod-shaped, 1.5 ± 0.5 μm long and 1.0 ± 0.1 μm wide and motile in the early exponential phase. Gram-negative and oxidase-positive. Growth is observed on MA, PYE agar and broth supplemented with 3 % sea salts, and moderately on R2A agar (Oxoid) at 28 °C, but no growth is observed on NA or Caso agar (both Oxoid). Growth on MA is observed at 20–40 °C. No growth is observed at 15 °C or below, or 45 °C or above. Growth is observed in R2A broth supplemented with 1–7 % (w/v) NaCl. The pH range of growth (also tested in R2A) was 6.5–10.5. No growth was observed in R2A broth at pH 5.5 or 11.5.

Able to utilize fumarate, DL-3 hydroxybutyrate and pyruvate as sole sources of carbon. No growth is observed with the following carbon sources: N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, l-arabinose, p-arbutin, cellobiose, D-fructose, D-galactose, D-glucose, trehalose, D-glucuronate, D-mannose, maltose, α-melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylene, D-adenitol, i-inositol, maltitol, D-mannitol, D-sorbitol, acetate, propionate, adipate, suberate, azelate, glutarate, oxoglutarate, L-aspartate, putrescine, cis-aconitate, trans-aconitate, citrate, DL-lactate, L-malate, itaconate, mesaconate, L-alanine, β-alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzate and phenylacetate. L-Alanine-pNA, bis-pNP-phosphate and proline-pNA are hydrolysed; aesculin, pNP-phenyl-phosphonate, ortho-nitrophenyl-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-xyloside, p-phosphoryl-choline and L-glutamate-γ-3-carboxy-pNA are not hydrolysed. In addition to the lipids listed in the genus description, minor amounts of another unidentified glycolipid (GL1) and three unidentified polar lipids are detectable. A β-peptidyl aminopeptidase gene (*bapA*) cannot be amplified by PCR with different pairs of conserved primers.

The type strain, CC-TBT-3 T (=DSM 24194 T =CCM 7794 T) was isolated on MA from water from a coastal hot spring of Green Island (Lutao), located off Taituang, Taiwan. The DNA G+C content of the type strain is 63.4 mol%.

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**References**


