Chryseobacterium taihuense sp. nov., isolated from a eutrophic lake, and emended descriptions of the genus Chryseobacterium, Chryseobacterium taiwanense, Chryseobacterium jejuense and Chryseobacterium indoltheticum

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Bacterial strain THMBM1T was isolated from decomposing algal scum that was collected during an algal bloom in Lake Taihu, Wuxi City, Jiangsu Province, China. Cells of strain THMBM1T were Gram-negative, facultatively anaerobic, non-motile rods. Colonies on tryptic soy agar were translucent and shiny with entire edges; yellow flexirubin-type pigments were produced. Growth was observed at 15–45 °C (optimum 30–37 °C), at pH 5.0–9.0 (optimum pH 8.0) and in the presence of 0–4.0 % (w/v) NaCl (optimum 0–1.0 %). Strain THMBM1T contained MK-6 as the sole respiratory quinone and sym-homospermidine as the predominant polyamine. The predominant cellular fatty acids were iso-C15 : 0 (53.2 %), iso-C17 : 0 3-OH (15.6 %) and iso-C17 : 1v9c (11.9 %). The polar lipid profile consisted of phosphatidylethanolamine and five unidentified lipids. The DNA G+C content was 36.8 mol% (Tm). Strain THMBM1T was closely related to members of the genus Chryseobacterium, with 16S rRNA gene sequence similarities ranging from 92.9 to 97.2 %, the highest sequence similarities being with Chryseobacterium taiwanense BCRC 17412T (97.2 %) and C. gambrini 5-ST1aT (97.1 %). DNA–DNA relatedness between strain THMBM1T and C. taiwanense JCM 21767T and C. gambrini DSM 18014T was 34.1 and 23.0 %, respectively. Based on these results, it is concluded that strain THMBM1T represents a novel species, for which the name Chryseobacterium taihuense sp. nov. is proposed. The type strain is THMBM1T (=CGMCC 1.10941T =NBRC 108747T). Emended descriptions of the genus Chryseobacterium and C. taiwanense, C. jejuense and C. indoltheticum are also proposed.

The genus Chryseobacterium (family Flavobacteriaceae, phylum Bacteroidetes) was first proposed by Vandamme et al. (1994). At the time of writing, 58 species with validly published names were included in the genus (http://www.bacterio.cict.fr/c/chryseobacterium.html). Members of the genus Chryseobacterium have been isolated from a variety of environments, including soil (Shen et al., 2005; Weon et al., 2008), wastewater (Kämpfer et al., 2003), fresh water (Kim et al., 2008), compost (Kämpfer et al., 2010), plant rhizosphere (Park et al., 2006), diseased fish (Bernardet et al., 2005; Ilardi et al., 2009), meat (De Beer et al., 2005), dairy environment (Hugo et al., 2003) and clinical samples (Bernardet et al., 2006, 2011; Vaneechoutte et al., 2007). Cells are Gram-negative, non-motile, non-spore-forming, aerobic rods. Colonies are translucent, circular with entire edges, smooth, shiny and typically pigmented from yellow to orange. MK-6 is the major or only respiratory quinone, branched-chain fatty acids (iso-C15 : 0, iso-C17 : 0 3-OH and iso-C17 : 1v9c) are the major fatty acids and sym-homospermidine is the major polyamine (Vandamme et al., 1994; Kämpfer et al., 2009a; Bernardet et al., 2006, 2011).

Algal blooms are a serious problem affecting aquatic ecosystems and environment sustainability (Paerl et al., 2003; Lehman, 2007). During post-blooming stage, large quantities of algal cells accumulate and decompose, resulting in serious environmental and ecological consequences.
Strain THMBM1T was isolated using the standard dilution-plating technique on R2A agar (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium830.pdf). Yellow-pigmented colonies formed after 3 days of incubation at 30 °C. The isolate was routinely maintained on R2A agar and preserved at −80 °C as a suspension in R2A broth supplemented with 17% (w/v) glycerol.

DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as previously described (Zhang et al., 2003). BLAST searches on NCBI (Altschul et al., 1990) showed that strain THMBM1T was phylogenetically related to members of the genus Chryseobacterium. Analysis of 16S rRNA gene sequences was performed using MEGA version 4.1 (Tamura et al., 2007). Multiple alignments with sequences of the genus Chryseobacterium were performed using CLUSTAL X (Thompson et al., 1997). Phylogenetic trees were drawn using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with Kimura's two-parameter calculation model (Tamura et al., 2007). Genetic distances between strain THMBM1T and strains of the genus Chryseobacterium species were calculated on the basis of pairwise alignment according to the p-distance algorithm. Analysis of 16S rRNA gene sequences confirmed that strain THMBM1T was phylogenetically related to members of the genus Chryseobacterium, the highest sequence similarities being with C. taiwanense BCRC 17412T (97.2%) (Tai et al., 2006), C. gambrini 5-1St1aT (97.1%) (Herzog et al., 2008), C. defluvii B2T (97.0%) (Kämpfer et al., 2003), C. ginsenosidimutans THG 15T (96.9%) (Im et al., 2011), C. jejuense JS17-8T (96.8%) (Weon et al., 2008) and C. indoltheticum LMG 4025T (96.7%) (Vandamme et al., 1994). The neighbour-joining and maximum-parsimony phylogenetic trees (Fig. 1) also indicated that strain THMBM1T clustered with the genus Chryseobacterium.

The morphological and physiological characteristics of strain THMBM1T were investigated with cells cultivated at...
30 °C on enriched R2A (E-R2A; five-strength enriched R2A). Growth was also tested in tryptic soy broth (TSB; Bacto), 1/2 strength TSB, R2A broth and Luria–Bertani broth (LB; laboratory-prepared). Gram-staining was performed using both a staining method (Smibert & Krieg, 1994) and a non-staining method (Buck, 1982) with cells grown on E-R2A agar at 30 °C for 2 days. Morphological observations were performed by transmission (JEM 1400; Jeol) and scanning (Quanta 200; FEI) electron microscopy. Motility was observed by the hanging-drop technique (Bernardet et al., 2002). Production of flexirubin-type pigments by strain THMBM1T was investigated by observation of a colour shift following exposure to 20% (w/v) KOH (Bernardet et al., 2002). The optimum and range of growth temperature were determined in E-R2A broth incubated for 3–7 days at 4, 8, 15, 20, 25, 30, 37, 40 and 45 °C. The pH range for growth was tested at pH 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11 by adjusting the pH of E-R2A broth with 5 M NaOH or HCl. Growth with NaCl was tested at 0, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11 by adjusting the pH of E-R2A broth supplemented with 0.05% L-cysteine monohydrate and 0.05% 1-cysteine monohydrochloride in Hungate tubes prepared under anaerobic conditions. Oxidase activity was assessed colorimetrically using N, N, N', N'-tetramethyl-p-phenylenediamine and catalase activity was determined by bubble production from 3% (v/v) H2O2. Hydrolysis of casein, aesculin, gelatin, starch, urea and Tween 80 was tested as described by Lanyi (1987) and Smibert & Krieg (1994). Voges–Proskauer reaction and hydrogen sulfide production were assessed according to Barrow & Feltham (1993). Additional biochemical and enzyme activities were tested using API 20 NE, API 50 CH (inoculated with cell suspensions in CHB medium) and API ZYM kits (bioMérieux) according to the manufacturer’s instructions. Strain THMBM1T and C. taiwanense JCM 21767T, C. gambrini DSM 18014T, C. jejuense NBRC 106406T and C. indoltheticum DSM 16778T were all tested under the same laboratory conditions for the above-mentioned biochemical tests as well as for chemotaxonomic studies.

Cells of strain THMBM1T were Gram-negative, non-motile rods approximately 1–2 μm in length and 0.2–0.3 μm in width (Fig. S1, available in IJSEM Online). Weak growth was observed under anaerobic conditions in E-R2A broth. Facultatively anaerobic growth has been reported for Chryseobacterium bovis and Chryseobacterium arotchii (Hantsis-Zacharov et al., 2008; Campbell et al., 2008). However, when C. arotchii was combined with Chryseobacterium hominis, anaerobic growth was not reported (Kämpfer et al., 2009b). Additional physiological and biochemical characteristics of strain THMBM1T are provided in the species description and properties differentiating the isolate from type strains of closely related species are detailed in Table 1.

For chemotaxonomic analyses, biomass of strain THMBM1T, C. taiwanense JCM 21767T, C. gambrini DSM 18014T, C. jejuense NBRC 106406T and C. indoltheticum

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<td>36.8</td>
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*Data in columns 2–5 were taken from Herzog et al. (2008), Weon et al. (2008) and Hugo et al. (2003).
†Different results were reported by Tai et al. (2006), Herzog et al. (2008) or Weon et al. (2008).
DSM 16778<sup>T</sup> was harvested from tryptic soy agar incubated at 30 °C for 2 days (stationary phase). Cellular fatty acids were extracted and methylated according to the standard protocol of Sherlock Microbial Identification System version 6.0 (MIDI), analysed by GC (model 6890; Agilent) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990). Isoprenoid quinones of strain THMBM1<sup>T</sup> were extracted with chloroform/methanol (2:1, v/v) and purified by TLC on Gel 60 F254 plates (10 × 20 cm, 0.25 mm thickness) using n-hexane/diethyl ether (85:15, v/v) as the solvent. The quinones were analysed by HPLC with an eclipse XDB-C18 column (4.6 × 150 mm; Agilent) and eluted with acetonitrile/isopropyl alcohol (2:1, v/v) at a speed of 1 ml min<sup>−1</sup>, (Collins, 1985; Wu et al., 1989; Zhang et al., 2011). Polar lipids were extracted, separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Komagata & Suzuki, 1987; Minnikin et al., 1984). Polyamines were extracted as described by Busse & Auling (1988) and analysed using HPLC equipment described by Busse et al. (1997).

The predominant cellular fatty acids of strain THMBM1<sup>T</sup> were iso-C<sub>15 : 0</sub> (53.2 %), iso-C<sub>17 : 0</sub> 3-OH (15.6 %) and iso-C<sub>17 : 1</sub> c (11.9 %). The complete fatty acid composition (Table 2) was similar to those of closely related *Chryseobacterium* species, although the proportion of summed feature 3 (comprising C<sub>16 : 1</sub>ω7c and/or C<sub>16 : 1</sub>ω6c) in strain THMBM1<sup>T</sup> was significantly lower. The only isoprenoid quinone detected in strain THMBM1<sup>T</sup> was menaquinone with six isoprene units (MK-6); this quinone is the major or only quinone found for all members of the family *Flavobacteriaceae*. The polar lipid profiles of strain THMBM1<sup>T</sup> and the four reference strains are shown in Fig. 2. The polar lipid profile of strain THMBM1<sup>T</sup> consisted mainly of phosphatidylethanolamine, with five unidentified polar lipids (two aminolipids, two glycolipids and one aminophospholipid). Strain THMBM1<sup>T</sup> and the four reference strains all contained phosphatidylethanolamine as the major polar lipid; these strains also contained other minor aminolipids or glycolipids that differentiated them from each other. Hence, our data confirms previous studies of other *Chryseobacterium* species (Kämpfer et al., 2003, 2009a; Kim et al., 2005, 2008; Herzog et al., 2008; Szoboszlay et al., 2008) demonstrating that all members of the genus *Chryseobacterium* contain phosphatidylethanolamine as the major polar lipid. The major polyamine of strain THMBM1<sup>T</sup> was sym-homospermidine, which is in line with previous results for the genus *Chryseobacterium* (Bernardet et al., 2006, 2011; Kämpfer et al., 2009a).

The DNA base composition was determined by the thermal denaturation method (Marmur & Doty, 1962). *Escherichia coli* K-12<sup>T</sup> was used as the reference. The DNA G + C content of strain THMBM1<sup>T</sup> was 36.8 mol%, which is within the range for the genus *Chryseobacterium* (Bernardet et al., 2011). DNA–DNA hybridization was performed by the thermal denaturation and renaturation method according to De Ley et al. (1970), with the modifications of Huss et al. (1983). Strain THMBM1<sup>T</sup> showed 34.1 and 23.0 % DNA–DNA relatedness with *C. taiwanense* JCM 21767<sup>T</sup> and *C. gambrini* DSM 18014<sup>T</sup>, respectively. These values are far below the threshold value of 70 % recommended by Wayne et al. (1987), indicating that strain THMBM1<sup>T</sup> represents a distinct genomic species.

On the basis of phylogenetic inference and genomic and phenotypic evidence, we conclude that strain THMBM1<sup>T</sup> represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium taihuense* sp. nov. is proposed. On the basis of new data obtained in this study, emended descriptions of the genus *Chryseobacterium* and *C. taiwanense*, *C. jejuense* and *C. indoltheticum* are also proposed.


The description is as given by Vandamme et al. (1994) and emended by Kämpfer et al. (2009a) with the following amendment. Phosphatidylethanolamine is the major polar lipid.
Emended description of *Chryseobacterium taiwanense* Tai et al. 2006

The description is as given by Tai *et al.* (2006), with the following amendment. In addition to phosphatidylethanolamine, the polar lipid profile contains six unidentified polar lipids (four aminolipids, one glycolipid and one aminophospholipid).

Emended description of *Chryseobacterium jejuense* Weon *et al.* 2008

The description is as given by Weon *et al.* (2008), with the following amendment. In addition to phosphatidylethanolamine, the polar lipid profile contains seven unidentified polar lipids (four aminolipids and three glycolipids).

Emended description of *Chryseobacterium indoltheticum* (Campbell and Williams 1951) Vandamme *et al.* 1994

The description is as given by Campbell & Williams (1951) and discussed by Bernardet *et al.* (2011) with the following amendment. In addition to phosphatidylethanolamine, the polar lipid profile contains six unidentified polar lipids (four aminolipids, one glycolipid and one aminophospholipid).

Description of *Chryseobacterium taihuense* sp. nov.

*Chryseobacterium taihuense* (tai.hu.en’se. N.L. neut. adj. *taihuense* pertaining to Lake Taihu in Jiangsu Province, China, where the type strain was isolated).

Cells are Gram-negative, facultatively anaerobic, non-endospore-forming, non-motile rods approximately 1–2 μm in length and 0.2–0.3 μm in width. Colonies on TSA are orange-yellow, translucent, shiny, circular with entire edges and 1.5–2.5 mm in diameter after 2 days at 30 °C. Grows well in TSB, 1/2 strength TSB, R2A broth and E-R2A broth; no growth occurs in LB broth. Flexirubin-type pigments are produced. Growth occurs at 15–45 °C (optimum 30–37 °C), at pH 5.0–9.0 (optimum pH 8.0) and in the presence of 0–4 % NaCl (optimum 0–1 %). Oxidase- and catalase-positive. Starch, casein and Tween 80 are hydrolysed. Hydrogen sulfide and acetoin are not produced. In the API 20 NE strip, nitrate is not reduced to nitrite, indole is produced, glucose is fermented, urease and arginine dihydrolase activities are present, aesculin and gelatin are hydrolysed, β-galactosidase activity is absent and glucose, mannose, mannitol, maltose, gluconate, caprate and malate are assimilated, but N-acetylglycosamine, L-arabinose, trisodium citrate, adipate and phenylacetate are not assimilated. In the API 50 CH strip, acid is produced from D-glucose, D-mannose, aesculin ferric citrate, cellobiose, maltose, trehalose, starch, glycerol and gentiobiose, but not from the other substrates. In the API ZYM strip, alkaline phosphatase, esterase lipase (C 8), lipase (C14), leucine arylamidase, α-chymotrypsin, cystine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl-β-glucosaminidase activities are present, but esterase (C4), α-mannosidase, β-fucosidase, β-glucuronidase, β-galactosidase and β-galactosidase activities are absent. The predominant cellular fatty acids (>11 %) are iso-C15:0, iso-C17:0 3-OH and iso-C17:1ω9c. MK-6 is the only respiratory quinone. In addition to phosphatidylethanolamine, the polar lipid profile contains one unidentified aminophospholipid, two unidentified aminolipids and two unidentified glycolipids. The major polyamine is *sym*-homospermidine.

The type strain is THMBM1T (=CGMCC 1.10941T =NBRC 108747T), isolated from Lake Taihu, PR China. The DNA G+C content of the type strain is 36.8 mol% (Tm).
Acknowledgements

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References


