Meiothermus terrae sp. nov., isolated from a geothermally heated soil sample

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A Gram-negative, aerobic bacterium, designated strain YIM 77755T, was isolated from a geothermally heated soil sample collected at Rehai National Park, Tengchong, Yunnan province, south-west China. Cells of the strain were rod-shaped and colonies were yellow and circular. Growth occurred in 0–1 % (w/v) NaCl, at pH 6.0–8.0 (optimum, pH 7.0) and at 35–55 °C (optimum, 50 °C). The predominant menaquinone was MK-8 and the DNA G+C content was 68.9 mol%. Major fatty acids (>10 %) were anteiso-C15 : 0 and iso-C15 : 0. The polar lipids consisted of an uncharacterized phospholipid and four glycolipids. Based on 16S rRNA gene sequence analysis, strain YIM 77755T formed a cluster with Meiothermus chliarophilus ALT-8T and showed the highest 16S rRNA gene sequence similarity to M. chliarophilus ALT-8T (98.23 %). DNA–DNA relatedness between YIM 77755T and M. chliarophilus DSM 9957T was 54.9 ± 4.1 %. On the basis of the morphological and chemotaxonomic characteristics as well as genotypic data, it is proposed that strain YIM 77755T represents a novel species of the genus Meiothermus, named Meiothermus terrae sp. nov. The type strain is YIM 77755T (=DSM 26712T=CCTCC AB 2012942T).

The species of the genus Meiothermus were formerly included in the genus Thermus, but the presence of moderate levels of 2-OH fatty acids showed clearly that these organisms belonged to a distinct genus; three species of the genus Thermus were reclassified within a separate genus Meiothermus by Nobre et al. (1996). At the time of writing, the genus Meiothermus comprised 10 species with validly published names: Meiothermus cateniformans, M. tawanensis, M. ruber, M. cerbereus, M. rufus, M. granaticius, M. hypogaeus, M. timidus, M. chliarophilus and M. silvanus (http://www.bacterio.net/m/meiothermus.html). The typical chemotaxonomic characters of the genus Meiothermus include MK-8 as the predominant menaquinone, iso- and anteiso-branched fatty acids as major fatty acids, high genomic DNA G+C content (60.8–69.9 mol%) and an uncharacterized phospholipid and glycolipids as major polar lipids (Tenreiro et al., 1995; Chung et al., 1997; Nobre & da Costa, 2001; Chen et al., 2002; Pires et al., 2005; Albuquerque et al., 2009, 2010; Zhang et al., 2010; Mori et al., 2012). During the course of an investigation of the culturable bacterial community at Rehai National Park in Yunnan Province, south-west China, a potential novel member of the genus Meiothermus was isolated. In this study, we report the accurate taxonomic characterization of strain YIM 77755T.

Strain YIM 77755T was isolated from a geothermally heated soil sample collected from Rehai National Park (24.56993°N 98.26291°E) by using a serial dilution technique. After 1 week of incubation on R2A agar (BD) at 50 °C, colonies were picked and repeatedly restreaked onto R2A agar at 50 °C until purity was confirmed. Strain YIM 77755T was...
routinely cultivated and stored as aqueous glycerol suspensions (20%, v/v) at −80 °C.

Cell morphology was determined with cultures grown for 6, 12, 24 and 48 h on R2A and Thermus agar (Williams & da Costa, 1992) at 50 °C. Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test method (Cerny, 1978). Morphological characteristics of the strain were observed by using light microscopy (model BH2; Olympus). Growth was tested at 20–75 °C in 5 °C steps on R2A agar plates. For NaCl tolerance experiments, R2A agar was used as the basal medium with NaCl being added at 0, 0.5, 1, 2, 3 and 4% (w/v). The pH growth range was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit by using the NE systems (bioMerieux) according to the manufacturer’s instructions.

Strain YIM 77755T grew well on R2A and Thermus agar. Colonies were yellow, smooth, entire and circular on all tested agar media for 2 days. Cells were Gram-negative, aerobic rods, 0.4–0.6 μm wide and 2.0–4.0 μm long (Fig. S1, available in IJSEM Online). Detailed morphological, physiological and biochemical characteristics of YIM 77755T are given in the species description. Phenotypic data for strain YIM 77755T and M. chliarophilus DSM 9957T are compared in Table 1. Strain YIM 77755T could be differentiated from M. chliarophilus DSM 9957T and other species of the genus Meiothermus on the basis of several properties, including ranges of temperature and NaCl concentration for growth, hydrolysis of starch, casein, gelatin and aesculin, activities of acid phosphatase and α-galactosidase and the ability to utilize maltose, xylose, glycerol and galactose as sole carbon sources (Table 1).

Biomass used for chemical studies (except for fatty acid analysis) was obtained from cultures grown on R2A agar plates for 3 days at 50 °C. Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1979; Collins & Jones, 1980). Quinones were isolated as described by Collins et al. (1980). Quinones were isolated as described by Collins et al. (1980). Quinones were isolated as described by Collins et al. (1980). Quinones were isolated as described by Collins et al. (1980). Quinones were isolated as described by Collins et al. (1980).

Table 1. Differential phenotypic characteristics of strain YIM 77755T and the type strains of species of the genus Meiothermus

<table>
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<tr>
<th>Characteristic</th>
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<td>Starch</td>
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<td>PL, GL</td>
<td>PL, 2GL</td>
<td>PL, 2GL</td>
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<td>PL, 2GL</td>
<td>PL, 2GL</td>
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*GL, Uncharacterized glycolipid; PL, uncharacterized phospholipid.
(1977) and separated by HPLC (Kroppenstedt, 1982). The genomic DNA G+C content was determined by using the HPLC method of Mesbah et al. (1989). Cellular fatty acid analysis was performed using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. The fatty acid methyl esters were identified by using the Microbial Identification Software package (Sherlock instructions. The fatty acid methyl esters were identified by using the MIDI database, TSBA6). Cultures of strain YIM 77755T and M. chliarophilus DSM 9957T for fatty acid analysis were collected under the same conditions (cultures grown on Thermus agar plates for 48 h at 50 °C).

The predominant menaquinone of strain YIM 77755T was determined to be MK-8. The polar lipids of strain YIM 77755T consisted of one uncharacterized phospholipid and four glycolipids (Fig S2), as observed for M. chliarophilus DSM 9957T, but in contrast to other species of the genus, which contain only one or two glycolipids (Table 1). Predominant fatty acids of strain YIM 77755T (>5%) were anteiso-C15:0 (26.31%), iso-C15:0 (14.04%), iso-C14:0 (8.41%), C16:0 (8.07%), iso-C16:0 (6.88%), iso-C17:0 (5.80%) and C17:0 (5.37%). This profile was also consistent with those described for other members of the genus Meiothermus, but the proportions of major fatty acids of YIM 77755T were different from those determined in this study for M. chliarophilus DSM 9957T (Table S1). The DNA G+C content of strain YIM 77755T was determined to be 68.9 mol%, which is in accordance with results reported previously for other members of the genus Meiothermus (60.8–69.9 mol%).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). The resulting 16S RNA gene sequence was compared with available 16S rRNA gene sequences of cultured species from GenBank via the BLAST program and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). Phylogenetic analyses were performed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods, by using the software package MEGA version 5.0 (Tamura et al., 2011). Kimura’s two-parameter model was used to calculate evolutionary distance matrices of the neighbour-joining and maximum-likelihood methods (Kimura, 1980). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 resamplings. DNA–DNA relatedness was studied by using the optical renaturation method (De Ley et al., 1970; Huss et al., 1983; Jahnke, 1992) on a UV–Vis spectrophotometer (model UV1601; Shimadzu) under optimal hybridization conditions.

The almost-complete 16S rRNA gene sequence (1480 bp) of strain YIM 77755T was obtained. By using EzTaxon-e, the novel isolate was shown to be closely related to M. chliarophilus ALT-8T, with a sequence similarity of 98.23%. Sequence similarities to the type strains of all other species studied were less than 94.0%. The tree reconstructed by the neighbour-joining method showed that the novel isolate formed a cluster with M. chliarophilus ALT-8T with 100% bootstrap support (Fig. 1). Similar results were obtained using the maximum-parsimony and maximum-likelihood algorithms (not shown). DNA–DNA relatedness between YIM 77755T and M. chliarophilus DSM 9957T was 54.9 ± 4.1%, which was significantly lower than the threshold value (70%) generally accepted for the recognition of genomic species (Wayne et al., 1987); thus, we suggest that strain YIM 77755T should be considered as a representative of a distinct genomic species of the genus Meiothermus.

**Fig. 1.** Neighbour-joining tree based on almost-complete 16S rRNA gene sequences (1427 bp) of strain YIM 77755T and members of the genus Meiothermus, showing the phylogenetic relationships between strain YIM 77755T and related taxa. Bootstrap values are given as percentages of 1000 replicates at branch points; only values >50% are shown. Asterisks denote nodes that were also recovered in the maximum-parsimony and maximum-likelihood trees. Bar, 0.02 substitutions per nucleotide position.
Phylogenetic analysis performed based on 16S rRNA gene sequences indicated that strain YIM 77755T belongs to the genus Meiothermus. The 16S rRNA gene sequence of strain YIM 77755T showed the highest similarity (98.23 %) to that of M. chliarophilus ALT-8T, but the DNA–DNA relatedness (54.9 ± 4.1 %) indicated that strain YIM 77755T represents a different genomic species of the genus Meiothermus. Strain YIM 77755T exhibited several phenotypic and chemotaxonomic characters of the genus Meiothermus; it was Gram-negative, aerobic and moderately thermophilic, possessed MK-8 as the predominant menaquinone and had a high DNA G+C content (68.9 mol%).

**Description of Meiothermus terrae sp. nov.**

Meiothermus terrae (ter’rae. L. gen. n. terrae of the soil).

Cells are Gram-negative, aerobic rods, 0.4–0.6 μm wide and 2.0–4.0 μm long. Colonies grown on R2A and Thermus agar plates for 2 days are yellow and circular. Grows at 35–55 °C (optimal 50 °C); no growth at 30 or 60 °C. Grows at pH 6.0–8.0 (optimal pH 7.0). Growth occurs in 0–1 % (w/v) NaCl. Oxidase-positive and catalase-negative. Degradates starch and casein, but not Tween 20, 40, 60 or 80, chitin, tyrosine or CM-cellulose. Positive for reduction of nitrate, starch and casein, but not Tweens 20, 40, 60 or 80, chitin, tyrosine or CM-cellulose. Positive for production of indole, arginine dihydrolase, urease and acid production from glucose (API 20 NE test strips). Positive in tests for alkaline phosphatase, esterase C4, leucine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase, but not for esterase lipase C8, β-glucuronidase, N-acetyl-β-glucosaminidase, α-chymotrypsin, lipase C14, valine arylamidase, cystine arylamidase, trypsin, α-mannosidase or α-fucosidase (API ZYM tests). Glucose, raffinose, trehalose, lactose, sucrose, mannose, inositol, cellobiose, mannitol, xylose and glycerol can be utilized as sole carbon sources, but maltose, fructose, galactose and L-arabinose are not utilized. The predominant menaquinone is MK-8. Major fatty acids (>10 %) are anteiso-C15 : 0 and iso-C15 : 0. The polar lipids consist of an uncharacterized phospholipid and four glycolipids.

The type strain, YIM 77755T (=DSM 26712T=CCTCC AB 2012942T), was isolated from a geothermally heated soil sample collected at Rehai National Park, Tengchong, Yunnan Province, south-west China. The DNA G+C content of the type strain is 68.9 mol%.

**Acknowledgements**

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