Meiothermus taiwanensis sp. nov., a novel filamentous, thermophilic species isolated in Taiwan

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Two novel filamentous bacterial isolates, strains WR-30^T and WR-220, with an optimum growth temperature of approximately 55–60 °C were isolated from Wu-rai hot springs in the northern part of Taiwan. These isolates were aerobic, thermophilic, non-sporulating, red-pigmented and heterotrophic and formed extremely long, filamentous trichomes from cells of different lengths. Phylogenetic analysis of 16S rDNA, DNA–DNA hybridization, morphological and biochemical features and fatty acid composition revealed that the isolates represent a novel species of the genus *Meiothermus*. The name *Meiothermus taiwanensis* sp. nov. is proposed for this novel species. The type strain of *M. taiwanensis* is strain WR-30^T (= ATCC BAA-399^T = CCRC 17170^T = DSM 14542^T); strain WR-220 (= ATCC BAA-400 = CCRC 17171 = DSM 14543) is a reference strain.

Keywords: filamentous bacterium, *Meiothermus*, hot springs, Taiwan

INTRODUCTION

Organisms associated with hot springs in geothermal areas have received considerable interest in recent years. Of these thermophilic micro-organisms, members of the genus *Thermus*, including its type species *Thermus aquaticus* (Brock & Freeze, 1969), are aerobic, non-sporulating, heterotrophic rods. However, species of the genus *Meiothermus*, formerly included in the genus *Thermus*, can be distinguished from those of the genus *Thermus* by their lower growth temperature range, the presence of moderate levels of 2-OH fatty acids and 14% 16S rDNA sequence divergence (Nobre *et al*., 1996). *Meiothermus* species form red- or yellow-pigmented colonies and have an optimum growth temperature in the range 50–65 °C. They are widely dispersed in hot, natural or aqueous, artificial environments such as domestic and industrial hot-water systems and thermally polluted streams. Four species of bacteria belonging to the genus *Meiothermus* have been described recently, *Meiothermus ruber*, *Meiothermus chliarophilus*, *Meiothermus silvanus* and *Meiothermus cerbereus* (Loginova *et al*., 1984; Tenreiro *et al*., 1995; Nobre *et al*., 1996; Chung *et al*., 1997).

This study presents two strains of a novel species of the genus *Meiothermus*, isolated from Wu-rai hot springs in the northern part of Taiwan. The novel species can be distinguished clearly from other species on the basis of its filamentous morphology and fatty acid composition. However, DNA–DNA hybridization values and phylogenetic analyses of the 16S rDNA sequences showed that these isolates have not been described previously. On the basis of the results presented in this study, the name *Meiothermus taiwanensis* sp. nov. is proposed for this novel species.

METHODS

Isolation and bacterial strains. Samples of hot spring water, solfataric soil and mud were collected from hot springs located in the Wu-rai area (121° 32′ 4″ E, 24° 51′ 52″ N), Taipei, Taiwan. Water samples were transported without temperature control and analysed within 24 h. Untreated, 100 µl samples of water were spread directly onto *Thermus* agar plates (Williams & da Costa, 1992), which were then sealed in plastic bags and incubated at 50 °C for 7 days. Colonies were purified by serial transfers. All isolates were preserved in *Thermus* medium containing 15% glycerol at −70 °C. Two isolates, designated WR-30^T and WR-220, were chosen for in-depth characterization and five strains of

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The GenBank accession numbers for the 16S rDNA sequences of strains WR-30^T and WR-220 are AF418001 and AF418002.

the genus *Meiothermus* were used for comparison. The type strains of *M. ruber* (DSM 1279^T), *M. cerbereus* (DSM 11376^T), *M. chliarophilus* (DSM 9957^T) and *M. silvanus* (DSM 9946^T) were obtained from the DSMZ. *M. ruber* NCIMB 11269 was obtained from the National Collection of Industrial, Food and Marine Bacteria, Aberdeen, UK.

Morphological and ultrastructural characteristics. Both light microscopy and TEM were used to observe the morphology of cells. For light microscopy, a Zeiss Axioscop microscope equipped with a Nikon Coolpix 990 digital camera was used routinely to obtain photomicrographs.

For TEM, bacterial strains were grown for 48 h and washed by centrifugation. For negative staining, 5 µl liquid culture was dropped on Formvar/carbon-coated grids (300 mesh) and stained with 1% phosphotungstic acid (pH 7·0). For ultrathin sections, cells were fixed in 2·5% (w/v) glutaraldehyde for 4 h, followed by 1% (w/v) osmium tetroxide in Veronal acetate buffer for 4 h and 2% uranyl acetate buffer for 1 h (Silva, 1973). Samples were dehydrated by a graded acetone series and specimens were embedded in Spurr resin (Spurr, 1969). Sections were cut with an ultramicrotome using a glass knife and stained for 5 min in post-staining solution (Kostman & Franceschi, 2000). Electron micrographs were generated using a Hitachi model H7100 electron microscope.

Phenotypic characteristics. All biochemical and tolerance tests were performed as specified previously (Santos *et al.*, 1989; Manaia & da Costa, 1991) in *Thermus* medium or on *Thermus* agar and incubated at 50 °C for 3 days. The pH range for growth was determined by measuring the turbidity (660 nm) of liquid cultures grown at 55 °C. Media with different pH values were prepared using the appropriate biological buffers (Chung *et al.*, 1997).

Filter-sterilized carbon sources (2·0 g l^{-1}), ammonium sulfate (0·5 g l^{-1}) and yeast extract (0·2 g l^{-1}) were added to *Thermus* basal salts for single-carbon-source assimilation tests. Growth was determined by measuring the turbidity (660 nm) of liquid cultures in 125 ml flasks for 4 days. Positive and negative control cultures were respectively grown in *Thermus* medium and minimal medium without a carbon source. All growth experiments were conducted in triplicate.

Lipoquinones and polar lipid composition. The cultures used for analysis were grown on *Thermus* agar at 55 °C for 48 h. Cells were collected from Petri dishes and lipids were extracted as described previously (Tindall, 1991). Polar lipids were analysed by one-dimensional TLC on silica gel 60 F254 plates (0·25 mm thickness; Merck) with a solvent system of chloroform/methanol/acetic acid/water (80:12:15:4 by vol.). TLC plates with separated polar lipid components were observed after staining with molybdophosphoric acid [10% (w/v) in ethanol] and heating at 121 °C for 30 min.

Lipoquinones were extracted from freeze-dried cells, purified by TLC (Tindall, 1989) and separated using a Hitachi model-L6200 HPLC, equipped with a C-18 reverse-phase column (250 × 4·6 mm; particle size 5 µm; Supelco), with methanol/heptane (10:2, v/v) as the mobile phase and detection at 269 nm.

Fatty acid composition. Cultures for fatty acid analysis were grown on *Thermus* medium plates in sealed plastic bags submerged in a water bath at 50 °C for 48 h. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation and extraction as described previously (Kuykendall *et al.*, 1988). The fatty acid methyl esters were separated using a Hewlett Packard model 5890 GC equipped with a flame-ionization detector fitted with a 5% phenylmethyl silicone capillary column (0·2 mm × 25 m; Hewlett Packard). The carrier gas was high-purity H_2, the column head pressure was 60 kPa, the septum purge was 5 ml min^{-1}, the column split ratio was 55:1 and the injection port temperature was 300 °C. The temperature program of the oven was 170 to 270 °C at a rate of 5 °C min^{-1}. Identification and quantification of the fatty acid methyl esters, as well as numerical analysis of the fatty acid profiles, were performed using the standard MIS Library Generation software (Microbial ID).

Determination of mean DNA base composition and DNA–DNA hybridization. DNA was isolated using a Qiagen DNAeasy tissue kit (Qiagen). The DNA G+C content was determined by HPLC, as described by Mesbah *et al.* (1989), and phage λ DNA was used as a control. DNA–DNA hybridization was elucidated by dot-blot hybridization and radioisotope detection. Probes were prepared using the random-prime labelling system (rediprime II; Amersham Pharmacia) with [α-^{32}P]dCTP. All hybridization procedures, including the optimal hybridization temperature and the buffer, followed the specification of Johnson (1984) and Kristjansson *et al.* (1994). The signals were generated by autoradiography with X-ray film (Kodak X-OMAT) and exposed for the time required to avoid film saturation. The signal produced by self-hybridization of the probe with homologous target DNA was taken as 100% and the hybridization percentages were calculated for the duplicated dots. Densitometric analyses of signals were processed using the program GEL-PRO version 3.0.

16S rDNA-based phylogenetic analysis. Extraction of genomic DNA, PCR-mediated amplification of the 16S rDNA and sequencing of the purified PCR products were performed according to Rainey *et al.* (1996). PCR-amplified products were purified and sequences were analysed by electrophoresis using a model 373A automatic sequencer (Applied Biosystems). The 16S rDNA sequences were compared with previously determined *Meiothermus* and *Thermus* sequences available from the EMBL database using BIOEDIT version 5.0.6 software (Maidak *et al.*, 1994). Evolutionary distances were calculated according to Jukes & Cantor (1969). The phylogenetic dendrogram was generated from evolutionary distances by the neighbour-joining method (Saitou & Nei, 1987) using BIOEDIT software.

RESULTS

Isolation of strains

Five samples, including hot spring water, solfataric soil and mud, were taken from Wu-rai hot springs, Taipei, Taiwan. The temperature range *in situ* was 50–80 °C and the pH values of the samples, determined at the ambient temperature, were between 6·2 and 7·9. Following incubation at 50 °C for 72 h, red- and yellow-pigmented colonies were observed on the surface of a *Thermus* agar plate. After serial transfer and purification, the yellow-pigmented colonies that grew at higher temperatures (over 70 °C) were considered to be members of the genus *Thermus* and red-pigmented

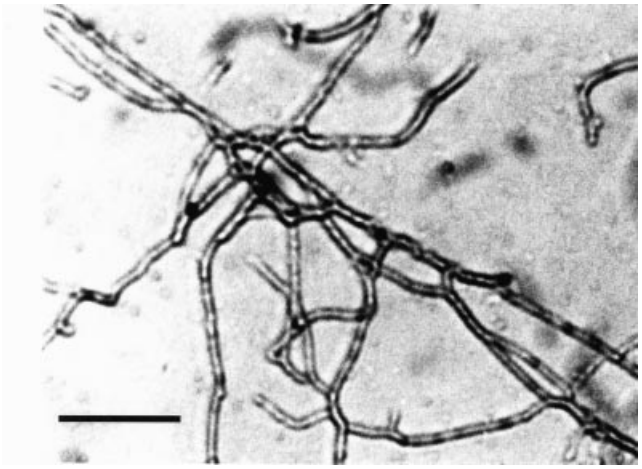

Fig. 1. Phase-contrast micrograph showing the filamentous morphology of strain WR-30^T. Bar, 10 μm.

colonies grown at lower temperature (about 50 °C) were considered to be members of the genus *Meiothermus*. Two isolates, WR-30^T and WR-220, which had different morphological properties, were selected for this study.

Morphological and ultrastructural characteristics

Cells of the isolates stained Gram-negative and formed long filaments; motility and spores were not observed. Septa were not visible by phase-contrast microscopy (Fig. 1). Colonies on *Thermus* medium were red- and red/orange-pigmented and formed a thin film on the surface of agar plates. TEM with negative staining showed that the morphology of strain WR-30^T was filamentous (Fig. 2a). Thin sections showed that the cell wall had a complex multilayered appearance. A thin electron-dense layer surrounded by a thick corrugated layer was adjacent to the cell membrane. The corrugated layer outside the cell wall ran continuously along the filaments and no septum formation was evident (Fig. 2b). Except for that of the outermost layer, the cell wall structure closely resembled that of the genus *Meiothermus* (Tenreiro *et al*., 1995).

Phenotypic and biochemical characteristics

The temperature range for growth of strain WR-30^T was 40–70 °C and the optimum growth temperature was 55 °C on *Thermus* medium. The optimum pH was approximately 8·0 at the optimum growth temperature.

Several biochemical characteristics, such as the presence of cytochrome oxidase and catalase, the utilization of a single carbon source and the hydrolysis of carbohydrate polymers, were identical in strains WR30^T and WR-220 and other type strains of the genus *Meiothermus*. The main differences in biochemical characteristics were observed during assimilation of single carbon sources. Three carbon sources, L-arabinose, ribitol and D-trehalose, could be utilized by strains WR-30^T and WR-220; these carbon sources cannot be utilized by other strains of the genus *Meiothermus*. The two isolated strains could also be distinguished from each other by the utilization of D-mannitol, D-raffinose and succinate. Table 1 presents the biochemical characteristics of strains WR-30^T and WR-220 and other *Meiothermus* species.

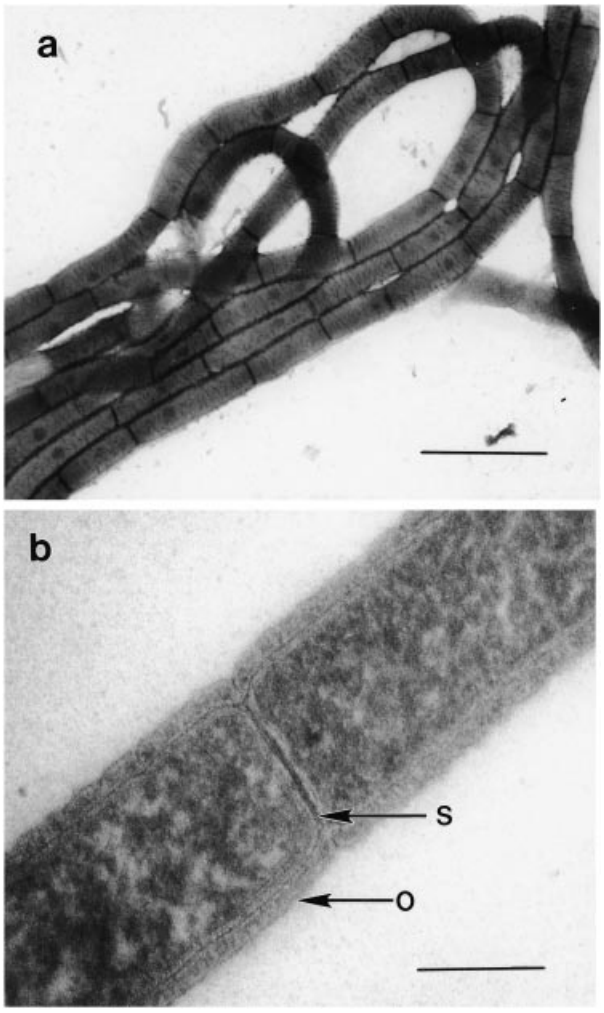

Fig. 2. TEM of strain WR-30^T. (a) Negatively stained multiple filaments of strain WR-30^T (bar, 2 μm); (b) thin-section electron micrograph of strain WR-30^T showing the septum (S) and the outer cell wall layer (O) running continuously along the filament (bar, 0·2 μm).

Lipoquinones and polar lipid composition

The polar lipids of the tested strains included one major phospholipid and two prominent glycolipids, designated GL-1a and GL-1b. GL-1a and GL-1b migrated close to each other near the origin of the TLC assay. Strains WR-30^T and WR-220 generally con-

Table 1. Biochemical features that distinguish species of the genus *Meiothermus*

Strains/species: 1, WR-30^T; 2, WR-220; 3, *M. ruber*; 4, *M. cerbereus*; 5, *M. chliarophilus*; 6, *M. silvanus*. All strains were grown at 55 °C except *M. chliarophilus*, which was grown at 50 °C. +, Positive result or growth; −, negative result or no growth; w, weak result. All strains hydrolysed elastin, fibrin, gelatin, casein and DNA and were positive for *β*-galactosidase and oxidase. D-Fructose, D-galactose, D-glucose, D-mannose, D-melibiose, lactose, L-glutamate, maltose and sucrose were utilized by all of the strains. None of the strains hydrolysed xylan or utilized citrate.

Characteristic	1	2	3	4	5	6
Pigmentation	Red	Red	Red	Red	Yellow	Red
Presence of:						
Catalase	+	+	+	−	−	−
α-Galactosidase	+	+	+	+	+	−
Hydrolysis of:						
Starch	−	−	−	−	+	−
Aesculin	+	+	+	+	+	−
Utilization of:						
D-Cellobiose	+	+	+	+	+	−
D-Mannitol	+	−	+	−	+	−
D-Raffinose	+	−	+	−	+	−
D-Sorbitol	+w	+	+	−	+	−
D-Trehalose	−	+	+	+	+	−
D-Xylose	+	+	+	−	+	+
Glycerol	−	−	+	−	+	−
L-Arabinose	+	+	−	−	−	−
L-Rhamnose	+w	−	−	−	−	−
Malate	−	−	+	−	−	−
myo-Inositol	+	+	+	−	+	−
Pyruvate	+	+	−	+	+	+
Ribitol	+	+	−	−	−	−
Succinate	+	−	+	−	−	−
L-Asparagine	+w	+	+	−	+	+
L-Glutamine	+	+	+	−	+	+
L-Serine	−	−	+	−	+	+
L-Arginine	+	+	+	−	+	+

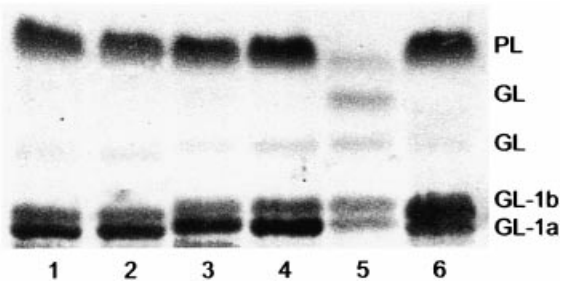

Fig. 3. One-dimensional TLC of polar lipids of *Meiothermus* strains. Components were revealed by staining the TLC plates with molybdophosphoric acid and heating at 121 °C for 30 min. Lanes: 1, WR-30^T; 2, WR-220; 3, *M. ruber* DSM 1279^T; 4, *M. silvanus* DSM 9946^T; 5, *M. chliarophilus* DSM 9957^T; 6, *M. cerbereus* DSM 11376^T. PL, Phospholipid; GL-1a, glycolipid-1a; GL-1b, glycolipid-1b; GL, minor glycolipid.

tained minor glycolipids. Fig. 3 depicts the polar lipids of tested strains of the genus *Meiothermus* assayed by one-dimensional TLC. The respiratory lipoquinone of strain WR-30^T, like that of other members of the genus *Meiothermus*, was menaquinone-8 (MK-8).

Fatty acid composition

Table 2 presents the composition of fatty acids of all tested organisms obtained following extraction, methylation and saponification of lipids and GC. The fatty acid composition of strains WR-30^T and WR-220 corroborates the assignment of these isolates to the genus *Meiothermus*, since both strains possessed 2-OH iso and anteiso fatty acids. The fatty acid composition of these strains was, however, very similar to those of the type strains of all the described species of the genus *Meiothermus*. The major fatty acids of strains WR-30^T and WR-220 were 15:0 iso and 17:0 iso; 15:0 iso fatty acids were present at higher levels than 15:0 anteiso fatty acids. The major distinguishing characteristic was relatively large amounts of 18:0 iso diol. The only other species that has this diol, in small amounts, is the type strain of *M. silvanus*, where it reached about 2% of the total fatty acids. Strains WR-30^T and WR-220

Table 2. Fatty acid composition of type strains of *Meiothermus* species and strains WR-30^T and WR-220 grown at 50 °C

Strain/species: 1, WR-30^T; 2, WR-220; 3, *M. ruber*; 4, *M. silvanus*; 5, *M. chliarophilus*; 6, *M. cerbereus*. Values are percentages of total fatty acids. –, Not detectable; ECL, equivalent chain length.

Fatty acid	ECL	1	2	3	4	5	6
13:0 iso	12·612	0·7	0·7	0·4	0·4	1·6	1·5
14:0 iso	13·618	0·7	0·7	1·3	0·6	1·9	2·6
13:0 iso 3-OH	14·110	1·1	1·1	0·4	1·0	–	–
15:1 iso F*	14·414	0·3	0·5	1·3	–	–	4·1
15:0 iso	14·621	38·4	31·8	30·9	25·9	39·0	35·5
15:0 anteiso	14·711	2·9	6·5	6·5	22·5	8·9	6·2
15:0	15·000	2·0	1·6	3·3	0·2	1·8	2·0
16:1ω7*t* alcohol	15·415	–	–	0·7	–	–	2·0
16:0 iso	15·626	2·6	2·7	4·8	1·6	2·2	4·1
15:0 iso 2-OH	15·847	0·7	1·1	0·5	0·4	0·5	0·4
16:0	16·000	6·1	6·5	4·9	5·5	8·2	5·1
Unknown	16·090	0·4	0·9	0·5	1·6	0·7	0·6
15:0 iso 3-OH	16·135	–	–	0·2	–	1·1	0·6
15:0 2-OH	16·217	0·3	–	0·9	–	0·4	0·4
17:1 iso ω9*c*	16·411	1·1	1·9	3·4	–	–	5·2
17:0 iso	16·629	17·4	16·3	16·5	12·7	13·4	6·0
17:0 anteiso	16·722	2·4	6·3	4·4	6·9	2·5	1·6
17:1ω8*c*	16·792	–	–	0·6	–	–	0·7
17:1ω6*c*	16·862	0·3	0·4	0·9	1·0	0·9	1·0
17:0	17·000	1·7	1·5	2·1	0·3	1·1	0·4
16:0 2-OH	17·235	1·0	1·2	0·6	0·4	0·7	0·7
17:0 iso 2-OH	17·872	12·0	10·4	6·8	9·6	10·9	5·7
17:0 anteiso 2-OH	17·968	0·2	0·7	0·3	3·0	0·8	–
17:0 iso 3-OH	18·164	–	0·6	1·5	–	0·2	4·7
17:0 2-OH	18·249	0·7	0·7	–	–	0·3	0·8
17:0 anteiso 3-OH	18·260	–	–	1·0	–	–	–
19:0 iso	18·633	0·3	–	–	1·8	0·2	–
19:0 anteiso	18·729	–	–	–	1·1	–	–
18:0 iso diol	19·060	4·5	3·1	0·7	2·2	–	–
18:0 anteiso diol	19·160	–	0·3	–	0·4	–	–

* The double bond position of this fatty acid is not known.

had higher levels of 18:0 iso diol, respectively reaching 4·5 and 3·1 % of the total fatty acids.

Mean base composition of DNA and DNA–DNA relatedness

The G+C content of the DNA of isolate WR-30^T was 61·4 mol % and that of WR-220 was 61·9 mol %, according to the HPLC method. To elucidate interrelatedness from DNA–DNA hybridization, all type strains of species of the genus *Meiothermus* and strains WR-30^T and WR-220 were used. Strain WR-30^T showed 57·4 % relatedness to *M. ruber* DSM 1279^T, 50·8 % relatedness to *M. cerbereus* DSM 11376^T, 19·5 % relatedness to *M. silvanus* DSM 9446^T, 28·7 % relatedness to *M. chliarophilus* DSM 9957^T and 51·2 % relatedness to *M. ruber* NCIMB 11269. Table 3 presents the DNA relatedness among strains WR-30^T, WR-220 and the other type strains of *Meiothermus* species.

16S rDNA-based phylogenetic analysis

Following PCR amplification and sequencing, 16S rDNA sequences of 1483 nt (strain WR-30^T) and 1482 nt (strain WR-220) were determined. A comparison of the 16S rDNA sequences of strains WR-30^T and WR-220 with reference sequence data in the EMBL database revealed that these strains belonged to the genus *Meiothermus*. A more detailed analysis based on a dataset consisting of at least 1400 unambiguous nucleotides between positions 28 and 1526 (*Escherichia coli* numbering; Brosius *et al.*, 1978) revealed that these isolates were most closely related to the *Thermus*/*Meiothermus* group. Strains WR-30^T and WR-220, which have identical 16S rDNA sequences

Table 3. DNA–DNA hybridization among strains WR-30^T, WR-220 and type strains of *Meiothermus* species

Strain	1	2	3	4	5	6	7
1. *M. taiwanensis* WR-30^T	100						
2. *M. taiwanensis* WR-220	94·3	100					
3. *M. ruber* DSM 1279^T	54·3	47·6	100				
4. *M. silvanus* DSM 9446^T	19·5	23·8	17·9	100			
5. *M. chliarophilus* DSM 9957^T	28·7	28·7	20·5	39·2	100		
6. *M. cerbereus* DSM 11376^T	50·8	47·1	49·6	24·3	28·9	100	
7. *M. ruber* NCIMB 11269	51·2	61·3	75·6	17·4	22·3	33·5	100

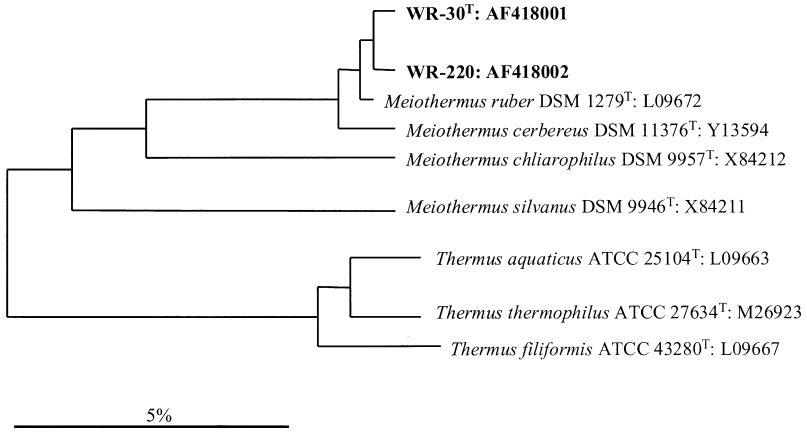

Fig. 4. 16S rDNA phylogenetic dendrogram showing relationships among various species of the genera *Thermus* and *Meiothermus*. Bar, 5 % evolutionary distance.

over all positions, exhibited the highest mutual similarity (99·46 %) and formed a sister cluster next to the type strain of *M. ruber*. The similarities between the 16S rDNA sequence of the WR-30^T/WR-220 cluster and those of *M. ruber* and *M. cerbereus* were respectively 98·4 and 97·0 %. The similarities between the 16S rDNA sequence of strains WR-30^T and WR-220 and those of other *Meiothermus* species were lower: 88·3 % for *M. silvanus* and 91·5 % for *M. chliarophilus*. Fig. 4 shows a 16S rDNA-based phylogenetic dendrogram generated by the neighbour-joining method.

DISCUSSION

Areas with geothermal springs are widely situated on the Earth's surface, but 'hot spots', where thermal environments are abundant, are restricted to areas associated with past or present volcanic activity such as the USA (Yellowstone National Park), the former USSR, Iceland, Italy, New Zealand and Japan. Many thermophilic micro-organisms have been isolated and studied from such places. Taiwan is in the circum-Pacific volcanic zone and exhibits many geothermal environments. Results obtained from preliminary studies (Chen *et al.*, 2000) have provided the basis for future investigation in the field of thermophilic micro-organisms in geothermal environments in Taiwan.

Bacteria of the genus *Meiothermus* are Gram-negative, heterotrophic, non-motile, pleomorphic rod-shaped cells with short filaments; they live aerobically at neutral pH and grow well at temperatures above 50 °C. These thermophiles are widely distributed in various areas such as geothermal hot springs and artificial thermal environments. 16S rDNA sequence analysis showed that strains WR-30^T and WR-220 are members of the genus *Meiothermus* and related to *M. ruber*. Strains WR-30^T and WR-220 had high 16S rDNA sequence similarity to the type strain of *M. ruber*, but the WR-30^T/WR-220 cluster was separate from *M. ruber*. Low DNA–DNA hybridization values showed that strains WR-30^T and WR-220 represent an independent species of the genus *Meiothermus*. Similar results were also observed in studies of *M. ruber* and *M. cerbereus* (Chung *et al.*, 1997).

The presence of 2-OH fatty acids in relatively moderate proportions, ranging between 8 and 14 %, is a chemotaxonomic characteristic of all species of the genus *Meiothermus* (Nobre *et al.*, 1996). Significant levels of 2-OH fatty acids were also present in strains of the novel species. The presence of higher levels of 18:0 iso

diol fatty acids appears to be a useful distinguishing characteristic. The only other species in the genus *Meiothermus* that has this diol, in small amounts, is the type strain of *M. silvanus*. Strains WR-30^T and WR-220 had higher levels of 18:0 iso diol fatty acids than did *M. silvanus*.

The morphology of the micro-organisms is constant and clear enough to distinguish these isolates from other species. Previous studies have revealed filamentous, thermophilic micro-organisms in different habitats. These include the photosynthetic bacterium *Chloroflexus aurantiacus* (Pierson & Castenholz, 1974), the phototrophic bacterium *Heliothrix oregonensis* (Pierson *et al*., 1985) and the heterotrophic bacteria *Geobacillus thermocatenulatus*, *Anoxybacillus flavithermus* (Golovacheva *et al*., 1975; Heinen *et al*., 1982; Pikuta *et al*., 2000) and *Thermus filiformis* (Hudson *et al*., 1987). In culture, most strains of the genera *Thermus* and *Meiothermus* form pleomorphic rod-shaped cells and short filaments (Brock, 1978). Species of *Thermus* and *Meiothermus* can become filamentous under certain culture conditions, such as addition of some D-amino acids to the growth medium (Brock & Freeze, 1969; Loginova *et al*., 1984). Most *Thermus* species produce rod-shaped cells, with the exception of the type strain of *T. filiformis*, which always produces very long intertwined filaments; rod-shaped cells have never been observed (Hudson *et al*., 1987). It has an extra wall layer, external to the corrugated layer, which runs interruptedly over zones of septum formation. Similar morphological features were observed in strains WR-30^T and WR-220. These novel *Meiothermus* isolates are morphologically filamentous, like the type strain of *T. filiformis*, but differences were also noted. Several morphological features such as mid-filament swellings of trichomes and small spheres between cells, evident in *T. filiformis*, were not observed in strains WR-30^T and WR-220.

Differences in morphological characteristics, 16S rDNA sequence divergence, the low DNA–DNA hybridization values with *M. ruber* and the distinctive fatty acid composition, with high proportions of 18:0 iso diol fatty acids, show that isolates WR-30^T and WR-220 represent a novel species belonging to the genus *Meiothermus*, for which the name *Meiothermus taiwanensis* sp. nov. is proposed.

Description of *Meiothermus taiwanensis* sp. nov.

Meiothermus taiwanensis (tai.wan.en′sis. N.L. fem. adj. *taiwanensis* of Taiwan, from the hot springs of Taiwan where the bacterium was first isolated).

Forms stable trichomes of indeterminate length (several hundred micrometres). Variable-length cells make filamentous trichomes and the cell lengths are 1·4–2·4 μm. Short filaments are also present. Septa are not visible by phase-contrast microscopy. Gram staining yields negative results. Cells are non-motile and do not form spores. Colonies on *Thermus* medium are red- and red/orange-pigmented, 2–3 mm in diameter after incubation for 72 h at 55 °C and form a thin film on the surface of agar plates. The temperature range for growth of the type strain is 40–70 °C and the optimum growth temperature is 55 °C. The optimum pH for growth is approximately 8·0. The major fatty acids are 15:0 iso and 17:0 iso; 2-OH iso and anteiso fatty acids such as 17:0 iso 2-OH are also present. The major distinguishing characteristic is the relatively high level of 18:0 iso diol fatty acid. Positive for α-galactosidase, β-galactosidase, oxidase and catalase, hydrolysis of elastin, fibrin, gelatin, casein and DNA and degradation of aesculin. The type strain can utilize D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-mannitol, L-rhamnose, D-melibiose, D-sorbitol, D-xylose, lactose, L-arabinose, L-glutamate, maltose, *myo*-inositol, pyruvate, ribitol, succinate, sucrose, L-asparagine, L-glutamine, L-proline and L-arginine as carbon sources. It does not hydrolyse starch or xylan and does not utilize citrate, D-trehalose, glycerol, malate or L-serine. The DNA G+C content of the type strain is 61·9 mol%.

The type strain is strain WR-30^T (= ATCC BAA-399^T = CCRC 17170^T = DSM 14542^T) and strain WR-220 (= ATCC BAA-400 = CCRC 17171 = DSM 14543) is a reference strain; both strains were isolated from the Wu-rai hot springs of Taipei in northern Taiwan.

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