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Meiothermus timidus sp. nov., a new slightly thermophilic yellow-pigmented species

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Abstract

Several yellow-pigmented isolates, with optimum growth temperatures between 55 and 60 °C, were recovered from hot springs in Central Portugal and the Azores. Phylogenetic analysis of the 16S rDNA showed that these organisms represented a new species of the genus *Meiothermus*. The new isolates could be distinguished from other strains of the species of the genus *Meiothermus* by biochemical characteristics and the fatty acid composition because they had very high levels of iso C15:0 and iso C17:0 and very low levels of anteiso C17:0 and iso C16:0. On the basis of the results presented here we propose the name *Meiothermus timidus* for the new species represented by strains SPS-243^T (=LMG 22897^T = CIP 108604^T), RQ-10 and RQ-12.

Keywords: *Meiothermus*; *Meiothermus timidus* sp. nov.; *Thermaceae*

1. Introduction

The species of the genera *Thermus* and *Meiothermus*, along with the recently described species of *Marinithermus* [1], *Vulcanithermus* [2] and *Oceanithermus* [3,4], the latter of which were isolated from abyssal hyperthermal vents, currently form the family *Thermaceae* [5]. The species *Meiothermus ruber* was initially included in the genus *Thermus*, but the description of other "low-temperature" species, clearly showed that these organisms belonged to a distinct genus, which was named *Meiothermus* [6]. The species of the genus *Meiothermus*, namely *M. ruber* [7], *Meiothermus silvanus* [8], *Meiothermus chliarophilus* [8], *Meiothermus cerbereus* [9] and *Meiothermus taiwanensis* [10] form, based on 16S rDNA sequence analysis, a separate line of descent within the genera of the family *Thermaceae* with which they share 85.2–86.6% sequence similarity [4,11]. One species named "*Meiothermus rosaceus*", isolated from hot springs in China, has not been validly described but appears to be extremely closely related to *M. ruber* [12].

The species of the genus *Meiothermus* have only been isolated from fresh water heated environments and have a lower growth temperature range than those of the genera *Thermus*, *Marinithermus* and *Vulcanithermus* [1–5,11]. The former have growth temperatures ranges between about 35 and 68 °C, while the latter grow between about 40 and 83 °C. Only a few strains of the genus *Thermus* possess 3-OH iso- or anteiso-fatty acids, but 2-OH fatty acids have never been encountered in strains of this genus [13]. The species of the genera

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Marinithermus [1], *Vulcanithermus* [2] and *Oceanithermus* [3,4] appear not to possess hydroxy-fatty acids. However, 2-OH and 3-OH fatty acids have always been detected in *Meiothermus* strains [14]. The strains of the species of the genus *Thermus* possess one major glycolipid designated GL-1 and a major phospholipid designated PL-2 [13], although all strains of the species of the genus *Meiothermus* examined possess two major glycolipid variants on thin-layer chromatography, designated GL-1a and GL-1b, that differ due to the presence of amide-linked 2-OH fatty acids in GL-1a and amide-linked 3-OH iso-branched or an iso-branched fatty acid in GL-1b [14]. The species of *Meiothermus* are generally red-pigmented, except for the three known strains of *M. chliarophilus*, which are yellow-pigmented. On the other hand, the other genera of the family *Thermaceae* consist of yellow- or non-pigmented strains.

The hydrothermal spring at São Pedro do Sul in Central Portugal has yielded many strains of the genus *Thermus* [5], as well as strains closely related to the type strain of *M. ruber* [6]. Recently, we isolated one yellow-pigmented strain of the genus *Meiothermus* that was clearly distinct from the strains of the other species of this genus. A few months later we also isolated several yellow-pigmented strains of the genus *Meiothermus* from the Island of São Miguel in the Azores that belonged to the same new species. Phylogenetic analysis of the 16S rRNA gene sequence, physiological and biochemical characteristics clearly indicate that strains SPS-243^T, RQ-10 and RQ-12 belong to a new species of the genus *Meiothermus* for which we propose the name *Meiothermus timidus*.

2. Materials and methods

2.1. Isolation and bacterial strains

Strain SPS-243^T (T = type strain), SPS-217, SPS-241 and SPS-242 were isolated from biofilm samples in the effluent water of the hot spring at São Pedro do Sul, in Central Portugal. Samples were transported without temperature control and filtered the same day through membrane filters (Gelman type GN-6; pore size 0.45 μm; diameter 47 mm). The filters were placed on the surface of *Thermus* medium solidified with agar [15] or buffered charcoal yeast extract (BCYE) which is normally used for the isolation and growth of *Legionella* spp. [16]. Strains RQ-10, RQ-12, RQ-18, RQ-21, TU-1 and TU-2, were isolated from the Island of São Miguel, in the Azores, by filtering water samples maintained for six days at room temperature. Filters were placed on the surface of *Thermus* agar. All plates, with the filters were wrapped in plastic bags and incubated at 50 °C for up to 4 days. Cultures were purified by sub-culturing on *Thermus* medium and the isolates stored at −70 °C in *Thermus* medium with 15% (w/v) glycerol. The type strains of *M. chliarophilus* ALT-8 (=DSMZ 9957) and *M. ruber* Loginova 21 (=DSMZ 1279) were used for comparative purposes.

2.2. Morphology, growth, biochemical and physiological characteristics

Cell dimensions and motility were determined by phase contrast microscopy during exponential growth in *Thermus* liquid medium. The growth temperature range of the strains was examined by measuring the turbidity (610 nm) of cultures incubated in 300-ml metal-capped Erlenmeyer flasks, containing 100 ml of *Thermus* medium in a reciprocal water-bath shaker. The pH range for growth was examined at 50 °C as described previously [8].

Unless otherwise stated, all biochemical and tolerance tests were performed, as described previously [8], in *Thermus* liquid medium or *Thermus* agar at 50 °C for up 5 days. Single-carbon source assimilation tests were performed in a minimal medium composed of *Thermus* basal salts with 0.1 g per litre of yeast extract containing filter-sterilized ammonium sulfate (0.5 g l^{-1}) and the carbon source (2.0 g l^{-1}). Growth was examined daily by measuring the turbidity of cultures incubated in 20 ml screw capped tubes containing 10 ml of medium for a total of 5 days. Positive and negative control cultures were grown in *Thermus* medium and the minimal medium without carbon source. Anaerobic growth was assessed in cultures grown in *Thermus* medium incubated in anaerobic chambers with an H_2/CO_2 atmosphere (BioMerieux, Marcy l'Etoile, France).

2.3. Lipid and fatty acid analyse

The cultures used for polar lipid analysis were grown in 1 l Erlenmeyer flasks containing 200 ml of *Thermus* medium at 50 °C in a reciprocal water-bath shaker until the late exponential phase of growth. Harvesting of the cultures, extraction of the lipids and single dimensional thin-layer chromatography were performed as described previously [17]. Lipoquinones were extracted from freeze-dried cells and were purified by thin-layer chromatography, and separated by high performance liquid chromatography as described previously [18]. Cultures for fatty acid analysis were grown on solidified *Thermus* medium, in sealed plastic bags submerged in a water bath at 50 °C for 24 h. Fatty acid methyl esters (FAMEs) were obtained from fresh wet biomass by saponification, methylation and extraction and the fatty acids identified and quantified with the standard MIS Library Generation Software (Microbial ID Inc., Newark, DE, USA) as described by the manufacturer.

2.4. Determination of G+C content of DNA and 16S rRNA gene sequence determination and phylogenetic analyses

The DNA for the determination of the G+C content of the DNA was isolated as described previously [19]. The G+C content of DNA was determined by high-performance liquid chromatography as described by Mesbah et al. [20].

The extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously [21]. Purified reactions were electrophoresed using a model 310 Genetic Analyzer (Applied Biosystems, Foster City, Ca.). The quality of 16S rRNA gene sequences was checked manually using the BioEdit sequence editor [22] and aligned against representative reference sequences of members of the family *Thermaceae*, obtained from EMBL, using the multiple-alignment CLUSTAL X software package [23]. The method of Jukes and Cantor [24] was used to calculate evolutionary distances; phylogenetic dendrograms were constructed using the neighbor-joining method [25], and trees topologies were evaluated by performing bootstrap analysis [26] of 1000 data sets using the MEGA2 package [27].

2.5. Nucleotide sequence Accession Nos.

The 16S rRNA gene sequences determined in this study were deposited in EMBL data library under the Accession No. SPS-243^T (AJ871168), RQ-10 (AJ871169), RQ-12 (AJ871171), RQ-18 (AJ871170), SPS-217 (AJ871174), SPS-241 (AJ871172) and SPS-242 (AJ871173).

3. Results

3.1. Isolation of strains

Buffered charcoal yeast extract medium was used to isolate organisms from biofilms, along runoffs (temp. 49.5 °C, pH 8.3) of the hot spring at São Pedro do Sul, in an attempt to isolate organisms that might not grow on some of the conventional media used for the isolation of thermophilic aerobes. All isolates recovered, during enrichments in BCYE at 50 °C, were subsequently found to grow very well on *Thermus* medium and did not have a requirement for any of the components of this medium. Strains SPS-241 and SPS-242 formed rod-shaped and filamentous cells, and red colonies. Strains SPS-243^T and SPS-217 were morphologically similar, but formed yellow-pigmented colonies. A large number of isolates from hot springs on the Is. of São Miguel in the Azores were obtained several months later after enrichments at 50 °C in an attempt to recover other yellow-pigmented *Meiothermus* strains. Several yellow-pigmented strains, namely RQ-10, RQ-12, RQ-18, RQ-21, TU-1 and TU-2 were recovered from thermal sites at Ribeira Quente and Furnas, where the water temperature varied between 47 and 60 °C and the pH was about 7.3. These organisms possessed nearly identical fatty acid profiles as well as 2-OH fatty acids and three strains (RQ-10, RQ-12 and RQ-18) were chosen for 16S rRNA gene sequence analysis and further characterization.

3.2. Biochemical and physiological characteristics

Strain SPS-243^T had an optimum growth temperature in the neighborhood of 55–60 °C and did not grow at 70 °C; the other red-pigmented isolates from São Pedro do Sul had slightly higher optimum growth temperatures of about 60 °C. The yellow-pigmented strain SPS-217 had an optimum growth temperature around 70 °C.

Table 1
Phenotypic characteristics of the new isolates and their phylogenetically closest relative *Meiothermus chliarophilus*

Characteristics[a]	Strains			
	SPS-243^T	RQ-10	RQ-12	*M. chliarophilus*^{T}
Pigmentation	Yellow	Yellow	Yellow	Yellow
Presence of:				
Catalase	+	+	+	–
Oxidase	+	+	+	+
Hydrolysis of:				
Elastin	+	+	+	+
Starch	+	+	+	+
Casein	+	+	+	+
Reduction of NO_3^- to NO_2^-	+	+	+	+
Utilization of:				
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Xylose	+	+	+	+
L-Arabinose	+	+	+	–
D-Trehalose	+	+	+	+
D-Cellobiose	+	+	+	+
D-Melibiose	+	+	+	+
D-Raffinose	+	+	+	+
L-Rhamnose	–	–	–	–
Sucrose	+	+	+	+
D-Sorbitol	+	+	+	+
D-Mannitol	+	+	+	+
Ribitol	–	–	–	–
Glycerol	–	–	–	+
Pyruvate	+	+	+	+
Succinate	+	+	+	–
L-Proline	+	+	+	+
L-Serine	+	+	+	+
L-Asparagine	+	+	+	+
L-Arginine	+	+	+	+
L-Glutamine	+	+	+	+

[a] +, Positive result; –, negative result.

The optimum pH of strain SPS-243^T was between 7.0 and 8.0. Strains SPS-243^T, RQ-10 and RQ-12 were oxidase positive and catalase positive (Table 1). These organisms, like other strains of the genus *Meiothermus*, used carbohydrates, organic acids and amino acids as single carbon and energy sources, however the carbon source assimilation patterns were almost identical to those of *M. chliarophilus*. Nitrate was reduced to nitrite, but anaerobic growth in the presence or absence of nitrate was not observed.

3.3. Polar lipids, respiratory quinones and fatty acids

The polar lipid pattern of strains SPS-241, SPS-242, and SPS-243^T was composed of one major phospholipid (PL-2) and two major glycolipid variants (GL-1a and GL-1b) migrating close to each other on TLC plates; the polar lipid pattern of strain SPS-217 had only one glycolipid (GL-1) similar to other *Thermus* strains (results not shown). The major respiratory quinone of all strains was menaquinone 8 (MK-8). The fatty acids were predominantly iso- and anteiso-branched fatty acids; 2-OH and 3-OH fatty acids were detected in the *Meiothermus* strains. Strains SPS-243^T and all the Ribeira Quente strains had higher levels of iso C15:0 and iso C17:0 and low levels of anteiso C17:0 and iso C16:0 than the strains of any of the other species of the genus *Meiothermus* (Table 2).

3.4. 16S rRNA gene sequence comparison and G+C content of DNA

Partial 16S rRNA gene sequences comprising 1455–1500 nucleotides were determined for strains SPS-243^T, RQ-10, RQ-12, RQ-18, SPS-217, SPS-241 and SPS-242. Comparison of these sequences with representatives of the main lines of descent within the domain *Bacteria* indicated that these strains were members of the family *Thermaceae* (Fig. 1). Isolate SPS-217 was clearly a member of the genus *Thermus* with highest sequence

Table 2
Mean faty acid composition of the strains belonging to the genus *Meiothermus* grown at 50 °C

Fatty acids[b]	Percentage of the total in:						
	M. ruber	*M. silvanus*	*M. chliarophilus*	*M. cerbereus*	*M. taiwanensis*	New isolates[a]	
	(13 strains)[c]	(5 strains)	(3 strains)	(6 strains)	(2 strains)	SPS 243^T	Azorean strains (6 strains)
13:0 Iso	0.6	0.8	1.5	1.4	0.7	1.3	1.7
14:0 Iso	0.7	0.7	1.7	2.7	0.7	–[d]	0.5
14:0	0.6	0.3	0.7	–	–	–	0.5
13:0 Iso 30H	0.4	0.8	–	–	1.1	0.5	0.4
15:1 Iso ω9c	2.7	–	–	3.8	0.3	–	–
15:0 Iso	33.0	25.6	42.1	34.6	38.4	46.5	49.3
15:0 Anteiso	5.5	26.5	8.1	11.1	2.9	3.0	3.3
15:0	1.8	0.4	2.1	1.6	2.0	0.5	1.1
16:1 Alcohol	0.8	–	–	1.9	–	–	–
16:0 Iso	2.9	1.5	2.5	4.0	2.6	0.8	0.6
15:0 Iso 2OH	0.7	0.9	0.5	–	0.7	1.1	0.7
16:0	7.6	6.4	9.1	4.5	6.1	6.4	6.8
Unknown diol	1.1	2.5	0.7	–	0.4	–	–
15:0 Iso 3OH	–	–	1.0	–	–	2.5	2.9
17:1 Iso ω9*c*	6.5	–	–	4.8	1.1	–	–
17:1 Anteiso ω9*c*	1.0	–	–	–	–	–	–
17:0 Iso	13.3	10.0	16.4	5.8	17.4	27.6	23.2
17:0 Anteiso	3.7	6.4	2.7	2.5	2.4	1.8	1.8
17:1 ω8*c*	0.8	–	–	–	–	–	–
17:1 ω6*c*	0.8	1.3	0.7	–	0.3	–	–
17:0	0.8	0.3	1.2	–	1.7	0.3	0.5
16:0 2OH	0.6	0.5	0.4	–	1.0	–	–
17:0 Iso 2OH	7.8	9.6	7.3	3.3	12.0	6.9	4.7
17:0 Anteiso 2OH	0.4	3.0	0.6	–	0.2	–	–
17:0 Iso 3OH	1.1	–	–	4.7	–	0.8	1.1
19:0 Iso	–	2.6	–	–	–	–	–
19:0 Anteiso	–	1.6	–	–	–	–	–
17:0 Anteiso 3OH	0.6	–	–	1.4	–	–	–
18:0 Iso diol	–	1.6	–	–	4.5	1.6	0.7

[a] The fatty acid composition of the type strain is listed separated from those of Azorean RQ and TU strains.
[b] Values for fatty acids present at levels of less than 0.5% in all strains are not shown.
[c] Number of strains examined.
[d] Not detected.

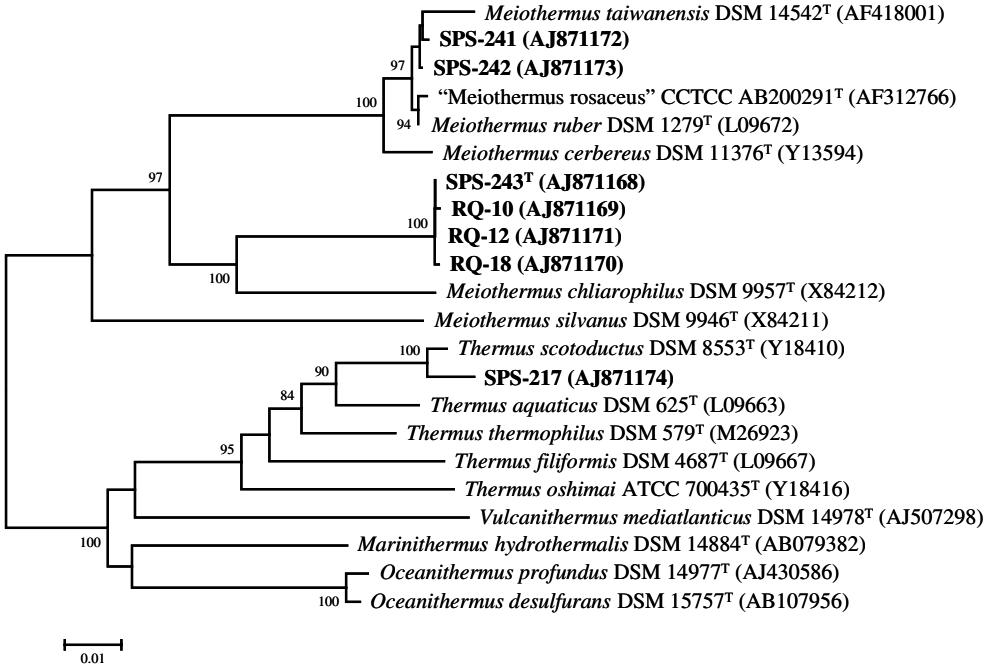

Fig. 1. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of strains SPS-243^T, RQ-10, RQ-12, RQ-18, SPS-241, SPS-242 and SPS-217 and the representative type strains of the family *Thermaceae*. The trees were created using the neighbour-joining method. The numbers on the tree indicate the percentages of bootstrap sampling, derived from 1000 replications. Isolates characterized in this study are indicated in bold. Scale bar, 10 inferred nucleotide substitutions per 100 nucleotides.

similarity to the type strain of *Thermus scotoductus* (98.8%), while the remainder of the isolates represented lineages of the genus *Meiothermus*. The pairwise 16S rRNA gene sequence similarity determined between strains SPS-241 and SPS-242 was 99.9% with each other and 98.4–99.8% within the *M. ruber*/*M. taiwanensis*/"*M. rosaceus*" clade (Fig. 1). The 16S rRNA gene sequence similarity between strains SPS-243^T, RQ-10, RQ-12 and RQ-18 varied from 99.8% to 100%. This group of strains showed highest pairwise similarity (93.0%) with the type strain of *M. chliarophilus*. The G+C content of the DNA of strain SPS-243^T was 65.1 mol%.

4. Discussion

Strains SPS-243^T, RQ-10 and RQ-12 clearly belong to the genus *Meiothermus* based on the phylogenetic analysis of 16S rRNA gene sequence, the low growth temperature range, the presence of two glycolipid variants and 2-OH fatty acids. The phylogenetic analysis shows that the new species represented by strain SPS-243^T, RQ-10, and RQ-12 is most closely related to *M. chliarophilus* although these two species share only about 93.0% 16S rRNA gene sequence similarity. Two red-pigmented isolates from the hot spring at São Pedro do Sul, namely SPS-241 and SPS-242 were most closely related to the *M. ruber*/*M. taiwanensis*/"*M. rosaceus*" clade, sharing 98.4–99.8% 16S rRNA gene sequence similarity. The two most closely related organisms of this clade were *M. ruber* and "*M. rosaceus*", which share 99.8% sequence similarity. However, *M. rosaceus* is reported to have a DNA:DNA reassociation value of 62.9% with the type strain of *M. ruber* indicating that this organism represents a distinct genomic species [12].

We initially thought that the yellow-pigmentation of the *M. chliarophilus* strains, which have only been isolated from the hot spring at Alcafache in Central Portugal, was not a stable characteristic of this species and that other strains from different geographical areas could be red-pigmented, like all other *Meiothermus* strains described previously [8–10]. The isolation of strain SPS-243^T from the hot spring at S. Pedro do Sul indicated that the genus *Meiothermus* comprises species where yellow-pigmentation could be a stable characteristic. The isolation of strain SPS-243^T induced us to attempt to isolate yellow-pigmented *Meiothermus* strains from hot springs on the Is. of S. Miguel, at an enrichment temperature of 50 °C, leading to the isolation of strains belonging to the same species as strain SPS-243^T and the demonstration that yellow-pigmented *Meiothermus* strains are more common than previously expected. It is very likely that yellow-pigmented isolates of the genus *Meiothermus* recovered from enrichments of water samples at 50–60 °C have been overlooked as belonging to species of the genus *Thermus* which also grow at these temperatures. We generally chose red-pigmented colonies for further examination of *Meiothermus*

spp. after enrichments at these low temperatures and discarded colonies with the normal yellow *Thermus* pigmentation.

The yellow-pigmentation of *M. chliarophilus* and strain SPS-243^T corroborate the phylogenetic analysis which indicates that these organisms are more closely related to each other than to the other species of the genus *Meiothermus*. The 16S rRNA gene sequence analysis argues for the division of the genus *Meiothermus* into three genera namely; one that would include *M. ruber*, *M. taiwanensis M. cerbereus* and "*M. rosaceus*", another genus that includes the species represented by strain SPS-243^T, RQ-10 and RQ-12 and *M. chliarophilus*, and finally a novel genus for *M. silvanus* strains. However, there are no phenotypic characteristics to distinguish these species along these ranks that justify such a massive change in the systematics of these organisms and we will, therefore, maintain the genus *Meiothermus* as defined by Nobre et al. [6].

Differences in carbon source assimilations, the distinctive fatty acid composition with high proportions of iso C15:0 and iso C17:0, yellow pigmentation and the 16S rRNA gene sequence analysis lead us to propose the new species *Meiothermus timidus* for strain SPS-243^T (= LMG 22897^T = CIP 108604^T).

5. Description of *Meiothermus timidus* sp. nov.

Meiothermus timidus (ti'mi. dus. L. adj. *timidus*, timid, shy; because only one strain was recovered from the hot spring at São Pedro do Sul after the isolation of so many organisms, over several years, from this site). *M. timidus* forms rod-shaped cells of variable length and are 0.5–0.8 μm wide. Long filaments are also present. Gram stain is negative. The cells are non-motile and spores are not formed. Colonies on *Thermus* medium are bright yellow-pigmented and 1–2 mm in diameter after 72 h of growth. The optimum growth temperature for strain SPS-243^T is about 55–60 °C. The optimum pH is about 7.5; growth does not occur at pH 5.0 or 10.5. The major respiratory quinone is menaquinone 8. Glycolipid variants GL-1a and GL-1b are present. The major fatty acids are 15:0 iso and 17:0 iso; 3-OH and 2-OH fatty acids are present. Aerobic and heterotrophic. All strains are oxidase positive and catalase positive. Nitrate is reduced to nitrite. Degradation of elastin, starch and casein is positive. Strains SPS-243^T, RQ-10 and RQ-12 utilize D-glucose, D-fructose, D-melibiose, D-cellobiose, sucrose, D-trehalose, D-raffinose, D-xylose, L- arabinose, D-sorbitol, D-mannitol, pyruvate, succinate, L-serine, L-asparagine, L- arginine, L-glutamine and L-proline.

The DNA of strain SPS-243^T has a G+C content of 65.1 mol%. This bacterium was isolated from hot spring at São Pedro do Sul in Central Portugal. The type strain, SPS-243^T, has been deposited in the Collection of the Institut Pasteur, Paris, France, as strain CIP 108604^T and in the BCCM/LMG Bacteria Collection, Ghent, Belgium as strain LMG 22897^T.

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References

[1] Sako, Y., Nakagawa, S., Takai, K. and Horikoshi, K. (2003) *Marinithermus hydrothermalis* gen. nov., sp. nov., a strictly aerobic, thermophilic bacterium from a deep-sea hydrothermal vent chimney. Int. J. Syst. Evol. Microbiol. 53, 59–65.

[2] Miroshnichenko, M.L., L'Haridon, S., Nercessian, O., Antipov, A.N., Kostrikina, N.A., Tindall, B.J., Schumann, P., Spring, S., Stackebrandt, E., Bonch-Osmolovskaya, E.A. and Jeanthon, C. (2003) *Vulcanithermus mediatlanticus* gen. nov., sp. nov., a novel member of the family *Thermaceae* from a deep-sea hot vent. Int. J. Syst. Evol. Microbiol. 53, 1143–1148.

[3] Miroshnichenko, M.L., L'Haridon, S., Jeanthon, C., Antipov, A.N., Kostrikina, N.A., Tindall, B.J., Schumann, P., Spring, S., Stackebrandt, E. and Bonch-Osmolovskaya, E.A. (2003) *Oceanithermus profundus* gen. nov., sp. nov., a thermophilic, microaerophilic, facultatively chemolithoheterotrophic bacterium from a deep-sea hydrothermal vent. Int. J. Syst. Evol. Microbiol. 53, 747–752.

[4] Mori, K., Kakegawa, T., Higashi, Y., Nakamura, K., Maruyama, A. and Hanada, S. (2004) *Oceanithermus desulfurans* sp. nov., a novel thermophilic, sulfur-reducing bacterium isolated from a sulfide chimney in Suiyo Seamount. Int. J. Syst. Evol. Microbiol. 54, 1561–1566.

[5] da Costa, M.S. and Rainey, F.A. (2001) Family *Thermaceae*, second ed (Boone, D.R. and Castenholtz, R.W., Eds.), Bergey's Manual of Systematic Bacteriology, vol. 1, pp. 403–420. Springer-Verlag, New York.

[6] Nobre, M.F., Trüper, H.G. and da Costa M.S. (1996) Transfer of *Thermus ruber* [7] , *Thermus silvanus* [8] and *Thermus chliarophilus* [8] to *Meiothermus* gen. nov. as *Meiothermus ruber* comb. nov., *Meiothermus silvanus* comb. nov., and *Meiothermus chliarophilus* comb. nov., respectively, and emendation of the genus Thermus. Int. J. Syst. Bacteriol. 46, 604–606.

[7] Loginova, L.G., Egorova, L.A., Golovacheva, R.S. and Seregina, L.M. (1984) *Thermus ruber* sp. nov., nom. rev. Int. J. Syst. Bacteriol. 34, 498–499.

[8] Tenreiro, S., Nobre, M.F. and da Costa, M.S. (1995) *Thermus silvanus* sp. nov. and *Thermus chliarophilus* sp. nov., two new species related to *Thermus ruber* but with lower growth temperatures. Int. J. Syst. Bacteriol. 45, 633–639.

[9] Chung, A.P., Rainey, F., Nobre, M.F., Burghardt, J. and da Costa, M.S. (1997) *Meiothermus cerbereus* sp. nov., a new slightly thermophilic species with high levels of 3-hydroxy fatty acids. Int. J. Syst. Bacteriol. 47, 1225–1230.

[10] Chen, M.Y., Lin, G.H., Lin, Y.T. and Tsay, S.S. (2002) *Meiothermus taiwanensis* sp. nov., a novel filamentous, thermo-

philic species isolated in Taiwan. Int. J. Syst. Evol. Microbiol. 52, 1647–1654.
[11] Nobre, M.F. and da Costa, M.S. (2001) The genus *Meiothermus*, second ed (Boone, D.R. and Castenholtz, R.W., Eds.), Bergey's Manual of Systematic Bacteriology, vol. 1, pp. 414–420. Springer-Verlag, New York.
[12] Chen, C., Lin, L., Peng, Q., Ben, K. and Zhou, Z. (2002) *Meiothermus rosaceus* sp. nov. isolated from Tengchong hot spring in Yunnan, China. FEMS Microbiol. Lett. 216, 263–268.
[13] Carreto, L., Wait, R., Nobre, M.F. and da Costa, M.S. (1996) Determination of the structure of a novel glycolipid from *Thermus aquaticus* 15004 and demonstration that hydroxy fatty acids are amide linked to glycolipids in *Thermus* spp. J. Bacteriol. 178, 6479–6486.
[14] Ferreira, A.M., Wait, R., Nobre, M.F. and da Costa, M.S. (1999) Characterization of glycolipids from *Meiothermus* spp.. Microbiology 145, 1191–1199.
[15] Williams, R.A.D. and da Costa, M.S. (1992) The genus *Thermus* and related microorganisms In: The Prokaryotes (Balows, A., Trüper, A.G., Dworkin, M., Harder, W. and Schleifer, K.-H., Eds.), second ed, pp. 3745–3753. Springer-Verlag, New York.
[16] Edelstein, P.H. (1981) Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. 14, 298–303.
[17] Prado, A., da Costa, M.S. and Madeira, V.M.C. (1988) Effect of growth temperature on the lipid composition of two strains of *Thermus* sp. J. Gen. Microbiol. 134, 1653–1660.
[18] Tindall, B.J. (1989) Fully saturated menaquinones in the archaebacterium *Pyrobaculum islandicum*. FEMS Microbiol. Lett. 60, 251–254.
[19] Nielsen, P., Fritze, D. and Priest, F.G. (1995) Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. Microbiology 141, 1745–1761.
[20] Mesbah, M., Premachandran, U. and Whitman, W. (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. Int. J. Syst. Bacteriol. 39, 159–167.
[21] Rainey, F.A, Ward-Rainey, N., Kroppenstedt, R.M. and Stackebrandt, E. (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. Int. J. Syst. Bacteriol. 46, 1088–1092.
[22] Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic. Acids. Symp. Ser. 41, 95–98.
[23] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.
[24] Jukes, T.H. and Cantor, C.R. (1969) Evolution of protein molecules In: Mammalian Protein Metabolism (Munro, H.N., Ed.), pp. 21–132. Academic Press, New York.
[25] Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
[26] Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
[27] Kumar, S., Tamura, K., Jakobsen, I.B. and Nei, M. (2001) MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 12, 1244–1245.