Transfer of *Thermus ruber* (Loginova et al. 1984), *Thermus silvanus* (Tenreiro et al. 1995), and *Thermus chliarophilus* (Tenreiro et al. 1995) 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*

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On the basis of phylogenetic, phenotypic, and chemotaxonomic distinctiveness, we formally propose that the species of the genus *Thermus* that have low optimum growth temperatures, *Thermus ruber*, *Thermus silvanus*, and *Thermus chliarophilus*, should be reclassified in the genus *Meiothermus* gen. nov. as *Meiothermus ruber* comb. nov., *Meiothermus silvanus* comb. nov., and *Meiothermus chliarophilus* comb. nov., respectively. An emended description of the genus *Thermus* is also presented.

There are currently eight validly described thermophilic (eu)bacterial species assigned to the genus *Thermus*. *Thermus aquaticus* (3), *Thermus filiformis* (10), *Thermus scotoductus* (11), *Thermus thermophilus* (14, 18, 28), and *Thermus Brockianus* (15, 28) form yellow or nonpigmented colonies and have optimum growth temperatures of about 70°C. All of the strains assigned to these species except the strains assigned to *T. thermophilus* have maximum growth temperatures below 80°C (13, 14). These species have been informally designated the high-temperature species of the genus *Thermus* to distinguish them from the species that have lower optimum growth temperatures.

Until recently, *Thermus ruber* was the only low-temperature *Thermus* species that had been described (12). The strains of this species form red-pigmented colonies, have optimum growth temperatures of about 60 to 65°C, do not grow at 70°C, and are widely distributed in hot springs and artificial thermal environments (9, 22, 24). Two new low-temperature species, *Thermus silvanus* and *Thermus chliarophilus*, were recently isolated from hot springs in Portugal and described (25). *T. silvanus* forms red-pigmented colonies and has an optimum growth temperature of about 55°C and a maximum growth temperature of about 65°C. *T. chliarophilus* forms yellow-pigmented colonies and has an optimum growth temperature of about 50°C and a maximum growth temperature of about 60°C.

The phenotypic diversity of the strains of the high-temperature species has hindered the taxonomy of these organisms. *T. thermophilus* is the only species that can be distinguished from the other species at this time because of its ability to grow at 80°C and its ability to grow in media containing more than 2% NaCl (13, 14). On the other hand, the low-temperature species can be distinguished from each other easily (25).

The high- and low-temperature species have several characteristics in common. Transmission electron microscopy has revealed that the high- and low-temperature *Thermus* species

all have a characteristic corrugated outer layer with regularly spaced invaginations connected to a thin peptidoglycan layer (2, 9, 25). Moreover, ornithine is the major diamino acid of the peptidoglycan of all strains (8, 9, 20), and menaquinone 8 is the major respiratory quinone (9, 25, 26).

Nevertheless, differences in other chemotaxonomic characteristics can be used to distinguish the high-temperature *Thermus* species from the low-temperature species. The polar lipid patterns of all high-temperature *Thermus* strains that have been examined consist of one major phospholipid, designated phospholipid 2, and one major glycolipid, designated glycolipid 1. A minor glycolipid (glycolipid 2) and a minor aminophospholipid (phospholipid 1) are also produced by most strains. This polar lipid pattern can be considered a diagnostic chemotaxonomic characteristic of high-temperature *Thermus* strains since the only exception is found in *T. scotoductus* X-1 colony type 1; glycolipid 1 is not synthesized by this organisms, and glycolipid 2 is the predominant glycolipid (4, 7, 17, 19, 21, 26). The polar lipid pattern of each low-temperature species consists of phospholipid 2 and two prominent glycolipids, designated glycolipids 1a and 1b, which are present in similar relative amounts, migrate close to each other, and are always detected (5, 25).

Odd-numbered iso and anteiso fatty acids are the predominant acyl constituents of the high- and low-temperature species. The amounts of the even-numbered iso branched-chain fatty acids are generally relatively low compared with the amounts of the odd-numbered fatty acids, and branched-chain monounsaturated fatty acids are detected only when the organisms are grown at low temperatures (4, 5, 7, 17, 21, 25, 26). Hydroxy fatty acids were not detected in the early studies of the fatty acid compositions of *Thermus* species because the transmethylation methods used led to degradation of these compounds (7). However, recent results have shown that iso 3-hydroxy fatty acids (10 to 17% of the total fatty acids) are present in all of the strains assigned to *T. aquaticus*, while anteiso 3-hydroxy fatty acids (about 10% of the total fatty acids) are present in the type strain of *T. filiformis* (7, 15a). Hydroxy fatty acids have not been detected in the other strains

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assigned to *T. filiformis* on the basis of the results of DNA-DNA hybridization studies (8) or in the strains of any of the other high-temperature species that have been described. On the other hand, all *T. ruber*, *T. silvanus*, and *T. chliarophilus* strains contain branched-chain 2-hydroxy fatty acids at concentrations that vary between 7 and 13% of the total fatty acids. 3-Hydroxy fatty acids are not found in *T. silvanus*, and the concentrations of 3-hydroxy fatty acids never exceed 1.5% of the total fatty acids in *T. ruber* and *T. chliarophilus* (25).

The genus *Thermus* belongs to an ancient phylum within the *Bacteria*, along with the distantly related mesophilic radioreistant species of the genus *Deinococcus* (27). Analyses of 16S rRNA gene sequences of the species of the genus *Thermus* led to the identification of two distinct phylogenetic lines (1, 6, 23). These analyses revealed that one phylogenetic line of descent included all of the high-temperature *Thermus* species, while the other line of descent contained the low-temperature species *T. ruber*. The level of sequence similarity between the two groups is about 86%, while the levels of sequence similarity among the high-temperature species are not less than about 94%. A 16S rRNA gene sequence analysis of the recently described low-temperature species *T. silvanus* and *T. chliarophilus* revealed that these species belong to the *T. ruber* line of descent and confirmed the evolutionary distinctiveness of the two groups of the genus *Thermus* (25). Moreover, two new 16S rRNA sequences of red-pigmented strains obtained from Yellowstone National Park showed that these organisms are very similar to the type strain of *T. ruber* (16). The three low-temperature species exhibit levels of 16S rRNA sequence similarity of about 88 to 91%, but share several unifying phenotypic and chemotaxonomic characteristics.

The phenotypic and chemotaxonomic differences between the high- and low-temperature *Thermus* species, along with the phylogenetic analysis results, support the argument that there are two groups in this genus. On the basis of the results of phylogenetic studies, as well as the different growth temperatures, the distinctive polar lipid patterns, and the hydroxy fatty acid compositions of the two groups of organisms, we propose that *T. ruber*, *T. silvanus*, and *T. chliarophilus* should be classified in the new genus *Meiothermus*.

Description of *Meiothermus* gen. nov. *Meiothermus* (Mei.o. ther'mus. Gr. *meio*- less, Gr. adj. *thermus* hot, M. L. masc. n. *Meiothermus*, to indicate an organism living in a less hot place). The description given below is based on data of Loginova et al. (12) and other authors (9, 24, 25). The cells are 0.5 to 0.8 μm in diameter; the cell length is variable, and short filaments are formed. The cells are gram negative and not motile. Red- or yellow-pigmented colonies are produced. Metabolism is respiratory and aerobic, but some species grow with nitrate as the terminal electron acceptor. Oxidase positive. Some species are catalase negative. The optimum growth temperature varies between 50 and 65°C. None of the species grows at 70°C. The optimum pH is about 8.0. Menaquinone 8 is the predominant respiratory quinone; ornithine is the principal diamino acid of the peptidoglycan. The polar lipid pattern is dominated by one major phospholipid and two prominent glycolipids that migrate close to each other. Additional phospholipids and glycolipids are minor components. The fatty acids are predominantly iso and anteiso branched; iso 2-hydroxy fatty acids are found in all strains.

Proteins and peptides are hydrolyzed by all strains. Starch is hydrolyzed by some species. Hexoses, a few pentoses and polyols, disaccharides, amino acids, and organic acids are used as sole carbon and energy sources. All strains require yeast extract or cofactors for growth. The G+C content of the DNA

ranges from 59 to 70 mol%. Strains of the genus *Meiothermus* have been isolated from terrestrial hot springs with neutral to alkaline pH values and fermentors incubated at elevated temperatures. The type species of the genus is *Meiothermus ruber* (12). Two other species of this genus have been described (25).

Description of *Meiothermus ruber* (Loginova, Egorova, Golovacheva, and Seregina 1984) comb. nov. The description of *Meiothermus ruber* below is based on data from references 9, 12, 24, and 25. The strains form red-pigmented colonies and are catalase positive. The optimum growth temperature is about 60 to 65°C; no growth occurs at temperatures above 70°C. In general, starch is not hydrolyzed, and nitrate reduction is very rare. The G+C content of the DNA ranges from 61 to 63 mol%. The type strain is strain ATCC 37498 (American Type Culture Collection, Rockville, Md.).

Description of *Meiothermus silvanus* (Tenreiro, Nobre, and da Costa 1995) comb. nov. The description of *Meiothermus silvanus* below is based on the data of Tenreiro et al. (25). The strains form red-pigmented colonies. Catalase negative. The optimum growth temperature is about 55°C; no growth occurs at temperatures above 65°C. Starch is hydrolyzed, and nitrate is reduced. Xylose and ribitol are utilized for growth. The G+C content of the DNA of the type strain is 63.6 mol%. The type strain is strain VI-R2 (= DSM 9946 [Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany]).

Description of *Meiothermus chliarophilus* (Tenreiro, Nobre, and da Costa 1995) comb. nov. The description of *Meiothermus chliarophilus* is based on the data of Tenreiro et al. (25). The strains of this species form yellow-pigmented colonies. Catalase negative. The optimum growth temperature is about 50°C; no growth occurs at temperatures above 60°C. Starch is hydrolyzed, and nitrate is reduced. Sucrose, trehalose, and cellobiose are used for growth. The G+C content of the DNA of the type strain is 69.9 mol%. The type strain is strain ALT-8 (= DSM 9957).

The exclusion of the low-temperature species from the genus *Thermus* means that the description of this genus must be emended. The description below is based on that of Brock and Freeze (3) and data from references 7, 10, 11, 14, 26, and 28.

Emended description of the genus *Thermus* Brock and Freeze 1969. The cells are 0.5 to 0.8 μm in diameter; the cell length is variable, and short filaments are formed by most strains. Long filaments are produced by a few strains. The cells are gram negative. Colonies are generally yellow pigmented, but nonpigmented strains are also found. Cells are nonmotile. Metabolism is oxidative and aerobic, but the strains of some species grow anaerobically with nitrate and nitrite as terminal electron acceptors. Oxidase positive and catalase positive. The optimum growth temperatures for the species of this genus range from about 65 to 75°C; most species have a maximum growth temperature below 80°C, but some species grow at higher temperatures. The optimum pH is about 7.8. Menaquinone 8 is the major respiratory quinone; ornithine is the principal diamino acid of the peptidoglycan. One major phospholipid and one major glycolipid are the principal polar lipids. Other phospholipids and glycolipids are minor components. The fatty acids are predominantly iso and anteiso branched; iso 3-hydroxy fatty acids are found in some species; and 2-hydroxy fatty acids are vestigial or absent.

Proteins and peptides are hydrolyzed by all strains. Monosaccharides, disaccharides, amino acids, and organic acids are used as sole carbon and energy sources. Many strains require yeast extract or cofactors for growth. The G+C content of the DNA ranges from 60 to 65 mol%. Strains of the genus *Thermus* have been isolated from terrestrial and marine hot springs and

artificial hot water systems with neutral to alkaline pH values. The type species is *T. aquaticus*. *T. filiformis*, *T. scotoductus*, *T. thermophilus*, and *T. Brockianus* are also assigned to this genus.

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