

acteristics of *D. proteolyticus* are almost identical to those described for *D. radiodurans*. The strains of this species are typically 1.0–2.0 µm in diameter. *cis*-Hexadecenoic (C_{16:1ω7c}) and *cis*-heptadecenoic acids (C_{17:1ω8c}) are the predominant fatty acids, each accounting for approximately 27% of the total fatty acid composition. The branched chain fatty acids (C_{17:0 iso}, C_{17:1ω9c iso}) are found, differentiating *D. proteolyticus* from *D. radiodurans* and *D. radiophilus*. The specific epithet implies that this species peptonizes proteins (milk, soy, and gelatin) as was observed by the original isolators (Kobatake et al., 1973). The other mesophilic *Deinococcus* species also show some activity but this species is the most active. Strain CCM 2703 was isolated from irradiated *Llama glama* feces.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: ATCC 35074, CCM 2703, DSMZ 20540, UWO 1056.

GenBank accession number (16S rRNA): Y11331.

6. **Deinococcus radiophilus** Brooks and Murray 1981, 357.^{VP} *ra.di.o'phil.us*. M.L. prefix *radio* radiation; Gr. adj. *philos* loving; M.L. adj. *radiophilus* radiation loving.

Morphological, biochemical, and chemotaxonomic characteristics of *D. radiophilus* are almost identical to those described for *D. radiodurans*. The strains of this species are typically 1.0–2.0 µm in diameter. Hexadecanoic (16:0) and *cis*-hexadecenoic acids (16:1ω7c) are the predominant fatty acids. Branched chain and cyclopropyl fatty acids are not detected.

D. radiophilus is distinguished from *D. radiodurans* by presence of significant levels of 12, 13, and 14 carbon saturated fatty acids. Strain UWO 1055 was isolated from irradiated Bombay duck (*Harpodon nehereus*).

The mol% G + C of the DNA is: 62 (T_m).

Type strain: ATCC 27603, DSMZ 20551, UWO 1055.

GenBank accession number (16S rRNA): Y11333.

7. **Deinococcus radiopugnans** Brooks and Murray 1981, 358.^{VP} *ra.di.o.pug'nans*. M.L. prefix *radio* radiation; L. part. adj. *pugnans* fighting or resisting; M.L. adj. *radiopugnans* radiation resisting.

Morphological, biochemical, and chemotaxonomic characteristics of *D. radiopugnans* are almost identical to those described for *D. radiodurans*. The strains of this species are typically 1.0–2.0 µm in diameter. The branched chain fatty acid (C_{17:1ω9c iso}) predominates. C_{13:0 iso}, C_{15:1 iso}, C_{15:0 iso}, and C_{17:0 iso} are also present, differentiating *D. radiopugnans* from other mesophilic deinococci. Smooth and rough variants as well as variants with less pigment may occur. The 15-carbon, saturated, branched chain fatty acid component may be absent. Nitrate is reduced to nitrite. Strain UWO 293 was isolated from irradiated haddock.

The mol% G + C of the DNA is: 70 (T_m).

Type strain: ATCC 19172, UWO 293.

GenBank accession number (16S rRNA): Y11334.

Order II. Thermales ord. nov.

FRED A. RAINEY AND MILTON S. DA COSTA

Ther.ma'les. M.L. masc. n. *Thermus* type genus of the order, *-ales* ending to denote an order; M.L. fem. pl. n. *Thermales* the order of *Thermus*.

Cells are straight rods of variable length, filaments are also present. **Nonmotile**; flagella are not present. Gram negative. Endospores are not observed. Most strains form **yellow- or red-pigmented colonies**; some strains are nonpigmented. Aerobic with a strictly respiratory type of metabolism, but some strains grow anaerobically with nitrate and nitrite as terminal electron acceptors. Oxidase positive; most strains are catalase positive. **Thermophilic**, with an optimum growth temperature range of ~50–75°C. **Menaquinone 8** is the predominant respiratory quinone; **ornithine** is the principal diamino acid of the peptidoglycan. One major phospholipid is present in all strains, one or two major

glycolipids are also present. Fatty acids are predominantly *iso*- and *anteiso*-branched; branched chain 2-hydroxy and/or 3-hydroxy fatty acids are present in many strains. **Heterotrophic**, some strains may be chemolithoheterotrophic oxidizing sulfur compounds. Isolated from and detected in **hydrothermal areas** with neutral to alkaline pH, also commonly isolated from man-made thermal environments.

The mol% G + C of the DNA is: 57–70.

Type genus: **Thermus** Brock and Freeze 1969, 295, emend. Nobre, Trüper and da Costa 1996b, 605.

Family I. Thermaceae fam. nov.

MILTON S. DA COSTA AND FRED A. RAINEY

Ther.ma'ce.ae. M.L. masc. n. *Thermus* type genus of the family; *-aceae* ending to denote a family; M.L. fem. pl. n. *Thermaceae* the *Thermus* family.

Cells are straight rods of variable length, filaments are also present. **Nonmotile**; flagella are not present. Gram negative. Endospores are not observed. Most strains form **yellow or red-pigmented colonies**, some strains are nonpigmented. Aerobic with a strictly respiratory type of metabolism, but some strains grow anaerobically with nitrate and nitrite as terminal electron acceptors. Oxidase positive; most strains are catalase positive. **Ther-**

philic, with an optimum growth temperature range of ~50–75°C. **Menaquinone 8** is the predominant respiratory quinone; **ornithine** is the principal diamino acid of the peptidoglycan. One major phospholipid is present in all strains, one or two major glycolipids are also present. Fatty acids are predominantly *iso*- and *anteiso*-branched; some strains of the genus *Thermus* possess 3-hydroxy fatty acids; all strains of the genus *Meiothermus* possess

2-hydroxy fatty acids, some strains also possess 3-hydroxy fatty acids. **Heterotrophic**, some strains may be chemolithoheterotrophic oxidizing sulfur compounds. Isolated from and detected in **hydrothermal areas** with neutral to alkaline pH, also commonly isolated from man-made thermal environments.

The mol% G + C of the DNA is: 57–70.

Type genus: **Thermus** Brock and Freeze 1969, 295, emend. Nobre, Trüper and da Costa 1996b, 605.

Genus I. Thermus Brock and Freeze 1969, 295,^{AL} emend. Nobre, Trüper and da Costa 1996b, 605

MILTON S. DA COSTA, M. FERNANDA NOBRE AND FRED A. RAINEY

Ther' mus. Gr. adj. *thermos* hot; M.L. masc. n. *Thermus* to indicate an organism living in hot places.

Straight rods, 0.5–0.8 µm in diameter; the cell length is variable. Short filaments are also formed under some culture conditions. Some strains have a stable filamentous morphology. **Nonmotile**; do not possess flagella. Endospores are not observed. Stain Gram-negative. Most strains form **yellow-pigmented colonies**, some strains are nonpigmented. **Aerobic** with a strictly respiratory type of metabolism, but some strains grow anaerobically with nitrate and nitrite as terminal electron acceptors. Oxidase positive and catalase positive. **Thermophilic**, with an optimum growth temperature of about 70–75°C; most strains have a maximum growth temperature below 80°C, but some strains grow at higher temperatures. The optimum pH is about 7.8. **Menaquinone 8** is the predominant respiratory quinone; **ornithine** is the principal diamino acid of the peptidoglycan. One major phospholipid and one major glycolipid dominate the polar lipid pattern on thin-layer chromatography. Additional phospholipids and glycolipids are minor components. Fatty acids are predominantly **iso- and anteiso-branched**; branched chain 3-hydroxy fatty acids are present in some strains. Proteins and peptides are hydrolyzed by all strains. Starch is hydrolyzed by some strains. Monosaccharides, disaccharides, amino acids, and organic acids are used as sole carbon and energy sources. The utilization of pentoses and polyols is very rare. Most strains require yeast extract or cofactors for growth. Found in **hydrothermal areas** with neutral to alkaline pH, also commonly isolated from man-made thermal environments.

The mol% G + C of the DNA is: 57–65.

Type species: **Thermus aquaticus** Brock and Freeze 1969, 295.

FURTHER DESCRIPTIVE INFORMATION

A number of early 5S rRNA studies of *T. aquaticus* and *T. thermophilus* indicated that these species had no clear phylogenetic relationship to any of the known bacterial groups for which 5S rRNA sequences were available for comparison (Erdmann et al., 1984; Pace et al., 1985; Vandenberghe et al., 1985). The later studies of Bakeeva et al. (1986) and Chumakov (1987) demonstrated a relationship between the genera *Thermus* and *Deinococcus* by 5S rRNA sequence analyses.

The first 16S rRNA studies of members of the genus *Thermus* were in the form of incomplete oligonucleotide catalogs (Hensel et al., 1986) but demonstrated a clear phylogenetic relationship between the genera *Thermus* and *Deinococcus* and concluded that *Thermus* was a member of the *Deinococcus* division as defined by Stackebrandt and Woese (1981). The degree of sequence similarity between full 16S rRNA sequences of *T. aquaticus* and *D. radiodurans* was first reported by Woese (1987) to be 81%, and it was suggested that this value was high enough to place these taxa in the same phylum, but in different subdivisions of the phylum "*Deinococcus-Thermus*". The same author also indicated

that the *Deinococcus-Thermus* lineage was the third deepest eubacterial branch at that time. The deep branching phylogenetic position of the genus *Thermus* was further investigated by Hartmann et al. (1989), who determined the full 16S rRNA sequence of *T. thermophilus* HB8. As new deep branching procaryotic lineages have been discovered, both as organisms isolated in pure culture or as environtaxa detected by molecular approaches in the environment, the order of branching within the procaryotic phylogenetic tree has changed, but the *Deinococcus-Thermus* lineage still represents a deep branching phylum (Rainey et al., 1997). The relationship of the genus *Thermus* and the genus *Deinococcus* was also demonstrated by Embley et al. (1993), and the study provided additional complete 16S rDNA sequences for *Thermus* strains VI-7a (erroneously designated Vi17) (EMBL Accession No. Z15061), YS38 (EMBL Accession No. Z15062), SPS-14 (EMBL Accession No. Z15060), and *Meiothermus (Thermus) ruber* ATCC 35948 (EMBL Accession No. Z15059).

The fine structure of the strains of the genus *Thermus* shows that the cells have an envelope consisting of a cytoplasmic membrane with a simple outline, a cell wall with an inner, electron-dense thin layer presumably representing the peptidoglycan connected by irregularly spaced invaginations to an outer corrugated "cobble-stone" layer (Brock and Edwards, 1970; Pask-Hughes and Williams, 1975; Kristjánsson et al., 1994) (Fig. B4.5). Unusual morphological structures, commonly called "rotund bodies", are occasionally seen in many strains by phase-contrast and transmission electron microscopy. These structures consist of several cells bound longitudinally by a common external layer of the cell envelope enclosing a large space between the cells (Brock and Edwards, 1970; Kraepelin and Gravenstein, 1980; Becker and Starzyk, 1984). The type strain of *Thermus filiformis* and one unclassified strain isolated from New Zealand (D. Cowan, personal communication) have, in contrast to other strains, a stable filamentous morphology and do not form rod-shaped cells on solid or in liquid media. *T. filiformis* forms septate cells within a continuous outer sheath, although published electron micrographs do not show if the cells separate completely or not. There also appears to be an additional layer surrounding the envelope of the type strain of *T. filiformis* (Hudson et al., 1987b). Several other strains form long filamentous cells resembling those of the type strain of *T. filiformis* when specific D-amino acids are added to the culture medium (Janssen et al., 1991). Most of these strains originated from New Zealand and some belong to *T. filiformis*; however, *T. scotoductus* strain NH also formed filamentous cultures, but *T. aquaticus* YT-1 and *T. thermophilus* HB8 did not.

A crystalline surface protein layer (S-layer) has been identified in *T. thermophilus* strains HB8 and HB-27. The S-layer of strain HB8 is composed of a major protein with a molecular weight of 100 kDa, designated P100 (Berenguer et al., 1988; Castón et al.,

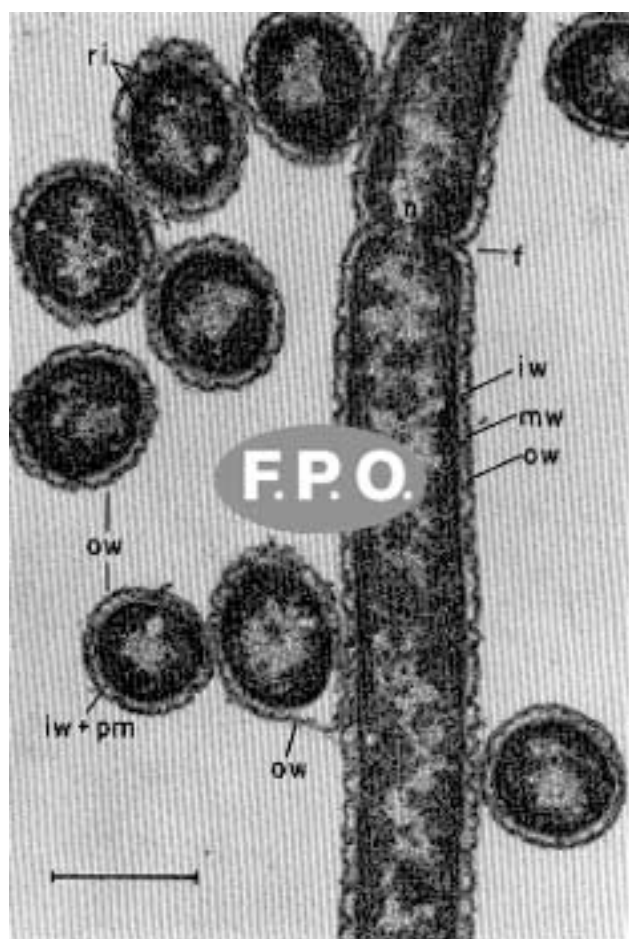


FIGURE B4.5. Transmission electron micrograph of *Thermus aquaticus* YT-1 showing the nucleoplasm (*n*) surrounded by numerous ribosomes (*ri*). The cell envelope comprises the plasma membrane (*pm*) and wall exhibiting an outer dense layer (*ow*), a middle light zone (*mw*), and a thin inner dense layer (*iw*). Note cell division furrow (*f*). Bar = 0.5 μ m. (Reprinted with permission from T.D. Brock and M. Edwards, *Journal of Bacteriology* 104: 509–517, 1970, ©American Society of Microbiology.)

1988; Faraldo et al., 1988). This protein forms oligomeric complexes stabilized by Ca^{2+} that interact with the peptidoglycan. The gene that codes for this protein has been cloned and sequenced, and mutants unable to produce P100 have been produced (Faraldo et al., 1992; Olabarriá et al., 1996b). Strain HB-27 has a similar S-layer, but the major protein has a molecular weight of 95 kDa (Fernández-Herrero et al., 1995). Mutants defective for the production of P100 and P95 grow slower than the wildtype strains and produce cells with altered morphology. For example, these mutants frequently form structures composed of groups of cells bound by a common envelope reminiscent of the “rotund bodies” seen in many wildtype strains of the genus, but no conclusions are yet possible on the relationship between both structures. Other outer envelope proteins also form an S-layer-like array in strain HB8 and may be minor S-layer components, but little is yet known about the function of these proteins (Olabarriá et al., 1996a; Fernández-Herrero et al., 1997).

The peptidoglycan of the strains of the genus *Thermus* contain L-ornithine as the diamino acid and glycylglycine as the interpeptide bridge (Merkel et al., 1978a; Pask-Hughes and Williams, 1978); this peptidoglycan composition is consistent with the A3 β

murein type of Schleifer and Kandler (1972) that is also found in the genera *Meiothermus* and *Deinococcus* (Hensel et al., 1986; Embley et al., 1987; Sharp and Williams, 1988). The structure of the peptidoglycan of *T. thermophilus* HB8 was recently examined by Quintela et al. (1995), who found that the N-terminal glycine was substituted by phenylacetic acid in a significant proportion of the interpeptide bridges.

The major respiratory quinone of strains of the genus *Thermus* is menaquinone 8 (MK-8) (Collins and Jones, 1981; Hensel et al., 1986; Williams, 1989). The presence of ornithine and MK-8 corroborate the phylogenetic interpretation that the genera *Thermus*, *Meiothermus*, and *Deinococcus* are related to each other, although the species of the *Thermus/Meiothermus* line of descent have few other characteristics in common with the species of the genus *Deinococcus*.

The polar lipid composition of the species of *Thermus* consists of one major phospholipid, designated phospholipid 2 (PL-2), and one major glycolipid, designated glycolipid 1 (GL-1), which comprise between 80% and 95% of the total polar lipid phosphorus and carbohydrate. Other minor polar lipids, namely phospholipid 1 (PL-1) and glycolipid 2 (GL-2), are also detected by thin-layer chromatography in most strains of the genus *Thermus* (Pask-Hughes and Shaw, 1982; Prado et al., 1988; Donato et al., 1990). The major glycolipid of several strains has been identified as a diglycosyl-(*N*-acyl)glycosaminyl-glucosyldiacylglycerol, which contains three hexose residues and one *N*-acylated hexosamine, giving a hexose/hexosamine/glycerol ratio of approximately 3:1:1. Depending on the strain, the polar head group of GL-1 contains *N*-acylglucosamine or *N*-acylgalactosamine, three glucose residues, two glucose residues plus one galactose, or one glucose plus two galactose residues (Oshima and Yamakawa, 1974; Pask-Hughes and Shaw, 1982; Prado et al., 1988; Wait et al., 1997). The innermost hexose bound to glycerol always appears to be glucose. The terminal galactose, present in GL-1 of several strains, such as *T. thermophilus* strain HB8, is in the rare furanose configuration instead of the more common pyranose configuration (Oshima and Yamakawa, 1974; Wait et al., 1997). In contrast to the usual polar head group, GL-1 from *Thermus aquaticus* strain YS 004 was recently shown to have *N*-acetylgalactosamine in place of the subterminal hexose residue resulting in a hexose/hexosamine/glycerol ratio of 2:2:1 (Carreto et al., 1996).

One exception to the canonical polar lipid pattern of the strains of the genus *Thermus* is found in one colony variant of *Thermus scotoductus* strain X-1 (ATCC 27978). This strain produces two colony types, designated t1 and t2 (Tenreiro et al., 1995b). The polar lipid composition of colony type t2 is typical of most *Thermus* strains, consisting of the major phospholipid (PL-2), the major glycolipid (GL-1), and traces of a minor glycolipid (GL-2), whereas in colony type t1 GL-2 is the major glycolipid and only trace amounts of GL-1 are detected (Tenreiro et al., 1995b). Glycolipid 2 (GL-2) of this strain, and the same minor glycolipid of *T. oshimai* strain SPS-11, was identified as a truncated version of GL-1 lacking the terminal hexose (Wait et al., 1997). The structure of the major phospholipid (PL-2) of the genus *Thermus* has never been identified, but recently it was shown to be identical to the major phospholipid found in *Deinococcus radiodurans*, reinforcing the phylogenetic interpretation that the *Thermus/Meiothermus* line of descent is related to the species of the genus *Deinococcus* (Hensel et al., 1986; Huang and Anderson, 1989; Rainey et al., 1997; Wait et al., unpublished results).

Unexpectedly, terminally and subterminally branched long chain diols, identified as 16-methylheptadecane-1,2-diol and 15-methylheptadecane-1,2-diol, were detected as major components of GL-1 and GL-2 of *T. scotoductus* X-1 and *T. filiformis* Tok A4 (Wait et al., 1997). Long chain diols had only been detected in *Thermomicrobium roseum* where they appeared to be the exclusive backbone structure of the polar lipids, which apparently lack the normal glycerol-based lipids (Pond et al., 1986; Pond and Langworthy, 1987). In the species of *Thermus*, long chain diol-based lipids never completely replace the normal glycerolipids, and the polar head group of both structural types of glycolipids is identical. The levels of diols vary with the strain, and some strains have only vestigial amounts. Long chain diols have also been identified in the glycolipids of one of the four species of the genus *Meiothermus* (Ferreira et al., 1999). The presence of long chain diols in *Thermomicrobium roseum*, *Thermus* and *Meiothermus* species, in conjunction with 16S rDNA phylogenies, leads to the hypothesis that these lipid backbones reflect a distant, but definite relationship between the *Deinococcus-Thermus* phylum and the Green Non-sulfur Bacteria (Weisburg et al., 1989; van de Peer et al., 1994).

Iso- and anteiso-branched $C_{15:0}$ and $C_{17:0}$ fatty acids are the predominant acyl chains of the strains of the genus *Thermus*. Straight chain saturated fatty acids and unsaturated branched chain fatty acids are minor components at the optimum growth temperature in the vast majority of the strains (Ray et al., 1971; Donato et al., 1990; Nobre et al., 1996a); nevertheless, straight chain $C_{16:0}$ reaches levels of about 20% of the total fatty acids in *T. thermophilus* AT-62 (Nobre et al., 1996a). Iso-branched fatty acids predominate over anteiso-branched fatty acids in the vast majority of the strains at the optimum growth temperature (Pask-Hughes and Shaw, 1982; Prado et al., 1988; Nobre et al., 1996a), although the type strain of *T. filiformis* possesses between 60 and 70% anteiso-fatty acids at all growth temperatures examined (Donato et al., 1990). Some strains of the genus *Thermus*, namely most strains of *T. aquaticus* and the type strain of *T. filiformis*, also contain moderate levels of branched chain 3-hydroxy fatty acids. 3-Hydroxy fatty acids are exclusively amide-linked to the galactosamine present in the glycolipids, but are never present in the strains where glucosamine replaces galactosamine (Carreto et al., 1996; Ferreira et al., unpublished results).

The majority of the isolates of the species of the genus *Thermus* form yellow-pigmented colonies, although the color varies considerably from deep yellow to very pale yellow. Many strains, isolated primarily from man-made environments that are maintained dark, are nonpigmented, although yellow-pigmented strains can also be isolated from these environments. Several nonpigmented *Thermus* strains have been isolated from abyssal hot springs, but even here, some isolates are yellow-pigmented (Marteinsson et al., 1995). Several novel carotenoids, designated thermozeaxanthins and thermobiszeaxanthins, were identified in *Thermus* strain HB-27 by Yokoyama et al. (1995, 1996), who also established the biosynthetic pathway of these compounds. In some strains, pigmentation appears to be an unstable characteristic because spontaneous nonpigmented mutants are frequently produced that never revert to yellow pigmentation. Moreover, the consistent isolation of nonpigmented strains from dark environments leads to the hypothesis that the yellow pigmentation of *Thermus* is favored in natural thermal areas exposed to sunlight where carotenoids would protect the cells from sunlight, whereas nonpigmented strains would have a selective advantage in non-illuminated environments because the production of ca-

rotenoids would be energetically expensive. This hypothesis has its roots in the observation that the nonpigmented strain X-1 had a higher growth rate than *T. aquaticus* YT-1 (Ramaley and Hixson, 1970). Recently, a gene cluster involved in the synthesis of carotenoids in strain HB-27 was located on a large plasmid, designated pTT27. The same authors reported that carotenoid over-producing mutants were more resistant to UV-irradiation than either the wild-type strain or the carotenoid under-producing mutants (Hoshino et al., 1994; Tabata et al., 1994). Nevertheless, the carotenoid over-producing mutants grew slower at supraoptimal temperatures than the wild-type strain. These results could explain why nonpigmented strains predominate in dark environments over pigmented strains, whereas pigmented strains constitute the predominant isolates in hot springs exposed to sunlight. The presence of carotenogenic genes on plasmids could also explain the high frequency of pigmentation loss in some strains due to curing of the plasmids under laboratory conditions.

The cardinal growth temperatures of the species of the genus *Thermus* range between about 45°C and 82–83°C. However, only a few strains, all closely related to *T. thermophilus* HB8, are capable of growth at 80°C or above (Manaia et al., 1994). The majority of the strains of the genus *Thermus* have, in fact, a maximum growth temperature slightly below 80°C (Brock and Freeze, 1969; Hudson et al., 1989; Santos et al., 1989; Manaia and da Costa, 1991). The optimum growth temperature of all strains is in the vicinity of 70°C, but in some strains the optimum growth temperature could be as high as 75°C. The type strain of *T. scotoductus*, however, was reported to have an optimum growth temperature of 65–70°C, and a maximum growth temperature of 73°C (Kristjánsson et al., 1994).

The strains of the species of the genus *Thermus* have a respiratory metabolism and are aerobic. Many strains are capable of growth under anaerobic conditions using nitrate as the electron acceptor; some strains also reduce nitrite (Munster et al., 1986; Hudson et al., 1989; Santos et al., 1989). None of the strains, however, appears to be capable of carrying out fermentation. The strains of the genus *Thermus* possess phosphofructokinase (Yoshida et al., 1971; Yoshida, 1972), fructose-1,6-bisphosphate aldolase (Freeze and Brock, 1970), glyceraldehyde-3-phosphate dehydrogenase (Fujita et al., 1976; Harris et al., 1980; Tanner et al., 1996), phosphoenolpyruvate carboxylase (Sundaram and Bridger, 1979), enolase (Barnes and Stellwagen, 1973), and lactate dehydrogenase (Machida et al., 1985), and it is presumed that these species have a complete Embden-Meyerhoff pathway for the initial catabolism of hexoses. Several enzymes of the tricarboxylic acid cycle (Nishiyama et al., 1986; Miyazaki, 1996), the glyoxylate bypass (Degryse and Glansdorff, 1976), and several components of the respiratory chain, such as menaquinone 8, NADH-quinone oxidoreductase (Yano et al., 1997), cytochromes ba_3 (Zimmermann et al., 1988; Oertling et al., 1994; Keightley et al., 1995), and cytochrome c_{552} (Soulimane et al., 1997), have been identified and characterized in these organisms. A V-type ATPase, found in *Archaea*, eucaryotic endomembrane systems, and some bacteria, was initially detected in *T. thermophilus* HB8 by Yokoyama et al. (1990), but later Radax et al. (1998) found that some strains, namely the type strains of *T. filiformis* and *T. scotoductus*, possessed an F-type ATPase found in other bacteria, mitochondria, and chloroplasts. Conversely, *T. aquaticus* TY-1, *T. thermophilus* HB-27, and *Meiothermus chliarophilus* possess V-type ATPase. The presence of two different types of ATPases in species of the same genus is not clearly understood,

although the authors speculate that horizontal gene transfer from other organisms may be responsible for the presence of the V-type ATPase in some strains. Alternatively, a common ancestor may have had both enzymes and only one or the other was retained during evolution.

All strains examined are catalase positive, in contrast to the strains of several species of the genus *Meiothermus* that do not have this enzyme under the conditions examined (Tenreiro et al., 1995b; Chung et al., 1997). A manganese superoxide dismutase has also been identified in *T. thermophilus* (Stallings et al., 1985; Lah et al., 1995). All strains are chemooorganotrophic and are capable of growth on amino acids, peptides and proteins, organic acids, and simple and complex carbohydrates (Table B4.4). With the exception of some strains of *T. thermophilus*, most other strains are not able to assimilate pentoses (Williams and da Costa, 1992). Recent results show that many of the strains appear to be able to hydrolyze Tween 20 to Tween 80, as well (Chung et al., unpublished results).

Thermus medium and Degryse medium 162 are the most frequently used media for growth of the strains of this genus (Degryse et al., 1978; Brock, 1984; Williams and da Costa, 1992). Both media contain yeast extract, and most strains require some cofactors for growth provided by this supplement, although yeast extract can be replaced by a complex mixture of amino acids, vitamins, and nucleotides (Sharp and Williams, 1988). Some strains, namely strain HB8, have been grown in a minimal salts medium containing a carbon source, biotin, and thiamine (Tanaka et al., 1981); strain YT-1 was grown in a minimal medium containing a carbon source, biotin, thiamine, and nicotinic acid (Yeh and Trela, 1976), whereas strain ZO5 has been grown in an inorganic medium without co-factors and containing pyruvate as the carbon source (van de Castele et al., 1997). Some caution is necessary when attempting to examine the growth of *Thermus* strains in these media, because it can be very difficult to reproduce.

To date, the genomic structure of the strains of the genus *Thermus* has not been extensively studied. A physical map of the chromosome of strain HB8 has been constructed using pulsed-field gel electrophoresis (PFGE) of macrorestriction fragments, and several genes have been located on the chromosome of this strain that is estimated to be about 1.74 Mb (Borges and Bergquist, 1993); the physical map of strain HB-27 was also constructed using several restriction endonucleases and was estimated at 1.82 Mb (Tabata et al., 1993). The chromosome size of several other strains of the genus *Thermus* was later estimated to range from about 1.8 to 2.5 Mb using PFGE (Rodrigo et al., 1994; Moreira et al., 1997).

The first plasmids from strains of the genus *Thermus* were isolated by Hishinuma et al. (1978), but plasmids have now been detected from the majority of the strains examined. For example, Munster et al. (1985) found plasmids in about 60% of the isolates from Yellowstone National Park, and Moreira et al. (1995) detected plasmids in about 80% of the strains examined. Plasmid pTT1 (plasmid pTT8 of Hishinuma et al., 1978) was the first to be characterized (Eberhard et al., 1981). Two other plasmids, one from *T. thermophilus* AT-62 and the other from *T. thermophilus* HB8, respectively designated pTF62 and pVV8, were also characterized by restriction endonucleases (Vasquez et al., 1981, 1983). Plasmid pVV8 is associated with cell aggregation of strain HB8 in rich medium (Mather and Fee, 1990), whereas, as stated above, plasmid pTT27 encodes carotenogenic genes in strain HB-

27 (Hoshino et al., 1994; Tabata et al., 1994). All other plasmids appear to be cryptic at this time (Raven, 1995).

The discovery that *Thermus* strains are naturally transformable, coupled to the ease of growth of these organisms in solid and liquid media, renders them excellent candidates for the development of thermophilic host-vectors systems (Koyama et al., 1986). In particular, strain HB-27, devoid of the *TaqI* restriction system found in many strains, has been used extensively as host for genetic manipulation (Koyama et al., 1986; Koyama and Furukawa 1990; Lasa et al., 1992b; Mather and Fee, 1992; Fernández-Herrero et al., 1995). Other strains, such as SPS-7 and SPS-10 are also naturally transformable (Peist, Marugg and da Costa, unpublished results). The plasmid vector pYK105 described by Koyama et al. (1989) constituted the first of a series of *Thermus*-specific and *Thermus*-*E. coli* shuttle vectors that have been constructed by several groups using *trpB* (Koyama and Furukawa, 1990), β -galactosidase (Koyama et al., 1990), or kanamycin resistance as selection markers (Lasa et al., 1992a; Mather and Fee, 1992; Wayne and Xu, 1997). For their replication in thermophiles, these vectors rely on cryptic *Thermus* vectors or on entire cryptic plasmids. Integration vectors have also been developed that have proved useful for stable expression and gene analysis (Lasa et al., 1992a; Fernández-Herrero et al., 1995; Weber et al., 1995; Tamakoshi et al., 1997).

Bacteriophage have also been found in strains of the genus *Thermus*, but have not been investigated in detail (Raven, 1995). The first *Thermus* phages were isolated by Sakaki and Oshima (1975) from *T. thermophilus* HB8 and other Japanese strains. Phage ϕ YS40 grew within the temperature range of *T. thermophilus*, where it formed clear plaques indicative of complete lysis of the culture. This phage has an icosahedral head and a tail terminated by a plate and tail fibers. The genetic material is double-stranded DNA of about 175 kb, and the mol% G + C of the DNA was estimated at 35%, unlike the mol% G + C value of the *T. thermophilus* DNA, which is about 64%. Other bacteriophage from strains of the genus *Thermus*, namely ϕ YB10, also have low G + C content, and some type of unknown thermostabilization mechanism must be necessary to prevent strand separation of the DNA of these viruses at high temperatures (Raven, 1995).

The exploitation of thermostable enzymes is a major goal of biotechnology; however, the main thrust is directed to the utilization of enzymes from organisms that grow at or near the boiling point of water, leaving out organisms in the growth range of the strains of the genus *Thermus*. Despite all the interest in the biotechnological exploitation of hyperthermophilic organisms, *T. aquaticus* YT-1 produces one of the most valuable enzymes in scientific and economic terms. This enzyme is, of course, *Taq* polymerase. This enzyme made the polymerase chain reaction (PCR) possible, and even other polymerases from hyperthermophiles, namely from *Thermococcus litoralis*, have not replaced the *Taq* polymerase.

Other useful enzymes have been isolated from *Thermus* strains, and some have been cloned in *E. coli*, but these have never really found a niche in the enzyme market because other, more stable enzymes are becoming available. Several proteases, such as Aqualysin I, Caldolysin, Caldolase, and PreTaq, have been purified and characterized, but there is very little biotechnological interest in them (Cowan and Daniel, 1982a, b; Matsuzawa et al., 1988; Saravani et al., 1989; Peek et al., 1992). An amylase has also been characterized from *T. filiformis* strain Ork A2 (Egas et al., 1998), but other more thermostable amylases from *Archaea* are known

TABLE B4.4. Characteristics of the type strains of the species of the genus *Thermus*^a

Characteristic	1. <i>T. aquaticus</i> YT-1 ^b	2. <i>T. brockianus</i> YS38 ^c	3. <i>T. filiformis</i> Wai33 A1 ^b	4. <i>T. oshimai</i> SPS-17 ^b	5. <i>T. scotoductus</i> SE-1 ^d	6. <i>T. thermophilus</i> HB8 ^b
Pigmentation	Deep yellow	Light yellow	Deep yellow	Light yellow	Colorless	Yellow
Colonies	Compact	Spreading	Compact	Compact	Compact	Compact
Optimum temperature, °C	70	70	70	70	65–70	70
Growth at 80°C	—	—	—	—	—	+
Growth in 1% NaCl	+	nd	—	—	nd	+
Growth in 3% NaCl	—	—	—	—	—	+
Anaerobic growth with NO ₃ [—]	—	+	—	+	+	—
Presence of:						
Oxidase	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
DNase	+	nd	—	—	nd	+
α-Galactosidase	+	nd	+	+	nd	+
β-Galactosidase	—	nd	+	+	nd	+
Hydrolysis of:						
Elastin	+	nd	—	+	nd	—
Fibrin	+	nd	—	+	nd	—
Casein	+	—	—	+	nd	+
Gelatin	+	—	+	+	—	+
Starch	+	—	—	—	—	—
Arbutin	—	nd	+	+	nd	+
Esculin	—	nd	+	+	nd	—
Utilization of:						
D-Glucose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
L-Rhamnose	—	nd	—	—	nd	—
L-Arabinose	—	nd	—	—	nd	—
D-Xylose	—	—	—	—	—	+
D-Galactose	+	+	+	+	nd	—
D-Mannose	+	nd	—	+	nd	+
D-Cellobiose	+	nd	—	—	nd	+
Lactose	+	+	+	+	nd	—
Sucrose	+	nd	+	+	nd	+
D-Trehalose	+	nd	+	+	nd	+
D-Melibiose	+	+	+	+	nd	+
D-Raffinose	+	nd	+	+	nd	—
Dextrin	—	nd	—	—	nd	—
Salicin	—	nd	+	+	nd	—
D-Mannitol	—	nd	—	—	nd	—
D-Sorbitol	—	nd	—	—	nd	—
myo-Inositol	—	nd	—	—	nd	—
Glycerol	—	nd	—	—	+	—
Pyruvate	+	+	+	+	+	+
Acetate	+	nd	+	+	—	+
Citrate	—	nd	—	—	nd	—
Lactate	—	nd	—	—	nd	—
Malate	+	nd	+	+	nd	—
Succinate	+	nd	+	+	—	—
Arginine	+	nd	+	+	nd	—
Proline	+	+	+	+	+	+
Ornithine	+	nd	+	+	nd	—
Serine	+	nd	—	—	nd	+
Acetamide	—	nd	—	—	nd	—
Mol% G + C	64	63	65	63	65	65

^aSymbols: +, positive result; —, negative result or no growth; nd, not determined.^bResults from Manaia and da Costa (1991).^cResults from Manaia et al. (1994).^dResults from Kristjánsson et al. (1994).

and are being examined for the conversion of starch to high glucose syrups.

Strains of the genus *Thermus* are ubiquitous in natural hydrothermal areas with neutral to alkaline pH; the first isolates of the genus *Thermus* were obtained from hydrothermal areas in Yellowstone National Park and Pacheteau's Calistoga in California by Brock and Freeze (1969). Isolates were later recovered from

many inland hydrothermal areas in Japan (Yoshida and Oshima, 1971; Saiki et al., 1972; Taguchi et al., 1982), Iceland (Pask-Hughes and Williams, 1977; Kristjánsson and Alfredsson 1983; Alfredsson et al., 1985; Hudson et al., 1987a), New Zealand (Hudson et al., 1986), New Mexico (Hudson et al., 1989), the island of São Miguel in the Azores, continental Portugal (Santos et al., 1989; Manaia and da Costa, 1991), the Australian Artesian Basin

(Denman et al., 1991), and the Kamchakta Peninsula (R. Sharp, personal communication). Furthermore, Yellowstone National Park continues to be the source of many more *Thermus* isolates (Munster et al., 1986; Hudson et al., 1989). In addition to continental hydrothermal areas where the concentration of sodium chloride is generally very low, strains of the genus *Thermus* have also been isolated from shallow marine hot springs off the coast of Iceland (Kristjánsson et al., 1986), on beaches on the island of Fiji (Hudson et al., 1989), and the island of São Miguel in the Azores (Manaia and da Costa, 1991), along the coast north of Naples, Italy (da Costa, unpublished results), and the island of Monserrat in the Caribbean (N. Raven, personal communication). Recently, isolates of the genus *Thermus* were also obtained from the most unique type of thermal environment—abyssal geothermal areas in the Mid-Atlantic Ridge and in the Guaymas Basin, Gulf of California, at depths of 3500 and 2000 m, respectively (Marteinsson et al., 1995), some of which belong to *T. thermophilus* (Marteinsson et al., 1999).

The isolation of strains of this genus is not restricted to natural hydrothermal areas. In fact, the isolation of strains from man-made environments was simultaneous with the description of *Thermus aquaticus* by Brock and Freeze (1969), who included yellow-pigmented strains isolated from cold and hot water taps at Indiana University, where no hot springs exist. Later, nonpigmented *Thermus* strains were isolated from hot water taps (Pask-Hughes and Williams, 1975; Stramer and Starzyk, 1981), domestic and industrial hot water systems (Brock and Boylen, 1973), and thermally polluted streams (Ramaley and Hixson, 1970; Brock and Yoder, 1971; Ramaley and Bitzinger, 1975; Degryse et al., 1978). In addition to these environments, and perhaps unexpectedly, strains of the genus *Thermus* have also been isolated from self-heating (thermogenic) compost piles in Switzerland and Germany (Beffa et al., 1996; G. Antranikian, personal communication).

In natural environments, these organisms are generally isolated from hydrothermal areas where the water temperature ranges from 55 to 70°C and the pH ranges from 5.1 to 10.5 (Kristjánsson and Alfredsson, 1983; Munster et al., 1986; Hudson et al., 1989; Santos et al., 1989). However, isolates have occasionally been recovered from water with temperatures as high as 95°C, and pH values as low as 3.9 (Kristjánsson and Alfredsson, 1983; Hudson et al., 1986; Munster et al., 1986). The recovery of strains of the genus *Thermus* from geothermal sites with low pH and very high temperatures, as well as from cold water sources, is believed to be due to dispersal from environments that these strains actually colonize (Williams and da Costa, 1992; Alfredsson and Kristjánsson, 1995).

Most of the isolates of the genus *Thermus* originate from terrestrial hot springs venting fresh water because these are more common than marine geothermal areas and because they are sampled more frequently. Some shallow marine hot springs contain less total salts than the surrounding sea (Kristjánsson et al., 1986), but other marine hot springs contain concentrations of salts similar to those of sea water, and all isolates from marine hot springs are, to our knowledge, halotolerant (Manaia and da Costa, 1991; Tenreiro et al., 1997). These organisms have higher growth rates in *Thermus* medium without added NaCl, but grow in this medium containing 3–4% NaCl, whereas the vast majority of the strains of *Thermus* isolated from inland hydrothermal areas will not grow at salinities above 1% NaCl (Kristjánsson et al., 1986; Hudson et al., 1989; Santos et al., 1989; Manaia and da Costa, 1991; Manaia et al., 1994). The halotolerant strains from

inland hot springs, namely strains HB8, AT-62, GK-24 from Japan, strain B from Iceland, and strain RQ-1 from the island of São Miguel in the Azores, are closely related to one another and to the isolates from marine hot springs (Manaia et al., 1994). However, the inland sites in Japan from which halotolerant strains were isolated are reported to be saline, and inland saline hot springs are also found in Iceland (Waring, 1965; Alfredsson and Kristjánsson, 1995). Nevertheless, the site on the island of São Miguel from which halotolerant strains RQ-1 and RQ-3 were isolated has a low salinity (Santos et al., 1989; Veríssimo et al., 1991), but is only about 5 km from shallow marine hot springs that have yielded similar halotolerant *Thermus* strains and *Rhodothermus marinus* (Manaia and da Costa, 1991; Nunes et al., 1992). On the other hand, none of the strains isolated from the Furnas area, about 1 km further inland, was halotolerant. These observations lead to the view that halotolerant isolates of *Thermus* are, as expected, primarily marine organisms that may occasionally colonize inland hot springs of low salinity. Moreover, all the halotolerant strains studied belong to the species *T. thermophilus*, or to other, closely related species that remain unclassified (Manaia and da Costa, 1991; Chung et al., unpublished results).

Studies based on whole DNA–DNA hybridization values and 16S rDNA sequence similarities of a large number of isolates show that some of the species appear to have a wide distribution in geothermal and man-made environments, whereas others have a restricted distribution. For example, *T. brockianus* strains have been isolated from Yellowstone National Park, and Iceland (Hudson et al., 1987a; Saul et al., 1993; Williams et al., 1995; Chung et al., unpublished results); strains belonging to *T. thermophilus*, or to species closely related to *T. thermophilus*, have been isolated from marine and inland hot springs in Japan, Iceland, the island of São Miguel, the island of Fiji, Naples, abyssal black smokers, and thermogenic composts; and strains of *T. scotoductus* have been isolated from Iceland, the Azores, continental Portugal, a thermally polluted stream in the United States, hot springs in New Mexico, and hot tap water in London. However, strains of *T. aquaticus* have only been isolated from Yellowstone National Park, and *T. filiformis* has been isolated only from New Zealand. Strains of *T. brockianus* and *T. aquaticus* are easily isolated from the same springs in Yellowstone National Park, and the absence of isolates of the latter species from other hydrothermal areas cannot be due to the difficulty in isolating strains of *T. aquaticus*. Moreover, many strains of *T. filiformis*, based on high DNA–DNA hybridization values and 16S rDNA sequence similarity, are known, but all originate from New Zealand (Georganta et al., 1993; Saul et al., 1993; Chung et al., unpublished results). In fact, with the exception of strains closely related to *T. thermophilus* from the South Island of New Zealand, strains belonging to other species have not been isolated from New Zealand. These results indicate that some species of the genus *Thermus* appear to be restricted to a limited number of sites or geothermal areas, whereas others are frequently isolated from widely dispersed hydrothermal areas. The lack of extensive sampling and characterization of isolates, and the lack of culture-independent phylogenetic studies of samples, is the most likely explanation for the inability to recover strains of some species from widely separated geothermal areas, but it is equally possible that physical, chemical, and biological parameters of the hydrothermal areas restrict the distribution of the strains of some species. It is interesting to note that only strains closely related to *T. brockianus* and *T. aquaticus* continue to be found in Yellowstone National Park. For example, Nold and Ward (1995) performed 16S rDNA

sequence analysis on a number of isolates from Octopus Spring in Yellowstone National Park; one isolate belonged to *T. Brockianus*, whereas the other strain was closely related to *T. aquaticus* YT-1 and to strain YSPID isolated several years earlier from the same area by Hudson et al. (1989). More intriguing are the recent results of Hugenholtz et al. (1998b), who used culture-independent phylogenetic methods to analyze the microbial community of Obsidian Pool in Yellowstone National Park. The only *Thermus* rRNA gene types found were all closely related to strain YSPID. Sequences closely related to *T. aquaticus* YT-1 or *T. Brockianus* YS38 were not found. These results indicate that, at least in some hot springs, only strains of one species can be detected, which somehow may be related to unknown factors governing colonization of the geothermal sites.

ENRICHMENT AND ISOLATION PROCEDURES

Brock and Freeze (1969) isolated the original strains of *Thermus aquaticus* on Castenholz D basal salts medium (Castenholz, 1969) supplemented with yeast extract (1.0 g/l) and tryptone (1.0 g/l). This medium has been used in most studies to isolate and to grow strains of the genus *Thermus*, and has been called Castenholz medium D for *Thermus* (Hudson et al., 1986) or simply *Thermus* medium (Munster et al., 1986; Williams and da Costa, 1992). Medium 162 of Degryse et al. (1978) is also commonly used to isolate and grow these organisms. The basal salts medium 162 is slightly different from Castenholz D medium, and generally 2.5 g/l of yeast extract and 2.5 g/l of tryptone are used. Both media are, nevertheless, adequate for the growth of all known strains of this genus.

The growth of the majority of the strains of *Thermus* is inhibited by levels of organic nutrients higher than about 1.0%. Hexoses are particularly inhibitory, apparently because of acidification of the medium. Some strains, particularly those closely related to *T. thermophilus* HB8, are more resistant to organic nutrients of the culture medium and are frequently grown in a medium containing (per liter water) Trypticase or polypeptone, 8.0 g; yeast extract, 4.0 g; and NaCl, 2.0 g (Oshima and Imahori, 1974). The review by Sharp et al. (1995) gives an extensive list of media, and their composition, used to grow the strains of this genus for several purposes, and should be consulted.

Most isolates of the genus *Thermus* have been obtained by enrichment in *Thermus* medium or in medium 162. Water or biofilm samples are inoculated into liquid medium and incubated at 70–75°C for 2–3 days. Turbid cultures are spread on the same medium solidified with agar (2–3%) and incubated at the same temperature until yellow or nonpigmented colonies appear and can be isolated. Alternatively, samples are directly spread on solid media (Hudson et al., 1986, 1989; Munster et al., 1986; Santos et al., 1989). Membrane filtration methods have also been used extensively for the isolation of strains of the genus *Thermus* and offer the advantage of recovering a larger number of different colonial types and minor populations, as compared to liquid enrichments that tend to select clones that grow better in the media used or that constitute the major populations of the samples. The membrane filtration method can be used with water or biofilms that have been macerated and shaken vigorously with small amounts of water from the same hot spring or phosphate-buffered saline (Kristjánsson and Alfredsson, 1983; Kristjánsson et al., 1986; Manaia and da Costa, 1991). Adequate volumes of the samples, or dilutions, are filtered through cellulose nitrate or acetate membrane filters (47 mm dia., 0.22–0.45 µm pore

size). The filters are placed onto the surface of plates of *Thermus* medium, or a similar, low nutrient medium solidified with 2–3% agar. The plates are then inverted, wrapped in plastic film, and incubated for 2–7 days at temperatures ranging from 70 to 75°C. Yellow or colorless colonies can easily be observed and picked for further purification. Other organisms may also be isolated under these conditions, namely aerobic spore-formers, although few of these are isolated at temperatures above 70°C. Identification of isolates of the genus *Thermus* can be easily accomplished by assessing the presence of cytochrome oxidase and catalase, cell morphology, fatty acid composition, and the characteristic polar lipid composition (Prado et al., 1988; Donato et al., 1990; Nobre et al., 1996a).

MAINTENANCE PROCEDURES

All strains grow well on *Thermus* medium or Degryse medium 162 solidified with 2% or 3% agar. Other solidifying agents such as Gelrite are not necessary at temperatures of 70–75°C. Nevertheless, glass plates may be useful at temperatures above 70°C because some plastic Petri plates can become deformed at high temperatures. Sometimes colonies of thermophilic spore-formers derived from the agar appear during cultivation of organisms at 70°C. Correct autoclaving procedures must be followed to insure that the medium is thoroughly brought to autoclaving temperature for the specified time. In our experience, it is really not necessary to sterilize the media at temperatures higher than 121°C. Sometimes it may be convenient to incubate uninoculated plates at high temperatures to insure that spore-forming strains did not survive sterilization. During incubation, the Petri plates should be wrapped in plastic film to prevent evaporation. Cultures on solid medium can be maintained for a few weeks in the dark at room temperature. Cultures can be stored for longer times by freezing at –70°C in cryotubes containing liquid media, mentioned above, supplemented with glycerol to yield a final concentration of 15% (v/v). Cultures have been maintained for several years without loss of viability by freeze-drying or by storage in liquid nitrogen.

TAXONOMIC COMMENTS

At this time, the genus *Thermus* consists of the species *T. aquaticus* (Brock and Freeze, 1969), *T. thermophilus* (Oshima and Imahori, 1974; Manaia et al., 1994), *T. filiformis* (Hudson et al., 1987b), *T. scotoductus* (Kristjánsson et al., 1994), *T. Brockianus* (Williams et al., 1995), and *T. oshimai* (Williams et al., 1996). The study of Saul et al. (1993) provided the first insight into the intragenetic phylogenetic relationships of the genus *Thermus*. The 20 strains for which the 16S rDNA sequence was determined in that study included the three species that were validly described at that time, namely *T. aquaticus* YT-1 (L09663), *T. filiformis* Wai33 A1 (L09667), and *T. ruber* (L09672) (later transferred to the genus *Meiothermus* [Nobre et al., 1996b]), the then-invalid species *T. thermophilus* HB8 (L09659) and "*T. flavus*" AT-62 (L09660), plus 14 strains from Iceland, New Zealand, and United States. This study demonstrated that the species of the genus *Thermus* could be differentiated using 16S rDNA sequence comparisons and provided some information on the biogeographical distribution of the *Thermus* species.

After the study of Saul et al. (1993), three new species of the genus *Thermus* were described, namely *T. Brockianus* (Williams et al., 1995), *T. oshimai* (Williams et al., 1996), and *T. scotoductus* (Kristjánsson et al., 1994). The descriptions of these species did not include phylogenetic analyses of 16S rDNA sequence data

nor did the authors demonstrate the phylogenetic position of these species within the radiation of the genus *Thermus*. Until recently (Chung et al., unpublished results), 16S rDNA sequences were not available for the type strains of *T. oshimai* and *T. scotoductus*.

Comparison of the 16S rDNA sequences of the type strains of each of the six validly described species of the genus *Thermus* shows the 16S rDNA sequence similarities within the genus *Thermus* to be in the range 91.2–96.4%. *Thermus oshimai* is the most distantly related of the species of the genus *Thermus* and this is reflected in the 16S rDNA sequence similarity values. The sequence of the type strain of *T. oshimai* has similarity values to *T. aquaticus* of 92.2%, to *T. Brockianus* of 91.7%, to *T. filiformis* of 91.2%, to *T. scotoductus* of 91.9% and to *T. thermophilus* of 93.0%. The latter five species of the genus *Thermus* have 16S rDNA sequence similarities in the range 94.1–96.4%.

Within each species the 16S rDNA similarity values are in the range 98.9–99.7% for *T. aquaticus*, 99.9–100% for *T. Brockianus*, 99.2–99.9% for *T. filiformis*, 99.8–100% for *T. oshimai*, 98.7–99.9% for *T. scotoductus*, and 99.4–100% for *T. thermophilus*. These values are based on the comparison of all published and a large number of unpublished full 16S rDNA sequences for strains of species of the genus *Thermus* (Fig. B4.6). These values clearly demonstrate the usefulness of 16S rDNA sequence data in the identification of new *Thermus* isolates or in determining their novelty and relatedness to the validly described *Thermus* species. Such data, however, are of little use in the differentiation of strains within a *Thermus* species.

The species *Meiothermus ruber* (Loginova et al., 1984), *M. silvanus*, and *M. chliarophilus* (Tenreiro et al., 1995a) were initially included in the genus *Thermus* despite their lower cardinal growth temperatures, because these species had many characteristics in common with the high-temperature strains and were phylogenetically closely related to them (Brock, 1984; Hensel et al., 1986; Sharp and Williams, 1988; Williams and da Costa, 1992; Embley et al., 1993). The description of *T. silvanus* and *T. chliarophilus* with higher 16S rDNA sequence similarity to *T. ruber* than to the high-temperature species of *Thermus*, coupled with the lower optimum growth temperature range and the presence of moderate levels of 2-OH fatty acids, led to the proposal of the genus *Meiothermus* and the emendation of the genus *Thermus*, which retained only the species with optimal growth temperatures around 70°C (Nobre et al., 1996b). 16S rDNA sequence similarity values of 84.9–86.7% are found between the species of the genera *Thermus* and *Meiothermus*, demonstrating the clear distinction between these genera. The large differences between

the 16S rDNA sequences of members of the genera *Thermus* and *Meiothermus* clearly allow differentiation of species of these two genera.

Two yellow-pigmented strains from Japan, designated “*Thermus flavus*” for strain AT-62 (Saiki et al., 1972) and “*Thermus caldophilus*” for strain GK-24 (Taguchi et al., 1982), were never validly described, and, in fact, have been shown to belong to the species *T. thermophilus* (Manaia et al., 1994; Williams et al., 1995). One strain, named “*Thermus lacteus*”, is a patent strain of unknown affinity deposited with the American Type Culture Collection (ATCC 31557).

Due to extremely variable biochemical and physiological characteristics and fatty acid composition, it is very difficult to define most of the species of the genus *Thermus* (Tables B4.4 and B4.5). The variability of biochemical and physiological parameters was noticed in early studies involving numerical taxonomy and has constituted one of the great hurdles for an adequate classification of most of the isolates of this genus (Cometta et al., 1982; Alfredsson et al., 1985; Hudson et al., 1986, 1987a, 1989; Munster et al., 1986; Santos et al., 1989). The phenotypic variability may be due to natural diversity within each species, but there is the possibility that it could also be the result of technical difficulties in assessing phenotypic characteristics. For example, it has been reported that glucose is not assimilated by many strains, but lowering the growth temperature to about 65°C results in assimilation of this carbon source by the majority of the strains (Manaia and da Costa, 1991). In an initial numerical classification of Portuguese and Azorean strains, the species later named *T. oshimai* formed two distinct clusters, yet all strains had high DNA–DNA hybridization values (Santos et al., 1989; Williams et al., 1995). In another study based primarily on strains isolated from Yellowstone National Park, one phenetic cluster contained most of the strains of *T. aquaticus*, whereas the other major cluster contained most of the strains of *T. Brockianus*. However, the latter cluster also contained, for example, strains of *T. thermophilus* (Munster et al., 1986).

The same type of variation was found in the fatty acid composition of a large number of strains belonging to all of the species of the genus; the species *T. thermophilus*, for example, had extremely variable fatty acid compositions, even though many strains share very high DNA–DNA hybridization values (Manaia et al., 1994; Nobre et al., 1996a).

To further complicate matters, most species have been described on the basis of a small number of isolates that have not been extensively characterized, so that interspecific diversity of phenotypic characteristics has not been assessed. For example, the species *T. filiformis* was described on the basis of one strain from New Zealand with a stable filamentous morphology (Hudson et al., 1987b). Other strains from New Zealand belong to this species on the basis of DNA–DNA hybridization values, but are not filamentous (Georganta et al., 1993). Moreover, the type strain of *T. filiformis* possesses very high levels of anteiso-fatty acids as well as 3-OH fatty acids, whereas the other strains have high levels of iso-fatty acids and lack 3-OH fatty acids (Ferraz et al., 1994; Nobre et al., 1996a). The biochemical and physiological diversity of *T. filiformis* strains that share high DNA–DNA hybridization values makes it difficult, if not impossible, to define a distinct phenotype for this species (Hudson et al., 1987b, 1989; Georganta et al., 1993).

The description of the species *T. scotoductus* was based on strains isolated from hydrothermally fed hot water taps in Iceland, as well as strain X-1 isolated from a thermally polluted

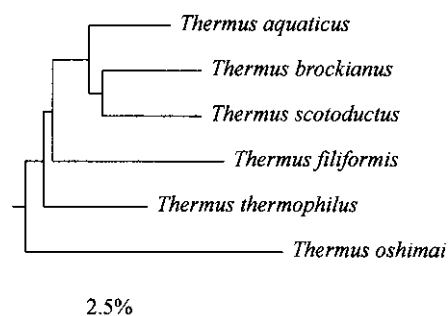


FIGURE B4.6. Phylogenetic dendrogram based on 16S rDNA sequence comparison of the type strains of the genus *Thermus*. Bar = 2.5 inferred nucleotide substitutions per 100 nucleotides.

TABLE B4.5. Fatty acid composition of the type strains of the genus *Thermus* grown at 70°C

Fatty acid	% of total in: ^a					
	1. <i>T. aquaticus</i> YT-1	2. <i>T. Brockianus</i> YS38	3. <i>T. filiformis</i> Wai33 A1	4. <i>T. oshimai</i> SPS-17	5. <i>T. scotoductus</i> SE-1	6. <i>T. thermophilus</i> HB8
C _{14:0} iso	0.9	1.6	0.9	0.5	—	0.8
C _{15:0} iso	17.6	33.5	4.1	37.7	15.8	32.4
C _{15:0} anteiso	1.9	3.1	18.9	3.8	16.1	4.7
C _{15:0}	—	0.6	—	3.7	1.1	—
C _{16:0} iso	13.0	12.1	8.4	3.1	3.8	5.8
C _{16:0}	16.3	9.2	3.5	7.2	8.0	8.0
UN ^b	—	—	2.9	—	2.5	—
C _{15:0} iso 3-OH	3.2	—	0.6	—	—	—
C _{15:0} anteiso 3-OH	—	—	1.1	—	—	—
C _{17:0} iso	27.0	34.1	6.6	36.1	25.3	41.9
C _{17:0} anteiso	2.7	3.3	36.7	3.7	25.8	5.7
C _{17:0}	—	—	—	2.1	1.2	—
C _{16:0} iso 3-OH	2.4	—	0.9	—	—	—
C _{16:0} 3-OH	2.5	—	—	—	—	—
C _{18:0} iso	0.6	0.5	0.9	—	—	—
C _{18:0}	0.9	—	—	—	—	—
C _{17:0} iso 3-OH	7.6	—	2.4	—	—	—
C _{17:0} anteiso 3-OH	0.7	—	8.8	—	—	—

^aValues for fatty acids present at levels of less than 0.5% in all strains are not shown. Under the conditions used to determine the fatty acid composition, diols were detected in low levels and were not included in the table.

^bUnknown fatty acid or alcohol with an equivalent chain length of 16.090.

stream in the United States (Ramaley and Hixson, 1970; Kristjánsson et al., 1994). The lack of pigmentation was one of the characteristics used to discriminate this species from other species of the genus, although all these strains came from nonilluminated or artificial environments, where nonpigmented strains would be expected to constitute the dominant populations. Two other nonpigmented strains, designated NH and DI, isolated from hot tap water in London, were later found to be practically identical to the type strain from Iceland (Pask-Hughes and Williams, 1975; Tenreiro et al., 1995b). Other strains from the hot spring at the end of a nonilluminated tunnel at Vizela in northern Portugal were found to be closely related to the type strain of *Thermus scotoductus* (Tenreiro et al., 1995b), and were, not surprisingly, colorless. However, yellow-pigmented strains from hot springs exposed to sunlight in the Azores were also found to be very closely related to the Vizela strains and to *T. scotoductus* (Santos et al., 1989; Tenreiro et al., 1995b; Williams et al., 1996). Moreover, only the strains from Iceland and from London have identical fatty acid compositions (Nobre et al., 1996a). These results also appear to leave the species *T. scotoductus* without distinct phenotypic characteristics.

The species *T. aquaticus* can be easily distinguished from *T. Brockianus*, but all of the strains extensively characterized originate from Yellowstone National Park and variability, therefore is limited (Munster et al., 1986; Williams et al., 1995). However, when biochemical and physiological characteristics of these organisms are compared to those of other species, the distinctiveness of *T. Brockianus* and *T. aquaticus* breaks down, even though there is no doubt that these two species constitute distinct genomic species. It is interesting to note that all of the *T. Brockianus* strains isolated from different hot springs at Yellowstone National Park have an identical fatty acid composition (Nobre et al., 1996a), and an identical genomic structure based on PFGE of large DNA fragments, leading us to believe that they constitute one clone (Moreira et al., 1997), whereas the *T. aquaticus* isolates have more variable fatty acid compositions and PFGE profiles. It is still possible to distinguish the strains of *T. aquaticus* from all other strains of the genus *Thermus* due to the presence of high

levels of iso-fatty acids coupled with the presence of 3-OH fatty acids (Nobre et al., 1996a). However, one strain from Yellowstone National Park that belongs to the species *T. aquaticus* by DNA-DNA hybridization values (Williams et al., 1996) does not possess 3-OH fatty acids or galactosamine in GL-1 (Ferreira et al., unpublished results) and may prove, after further examination, to constitute a nasty little exception to the classification and identification of *T. aquaticus* by fatty acid analysis. The description of the species *T. oshimai* was based on strains isolated from continental Portugal, the Azores, and Iceland, and appears to have a fairly homogeneous phenotype despite the formation of two phenotypic clusters in the original characterization of these strains (Santos et al., 1989; Nobre et al., 1996a; Williams et al., 1996).

Two characteristics, growth of the strains at 80°C or higher and halotolerance, appear to distinguish *T. thermophilus* from other species of this genus (Manaia et al., 1994). Several strains are very closely related to strain HB8 on the basis of DNA-DNA hybridization results and phylogenetic analysis, and can clearly be assigned to *T. thermophilus*. These are strains AT-62, HB-27, B, GK-24, RQ-1, and RQ-3. Other strains isolated from shallow marine hot springs are also closely related to strain HB8, sharing about 60% DNA-DNA hybridization values. Several strains recovered from shallow marine hot springs were also assigned to *T. thermophilus* because they could not be distinguished from this species (Manaia et al., 1994), but we are now of the opinion that the taxonomic status of strains such as Fiji A3 should be reassessed (Manaia et al., 1994; da Costa, unpublished results).

The species *T. thermophilus* was validly described by Oshima and Imahori (1974) based on strain HB8, previously named "*Flavobacterium thermophilum*" (Yoshida and Oshima, 1971). However, this species was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980) because it could not be distinguished from *T. aquaticus*. Williams (1989) showed that strain HB8 had high DNA-DNA hybridization values with strains AT-62, GK-24, and B, but did not attempt to revive the name *T. thermophilus* due to the lack of phenotypic characteristics that could distinguish this species from the other species of the genus. The isolation of halotolerant strains from marine hot springs on

the island of São Miguel in the Azores led to a detailed phenotypic characterization, and DNA–DNA hybridization study of strains belonging to this species (Manaia and da Costa, 1991; Manaia et al., 1994). The results showed that several marine and inland strains shared the ability to grow at temperatures above 80°C and were halotolerant. These characteristics, coupled with high DNA–DNA hybridization values, led to the revival of the species *T. thermophilus*. The revival of the name *T. thermophilus* by Manaia et al. (1994) appeared in the Validation List several pages after the publication of Williams et al. (1995) where the revival of the name *T. thermophilus* was also proposed. However, Williams et al. (1995) proposed the name *T. thermophilus* as valid only in the abstract of the publication without a formal proposal in the text, and did not present phenotypic results showing that the species could be distinguished from other species of the genus

Thermus. For these reasons the publication by Manaia et al. (1994) is considered the effective publication for the revival of the species *T. thermophilus* (H. Trüper, B. Tindall, N. Weiss, personal communication).

ACKNOWLEDGMENTS

Until Brock and Freeze described *Thermus aquaticus* in 1969, the interest in thermophilic bacteria was negligible. Other thermophiles were known at that time, but the lack of an ecological perspective diverted our minds from the possibility that many organisms could inhabit natural environments with extremely high temperatures. It was the insight of Thomas Brock on life at high temperatures that led to the intense research now conducted on thermophilic bacteria, and it was *Thermus aquaticus* that led the way. For this reason, and many others, we wish to dedicate this chapter to Thomas D. Brock.

List of species of the genus *Thermus*

1. ***Thermus aquaticus*** Brock and Freeze 1969, 295.^{AL}
a.qua'ti.cus. L.masc. adj. *aquaticus* living in water.

The strains of this species form rod-shaped cells 0.5–0.8 µm in diameter with variable length. Short filaments are present. Colonies are about 1 mm in diameter after 48 h growth on *Thermus* medium at 70°C and are bright yellow. The strains of this species generally hydrolyze casein, gelatin, and starch; the strains do not utilize lactose or melibiose, and do not reduce nitrate or nitrite. With one possible exception, all other strains examined have iso 3-OH fatty acids. Strains YS 004, YS 013, YS 025, YS 031, and YS 041 of Munster et al. (1986) can be assigned to this species (Williams, 1989; Williams et al., 1995; Nobre et al., 1996a). These strains have mol% G + C of the DNA between about 60% and 64%. The strains of this species have only been isolated from Yellowstone National Park.

The mol% G + C of the DNA is: 64 (T_m) (type strain).

Type strain: YT-1, ATCC 25104, DSMZ 625.

GenBank accession number (16S rRNA): L09663.

2. ***Thermus brockianus*** Williams, Smith, Welch, Micallef and Sharp 1995, 498.^{VP}
brock'i.a.nus. M.L. masc. adj. *brockianus* named after Thomas D. Brock.

Strains of this species form rod-shaped cells and short filaments. The colonies of the strains from Yellowstone National Park are pale yellow, and spread on *Thermus* agar. These strains do not hydrolyze casein, and only a few hydrolyze gelatin or starch; nitrate and nitrite are reduced; lactose, trehalose and melibiose are utilized as single carbon sources. The strains do not possess 3-hydroxy fatty acids. All isolates from Yellowstone National Park are very closely related, and appear to constitute one clone. Several strains of phenetic group 2 of Munster et al. (1986), namely strains YS 07, YS 11, YS 19, YS 30, YS 40, and YS 44, belong to this species (Williams et al., 1995; Nobre et al., 1996a). Strain ZHG1 A1 from Iceland (Hudson et al., 1986) also belongs to this species (Williams et al., 1995).

The mol% G + C of the DNA is: 63 (T_m).

Type strain: YS38, NCIB 12676.

GenBank accession number (16S rRNA): Z15062.

3. ***Thermus filiformis*** Hudson, Morgan and Daniel 1987b, 435.^{VP}

fi.li.for'mis. L. neut. n. *filum* thread; L. fem. n. *forma* shape; L. masc. adj. *filiformis* thread shaped.

The strains of this species form bright yellow colonies. The type strain of this species has a stable filamentous morphology, but other strains assigned to this species produce rod-shaped cells. The type strain possesses very high levels of anteiso-fatty acids and anteiso 3-hydroxy fatty acids. Anteiso fatty acids are present in low levels in other strains and 3-hydroxy fatty acids are absent. It appears that this species has no phenotypic characteristics that distinguishes it from other species of the genus *Thermus*. Several isolates of Hudson et al. (1986), namely strains T351, Rt358, Tok22, and Rt6 A1 belong to this species due to high DNA–DNA hybridization values (Georganta et al., 1993). Strains of this species have been isolated only from hot springs in New Zealand.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: Wai33 A1, ATCC 43280, DSMZ 4687.

GenBank accession number (16S rRNA): L09667, X58345.

4. ***Thermus oshimai*** Williams, Smith, Welch and Micallef 1996, 406.^{VP}

o.shi'ma.i. M.L.gen. n. *oshimai* named after Tairo Oshima.

Strains of this species form rod-shaped cells and short filaments. The colonies of most strains are pale yellow; some strains are not pigmented. The strains of this species hydrolyze casein and fibrin. Most strains reduce nitrate and nitrite. The strains of this species utilize sucrose, maltose, trehalose, lactose, and melibiose, and possess α- and β-galactosidase. This species includes strains of phenetic clusters E and F of Santos et al. (1989) isolated from the hot spring at São Pedro do Sul, continental Portugal, and the island of São Miguel, the Azores, and strains JK-66, JK-90, and JK-91 from Iceland (Williams et al., 1996).

The mol% G + C of the DNA is: 63 (T_m).

Type strain: SPS-17, ATCC 700435, NCIB 13400.

5. ***Thermus scotoductus*** Kristjánsson, Hjörleifsdóttir, Marteinsson and Alfredsson 1995, 418^{VP} (Effective publication: Kristjánsson, Hjörleifsdóttir, Marteinsson and Alfredsson 1994, 49.)

sco.to.duc'tus. Gr. n. *scotos* darkness; L. masc. n. *ductus* Roman water duct; M.L. masc. n. *scotoductus* living in pipes and producing a dark pigment.

Cells are about 0.5 µm × 1.5 µm; filaments are present.

Old cultures of some strains produce a dark water-soluble pigment. The type strain and strains X-1 (ATCC 27978), NH (NCIB 11245) and DI (NCIB 11246), are nonpigmented. The optimum growth temperature of the type strain is between 65 and 70°C, but strain X-1 has a higher optimum growth temperature. Strain X-1 is composed of two colony types; in colony type t1 glycolipid 2 (GL-2) is the major glycolipid, and glycolipid 1 (GL-1) is not detected or is present in vestigial concentrations. Strains of this species have been isolated from hot water systems in Iceland and London, and a thermally polluted stream in the United States. Nonpigmented strains from Vizela, continental Portugal, yellow-pigmented strains of phenetic groups A and B from the island of São Miguel in the Azores (Santos et al., 1989), and strain NMX2 A1 from New Mexico (Hudson et al., 1989) probably belong to this species.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: strain SE-1, ATCC 51532, DSMZ 8553.

GenBank accession number (16S rRNA): AF032127.

6. ***Thermus thermophilus*** (ex Oshima and Imahori 1974) Manaia, Hoste, Gutierrez, Gillis, Ventosa, Kersters and da

Costa 1995, 619^{VP} (Effective publication: Manaia, Hoste, Gutierrez, Gillis, Ventosa, Kersters and da Costa 1994, 530.) *ther.mo'phi.lus*. Gr. n. *therme* heat; Gr. adj. *philos* loving; M.L. masc. adj. *thermophilus* heat-loving.

The cells are rod shaped, and short filaments are also formed. The strains of this species form light yellow non-spreading colonies on *Thermus* medium. All the strains can grow at temperatures as high as 80–82°C and in *Thermus* medium containing 3.0% NaCl. Strains B (NCIB 11247), AT-62 (ATCC 33923), RQ-1 (DSMZ 9247), GK-24 (Taguchi et al., 1982), IB-21, and HB-27 can be assigned to this species (Manaia et al., 1994; Williams et al., 1995). In the absence of other distinguishing characteristics, strains Fiji3, A1 (Hudson et al., 1989), and several halotolerant strains from marine hot springs on the island of São Miguel also should be included in this species (Manaia et al., 1994). This species has been isolated from inland and marine hot springs Japan, Iceland, and the island of São Miguel, Azores.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: HB8, ATCC 27634, DSMZ 579, NCIB 11244.

GenBank accession number (16S rRNA): M26923, X07998, X58342.

Genus II. Meiothermus Nobre, Trüper and da Costa 1996b, 605^{VP}

M. FERNANDA NOBRE AND MILTON S. DA COSTA

Mei.o.ther'mus. Gr. prefix *meio-* less; Gr. adj. *thermos* hot; M.L. masc. n. *Meiothermus* to indicate an organism in a less hot place.

Straight rods, 0.5–0.8 µm in diameter; the cell length is variable. Long filaments are also formed under some culture conditions. **Nonmotile**; do not possess flagella. Endospores are not observed. Stain Gram-negative. Most strains form **red- or orange-pigmented colonies**, some strains are bright **yellow**. Aerobic with a strictly respiratory type of metabolism, but some strains use nitrate as terminal electron acceptor. Oxidase positive; the strains of one species are catalase positive, whereas the strains of the other species are catalase negative. **Slightly thermophilic**, with optimum growth temperatures of 50–65°C; strains do not grow at 70°C. The optimum pH ranges from 7.5 to 8.0. **Menaquinone 8** is the predominant respiratory quinone; **ornithine** is present in the peptidoglycan. One major phospholipid and two prominent glycolipids migrating close to each other dominate the polar lipid pattern. Additional phospholipids and glycolipids are minor components. Fatty acids are predominantly **iso- and anteiso-branched**. **Branched-chain 2-hydroxy fatty acids** are present in all strains. Proteins and peptides are hydrolyzed by all strains. Starch is hydrolyzed by some strains. Hexoses, a few pentoses and a few polyols, disaccharides, amino acids, and organic acids are used as sole carbon and energy sources. Most strains require yeast extract or cofactors for growth. Found in **hydrothermal areas** with neutral to alkaline pH, also isolated from fermentors.

The mol% G + C of the DNA is: 59–70.

Type species: ***Meiothermus ruber*** (Loginova, Egorova, Golovacheva and Seregina 1984) Nobre, Trüper and da Costa 1996b, 605 (*Thermus ruber* Loginova, Egorova, Golovacheva, Seregina 1984, 498.)

FURTHER DESCRIPTIVE INFORMATION

Phylogenetic analysis based on 16S rDNA sequence analysis shows that the species of the genus *Meiothermus* form a sister line of descent with the species of the genus *Thermus* with which they share only about 86% sequence similarity (Nobre et al., 1996b). These two closely related genera constitute the order *Thermales*, which, along with the distantly related species of the order *Deinococcales*, constitute the *Deinococcus-Thermus* phylum within the domain *Bacteria* (Weisburg et al., 1989; Embley et al., 1993; Nobre et al., 1996b; Rainey et al., 1997). It had been noted for some time that the red-pigmented "low-temperature" species designated *Thermus ruber* formed a separate line of descent from the "high-temperature" species of the genus *Thermus* (Weisburg et al., 1989; Bateson et al., 1990; Embley et al., 1993), but the species was nevertheless maintained in this genus. The description of two new, slightly thermophilic species that, based on 16S rRNA sequence analysis and chemotaxonomic parameters, were more closely related to *Thermus ruber* than to the other species of the genus *Thermus* led to the proposal of the genus *Meiothermus* for the species with low growth temperatures (Tenreiro et al., 1995a; Nobre et al., 1996b).

Transmission electron microscopy shows that the cells of the genus *Meiothermus* have an envelope consisting of a cytoplasmic membrane with a simple outline, a cell wall with an inner, electron-dense thin layer, presumably representing the peptidoglycan connected to an outer corrugated "cobble-stone" layer by irregularly spaced invaginations. The species of the genus *Meiothermus* are morphologically indistinguishable from the species of *Ther-*

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