Thermus silvanus sp. nov. and *Thermus chliarophilus* sp. nov., Two New Species Related to *Thermus ruber* but with Lower Growth Temperatures

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Strains of *Thermus silvanus* sp. nov. and strains of *Thermus chliarophilus* sp. nov. were isolated from the hot spring at Vizela in northern Portugal and the hot spring at Alcafache in central Portugal, respectively. The strains of *T. silvanus* produce orange-red-pigmented colonies and have an optimum growth temperature of about 55°C, while the strains of *T. chliarophilus* produce yellow-pigmented colonies and have an optimum growth temperature of about 50°C. The strains of both species are catalase negative. These species can be distinguished from each other and from *Thermus ruber* by biochemical characteristics, fatty acid composition data, and 16S rRNA gene sequence data. Our phylogenetic analysis showed that strains VI-R2^T (T = type strain) and ALT-8^T belong to the *T. ruber* line of descent. The type strain of *T. silvanus* is strain VI-R2 (= DSM 9946), and the type strain of *T. chliarophilus* is strain ALT-8 (= DSM 9957).

Five species of bacteria belonging to the genus *Thermus* have been validly described previously. *Thermus aquaticus* (1) was described on the basis of strains isolated at Yellowstone National Park; the description of *Thermus filiformis* (7) was based on one filamentous strain from New Zealand; *Thermus scotoductus* (9) was described on the basis of nonpigmented strains from Iceland and the United States; and *Thermus thermophilus* was validly described (16) but was not included on the Approved Lists of Bacterial Names (26) or on subsequent validation lists because it could not be distinguished from *T. aquaticus*. However, it is now possible to distinguish *T. thermophilus* from the other *Thermus* species by using DNA homology data and phenotypic characteristics (13, 14, 31). The species mentioned above produce yellow-pigmented or nonpigmented colonies, have optimum growth temperatures between 70 and 75°C, and are commonly designated the high-temperature species of the genus *Thermus*.

Thermus ruber strains (11) have optimum growth temperatures of 60 to 65°C and maximum growth temperatures of just less than 70°C and form red-pigmented colonies. Despite the extensive geographical distribution of this species and its phenotypic diversity, all of the strains that have been examined exhibit high levels of DNA homology (6, 20, 23).

Recently, we isolated several orange-red-pigmented *Thermus* strains from the hot spring at Vizela in northern Portugal and yellow-pigmented *Thermus* strains from the hot spring at Alcafache in central Portugal and found that these organisms had low optimum growth temperatures. In this paper we describe two new *Thermus* species that are related to *T. ruber* on the basis of biochemical and tolerance characteristics, chemotaxonomic parameters, and 16S rRNA gene sequences. We propose the names *Thermus silvanus* and *Thermus chliarophilus* for these new species.

MATERIALS AND METHODS

Isolation and bacterial strains. Strains VI-R1, VI-R2^T (T = type strain), VI-R7, VI-R11, and VI-R12 (the VI-R strains) were isolated from the hot spring

(vent temperature, 56°C; pH 8.9) located at the end of a 450-m tunnel and from thermal water (temperature, 33°C; pH 8.8) piped to a spa at Vizela in northern Portugal. Strains ALT-8^T, ALT-9, and ALT-10 (the ALT strains) were isolated from the hot spring at Alcafache (vent temperature, 50°C; pH 8.0) in central Portugal. Water samples were filtered through membrane filters (Gelman type GN-6; pore size, 0.45 µm; diameter, 47 mm); the filters were placed on the surfaces of *Thermus* agar plates (32), and these preparations were wrapped in plastic bags and incubated at 50°C for up to 7 days. Strain VI-R1 was recovered 3 years before the other strains from plates incubated at 60°C by using the methods described above. Cultures were purified by subculturing and were kept at –80°C in *Thermus* medium containing 15% glycerol.

Several *T. ruber* strains were used for comparative purposes. *T. ruber* DSM 1279^T and “*Thermus rubens*” ATCC 31556 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, and the American Type Culture Collection, Rockville, Md., respectively. Strains MTL-16501 and MTL-16503 from the Azores, strains MTL-16105 and MTL-16106 from Iceland, strains MTL-16234 and MTL-16294 from New Zealand, and strains MTL-15056 and MTL-15057 from the United States were kindly donated by R. Sharp, Microbial Technology Laboratory, Centre for Applied Microbiology & Research, Salisbury, United Kingdom. Strains SPS-R1, SPS-R4, and SPS-R7 were isolated from the hot spring at São Pedro do Sul, Portugal, and have been described previously (4).

Biochemical and tolerance characteristics. Unless otherwise stated, all biochemical and tolerance tests were performed as described previously (13, 21) in *Thermus* medium or on *Thermus* agar incubated at 50°C for up to 5 days. Catalase activity was examined by using the methods described by Vesey et al. (29) and Smbert and Krieg (27). The growth temperature ranges of *T. ruber* DSM 1279^T, VI-R2^T, and ALT-8^T were determined by incubating 300-ml metal-capped Erlenmeyer flasks containing 100 ml of *Thermus* medium in a reciprocal water bath shaker. The pH ranges for growth were determined in *Thermus* medium as described above by using 20 mM MES [2-(N-morpholino)ethanesulfonic acid] for pH values between 5.0 and 6.5, Tris for pH values between 7.0 and 8.5, and CAPSO [3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid] for pH values between 9.0 and 10.5; the pH of each buffer was adjusted with HCl or NaOH. The pH values were always determined at room temperature.

Single-carbon-source assimilation tests were performed in a minimal medium composed of *Thermus* basal salts medium without nitrate to which filter-sterilized carbon sources (2.0 g liter⁻¹), ammonium sulfate (0.5 g liter⁻¹), and yeast extract (0.2 g liter⁻¹) were added. Growth was examined by measuring the turbidity of cultures incubated at 50°C with shaking in 300-ml metal-capped Erlenmeyer flasks containing 100 ml of medium every 12 h for 3 days. Positive and negative control cultures were grown in *Thermus* medium and in minimal medium without a carbon source, respectively.

Electron microscopy. Cultures were grown for 24 h in *Thermus* medium at 50°C, washed by centrifugation, prefixed for 16 h at room temperature in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, washed in the same buffer, and fixed in 1% OsO₄ in Veronal-acetate buffer (pH 6.2) containing 10.0 mM NaCl and 10.0 mM CaCl₂ for 16 h at room temperature (25). The cells were washed in water and postfixed for 30 min at room temperature in 1.0% uranyl acetate. The pellets were dehydrated with ethanol and embedded in Epon. Ultrathin sections were double contrasted by using uranyl lead (25) or a modification of the Thiéry method (24).

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Polar lipid, lipoquinone, and fatty acid compositions. The cultures used for polar lipid analysis were grown in 1-liter 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 two-dimensional thin-layer chromatography were performed as described previously (18).

Lipoquinones were extracted from freeze-dried cells and were purified by thin-layer chromatography as described by Tindall (28). The lipoquinones were separated with a Gilson high-performance liquid chromatography apparatus by using a reverse-phase (RP18) Spherisorb S5 ODS2 column and methanol-heptane (10:2, vol/vol) as the mobile phase and were detected at 269 nm.

The cultures used for fatty acid analysis were grown on *Thermus* agar plates in sealed plastic bags submerged in a water bath at 50°C for 24 h. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation, and extraction as described previously (10). The fatty acid methyl esters were separated by using a Hewlett-Packard model 5890 gas chromatograph equipped with a flame ionization detector and a 5% phenylmethyl silicone capillary column (0.2 mm by 25 m; Hewlett-Packard). The carrier gas was high-purity H₂; the column head pressure was 60 kPa; the septum purge rate was 5 ml/min; the column split ratio was 55:1; and the injection port temperature was 300°C. The temperature of the oven was programmed to increase from 170 to 270°C at a rate of 5°C/min. Identification and quantification of the fatty acid methyl esters, as well as a numerical analysis of the fatty acid profiles, were performed by using the standard MIS library generation software (Microbial ID, Inc., Newark, Del.).

DNA base composition and 16S rDNA sequence analysis. The DNAs used to determine DNA base compositions were isolated as described by Cashion et al. (2). The G+C content of the DNA was determined by high-performance liquid chromatography as described by Mesbah et al. (15). A 16S ribosomal DNA (rDNA) sequence analysis was performed by using the methods for genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, and purification of the PCR products described previously (18, 19). Purified PCR products were sequenced by using a *Taq* Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. Sequence reaction mixtures were electrophoresed by using an Applied Biosystems model 373A DNA sequencer. The 16S rDNA sequences were manually aligned with representative sequences from members of the *Thermus* line of descent (22). Pairwise evolutionary distances were compared by using the correction of Jukes and Cantor (8). The least-squares distance method of DeSoete (3) was used to construct a phylogenetic dendrogram from the distance matrices.

Nucleic acid sequence accession numbers. Our 16S rDNA sequence data are available from the EMBL data library under accession number X84211 for strain VI-R2^T and accession number X84212 for strain ALT-8^T.

RESULTS

Isolation of strains. Strain VI-R1 was isolated at 60°C, but later it was found that this strain grows slowly at this temperature in a liquid medium and that its fatty acid composition is different from the fatty acid compositions of other *T. ruber* strains (4). These observations led to subsequent isolation attempts in which samples obtained from Vizela in northern Portugal and Alcafache and São Gemil in central Portugal were incubated at 50°C. *Thermus* strains were isolated from the water samples obtained from Vizela and Alcafache after membrane filters were incubated at 50°C for 3 days. Orange-red-pigmented colonies were isolated from Vizela, and yellow-pigmented colonies were isolated from Alcafache. On the basis of polar lipid and fatty acid analysis data none of the other organisms isolated, except the nonpigmented high-temperature strains obtained from the Vizela samples, was considered a member of the genus *Thermus*.

Morphological, biochemical, and tolerance characteristics. All of the strains formed rod-shaped cells of various lengths and short filaments in liquid media and on solid media. Transmission electron microscopy showed that the cells of strains VI-R2^T and ALT-8^T had an envelope consisting of a three-layer, symmetric cytoplasmic membrane and a cell wall with an inner, electron-dense thin layer, which presumably represented the peptidoglycan. An outer corrugated layer was connected to the peptidoglycan by irregularly spaced invaginations (Fig. 1a). Strain VI-R2^T was shown by Thiéry staining to have polysaccharide inclusions (Fig. 1b).

The optimum growth temperatures were about 55°C for

strain VI-R2^T and about 50°C for strain ALT-8^T. No growth occurred at or below 35°C; strain ALT-8^T did not grow at temperatures above 60°C, while strain VI-R2^T did not grow at temperatures above 65°C. On the other hand, *T. ruber* DSM 1279^T had an optimum growth temperature of about 65°C but did not grow at 70°C (Fig. 2a). Growth of strains VI-R2^T and ALT-8^T was not observed at pH values below 5.5 or above 10.0 (Fig. 2b). Growth of these organisms did not occur in *Thermus* basal salts medium containing glucose and (NH₄)₂SO₄; yeast extract was necessary for the growth of all of the VI-R and ALT strains. None of the strains grew under anaerobic conditions in the absence of nitrate; deep stab cultures in soft *Thermus* agar resulted in profuse growth only at the surface.

All of the strains were oxidase positive; the VI-R and the ALT strains were catalase negative as determined by the two methods used to assess the presence of this enzyme, while all of the strains assigned to *T. ruber* were catalase positive as determined by both methods. The VI-R and the ALT strains reduced nitrate to nitrite, while *T. ruber* DSM 1279^T did not. Assimilation of single carbon sources could not be assessed in static tubes or on petri plates containing minimal medium because of erratic growth. However, it was possible to measure growth in shake cultures. Other biochemical and tolerance characteristics that distinguish the strains are shown in Table 1. The only differences in the assimilation of the single carbon sources tested were the utilization of xylose and ribitol by VI-R1 and VI-R2^T and the utilization of sucrose and trehalose by ALT-8^T and ALT-10.

Polar lipids, respiratory quinones, and fatty acids. The polar lipids of the VI-R strains, the ALT strains, and the *T. ruber* strains consisted of one major phospholipid and two prominent glycolipids that migrated close to the origin. Two additional minor glycolipids and one minor phospholipid were also present in all of the strains (data not shown). Menaquinone 8 was the only respiratory lipoquinone detected in all strains.

The major fatty acids of all of the organisms which we examined were iso- and anteiso-branched acids (Table 2). All of the strains examined contained a distinctive 17:0 2OH iso fatty acid in moderate amounts. However, major differences in the fatty acid compositions of the strains of the three species were observed after growth at 50°C; in the *T. ruber* and ALT strains 15:0 iso and 17:0 iso acids were present in higher proportions than the corresponding anteiso fatty acids, while in the VI-R strains 15:0 iso and 15:0 anteiso acids were the major fatty acids and 17:0 iso and 17:0 anteiso acids were minor components. At this growth temperature, branched-chain monounsaturated fatty acids, which were present in moderate amounts in the *T. ruber* strains, were virtually absent in the ALT and the VI-R strains. At the optimum growth temperatures, the *T. ruber* strains could not be distinguished from the ALT strains because of the absence of 17:1 ω₉c iso acid in *T. ruber*.

16S rRNA gene sequence analysis. The 16S rDNA similarity matrix in Table 3 shows that strains ALT-8^T and VI-R2^T are more closely related to *T. ruber* DSM 1279^T than to the high-temperature *Thermus* species. The level of 16S rRNA gene sequence similarity for strains ALT-8^T and VI-R2^T was 88.5%. Moreover, strains VI-R2^T and ALT-8^T exhibited levels of sequence similarity of 90.0 and 91.2% with *T. ruber*, respectively. Lower levels of sequence relatedness were observed with the high-temperature *Thermus* species (approximately 85 to 86%). Our phylogenetic tree showed that strains ALT-8^T and VI-R2^T

FIG. 1. Ultrastructure of strain VI-R2^T. (a) Thin section contrasted with uranyl lead showing the outer layer (OL), peptidoglycan (PG), and cytoplasmic membrane (CM). Bar = 0.2 μ m. (b) Thin section stained by the Thiéry method showing polysaccharide inclusions (P). Bar = 0.2 μ m.

diverged from the *T. ruber* line of descent and that yellow-pigmented strain ALT-8^T was more closely related to *T. ruber* DSM 1279^T than VI-R2^T was (Fig. 3).

DISCUSSION

The 16S rRNA gene sequences of red-pigmented strain VI-R2^T and yellow-pigmented strain ALT-8^T show that these organisms represent two new species that are related to but distinct from *T. ruber*. The evolutionary distances that separate the three species in the *T. ruber* line of descent are, in fact, greater than the evolutionary distances that separate the *Thermus* species with optimum growth temperatures of 70 to 75°C (22). The *Thermus* and *Deinococcus* species form one deeply branching phylum in the evolution of the bacteria (30). Within the genus *Thermus*, one line of descent appears to have

given rise to species with high optimum growth temperatures, and the other appears to have given rise to species with low optimum growth temperatures. Our results also show that the diversity of the "low-temperature" *Thermus* species may be as great as the diversity of the high-temperature species of this genus.

The major physiological and biochemical characteristics that distinguish the ALT and VI-R strains from *T. ruber* are the lower growth temperature range, the reduction of nitrate to nitrite, and the absence of catalase activity in the ALT and VI-R strains. Strain VI-R2^T and VI-R1 can be distinguished from strains ALT-8^T and ALT-10 on the basis of pigmentation, the presence of α -galactosidase in strains ALT-8^T and ALT-10, the assimilation of xylose and ribitol by strains VI-R2^T and VI-R1, and the assimilation of sucrose and trehalose by strains ALT-8^T and ALT-10.

TABLE 1. Biochemical and tolerance characteristics that distinguish strains after growth at 50°C

Characteristic	<i>T. ruber</i> DSM 1279 ^T	Strain VI-R1	Strain VI-R2 ^T	Strain ALT-8 ^T	Strain ALT-10
Pigmentation	Red	Orange-red	Orange-red	Yellow	Yellow
Growth in the presence of:					
1.0% NaCl	+	—	—	+	+
2.0% NaCl	+	—	—	—	—
Hydrolysis of starch (5 days)	—	+	+	+	+
Presence of:					
α-Galactosidase	+	—	—	+	+
Catalase	+	—	—	—	—
Degradation of <i>p</i> -nitrophenyl-β-glucopyranoside	+	—	—	+	+
Utilization of:					
D-Xylose	+	+	+	—	—
Sucrose	+	—	—	+	+
D-Cellobiose	+	+	—	+	+
D-Trehalose	+	—	—	+	+
Glycerol	+	—	+	—	—
Ribitol	—	+	+	—	—
<i>myo</i> -Inositol	+	—	—	—	—
Salicin	+	—	—	—	—

^a +, positive result or growth; —, negative result or no growth. All of the organisms hydrolyzed elastin, fibrin, casein, gelatin, and hide powder azure, degraded arbutin, esculin, hippurate, and *p*-nitrophenyl-α-glucopyranoside, reduced tellurite (0.1%, wt/vol), and were β-galactosidase positive. D-Glucose, D-fructose, D-mannose, D-galactose, maltose, lactose, D-melibiose, D-mannitol, D-sorbitol, pyruvate, L-glutamate, L-asparagine, L-serine, L-glutamine, and L-proline were assimilated by all of the strains. None of the strains utilized L-arabinose, L-rhamnose, D-raffinose, meso-erythritol, acetate, succinate, citrate, or acetamide.

FIG. 2. Effects of temperature (a) and pH (b) on the growth of *T. ruber* DSM 1279^T (■), strain ALT-8^T (○), and strain VI-R2^T (●).

Electron microscopy showed that strains VI-R2^T and ALT-8^T have an ultrastructure identical to that of *T. ruber*, with the distinctive outer wall layer possessing frequently spaced invaginations to the peptidoglycan (6).

The chemotaxonomic parameters which we examined indicated that the ALT and VI-R strains are related to *T. ruber*. Strains of the high-temperature species produce only one major glycolipid, while all *T. ruber* strains and the VI-R and ALT strains produce two prominent glycolipids which migrate close to each other (4). The fatty acid compositions of the species

TABLE 2. Mean fatty acid compositions of the strains examined after growth at 50°C

Fatty acid ^a	Equivalent chain length	% in:		
		<i>T. ruber</i> (n = 13) ^b	VI-R strains (n = 5)	ALT strains (n = 3)
13:0 iso	12.612	0.6	0.8	1.5
14:0 iso	13.617	0.7	0.7	1.7
15:1 iso F ^c	14.413	2.7		
15:0 iso	14.623	33.0	25.6	42.1
15:0 anteiso	14.713	5.5	26.3	8.1
15:0	15.000	1.8	0.4	2.1
16:1 ω7t alcohol	15.408	0.8		
16:0 iso	15.626	2.9	1.5	2.5
16:0	15.997	7.6	6.4	9.1
Unknown 1	16.090	1.1	2.5	0.7
iso 17:1 ω9c	16.417	6.5		
17:0 iso	16.630	13.3	10.0	16.4
17:0 anteiso	16.723	3.7	6.4	2.7
17:1 ω6c	16.863	0.8	1.3	0.7
17:0	16.999	0.8	0.3	1.2
17:0 iso 2OH	17.875	7.8	9.6	7.3
17:0 anteiso 2OH	17.971	0.4	3.0	0.6
17:0 iso 3OH	18.161	1.1		
Unknown 2	19.067	0.6	1.6	

^a Data for several fatty acids that were present at levels of less than 1.0% in all strains are not shown.

^b n is the number of strains examined.

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

TABLE 3. Levels of 16S rDNA similarity for strains ALT-8^T and VI-R2^T and related taxa

Strain	% 16S rDNA similarity to:											
	ALT-8 ^T	VI-R2 ^T	<i>Thermus ruber</i> DSM 1279 ^T	<i>Thermus</i> sp. strain NMX2	<i>Thermus</i> sp. strain ZHGIB	<i>Thermus aquaticus</i> YT-1 ^T	<i>Thermus</i> sp. strain HS1	<i>Thermus thermophilus</i> HB-8 ^T	<i>Thermus filiformis</i> Wai 33A1 ^T	<i>Thermus</i> sp. strain Rt41 A	<i>Thermus</i> sp. strain Tok3	<i>Fervidobacterium nodosum</i> DSM 5306 ^T
VI-R2 ^T	88.5											
<i>Thermus ruber</i> DSM 1279 ^T	91.2	90.0										
<i>Thermus</i> sp. strain NMX2	85.8	85.3	86.0									
<i>Thermus</i> sp. strain ZHGIB	85.9	86.4	86.3	96.3								
<i>Thermus aquaticus</i> YT-1 ^T	85.8	86.2	86.6	96.8	96.8							
<i>Thermus</i> sp. strain HS1	86.5	85.4	86.5	95.0	95.3	96.2						
<i>Thermus thermophilus</i> HB-8 ^T	86.7	85.7	86.8	95.3	95.6	96.5	99.6					
<i>Thermus filiformis</i> Wai 33A1 ^T	86.3	85.6	86.5	94.1	94.3	94.9	94.5	94.9				
<i>Thermus</i> sp. strain Rt41 A	86.0	85.3	86.1	93.7	93.9	94.5	94.3	94.7	99.5			
<i>Thermus</i> sp. strain Tok3	86.2	85.4	86.6	94.1	94.1	94.7	94.7	95.1	99.5	99.3		
<i>Fervidobacterium nodosum</i> DSM 5306 ^T	76.5	75.4	74.8	78.5	77.5	77.2	77.6	77.7	78.7	78.6	78.8	
<i>Thermotoga maritima</i> MSB-8 ^T	78.5	77.8	76.5	80.8	80.0	79.9	80.8	80.8	80.5	80.1	80.5	86.3

related to *T. ruber* are also distinctive because of the presence of a branched-chain 2-hydroxy fatty acid, 17:0 2OH iso. This fatty acid is not found in any of the high-temperature strains, although branched-chain 3-hydroxy fatty acids are present in moderate amounts in all *T. aquaticus* strains and in *T. filiformis* Wai33 A1^T (= ATCC 43280^T) (5). Moreover, the ALT and VI-R strains can be easily distinguished from each other and from *T. ruber* by their fatty acid compositions after growth at 50°C.

T. ruber strains occur worldwide in neutral to alkaline hot springs and artificial hot water environments and form a ho-

mogeneous species as determined by DNA homology and fatty acid composition data (4, 6, 20, 23). The red-pigmented VI-R strains were isolated only at Vizela, and the species represented by the ALT strains, despite efforts to isolate similar organisms from another hot spring located nearby with a water temperature of 48°C and pH 7.9, was encountered only at Alcafache, where the ALT strains were the only *Thermus* strains that could be isolated at 50°C. Moreover, strains of *T. ruber* designated SPS were consistently recovered from the warm runoff of the hot spring (vent temperature, 68°C; pH 8.6) at São Pedro do Sul, about 30 km from Alcafache. Therefore, we assume that physicochemical and/or biological parameters are major factors that affect colonization by these organisms and that some species could have very restricted distributions. On the other hand, the two new species of the genus *Thermus* represented by the VI-R and ALT strains may not have been recovered previously because most isolation procedures used for *T. ruber*-like organisms are usually performed at a temperature of 60 or 65°C (6, 23, 32).

Isolation of the yellow-pigmented ALT strains related to *T. ruber* was not expected, although yellow-orange-pigmented isolates with an optimum growth temperature of 65°C that were presumed to belong to *T. ruber* had been recovered previously from soil in the Togo Republic. The latter organisms had morphological and physiological characteristics of the genus *Thermus* but to our knowledge were not studied further (12).

On the basis of the results presented above, the name *Thermus silvanus* is proposed for the VI-R strains (type strain, strain VI-R2) and the name *Thermus chliarophilus* is proposed for the ALT strains (type strain, strain ALT-8).

Description of *Thermus silvanus* sp. nov. *Thermus silvanus* (sil. va' nus. L. adj. *silvanus*, in honor of Manuel T. Silva, a Portuguese microbiologist and immunologist). *T. silvanus* strains form rod-shaped cells; cell length is variable, and the cells are 0.5 to 0.8 µm wide. Long filaments are also present. Gram negative. The cells are nonmotile, and spores are not formed. Colonies on *Thermus* medium are orange-red pig-

FIG. 3. Phylogenetic dendrogram showing the positions of strains ALT-8^T and VI-R2^T within the radiation of the genus *Thermus*. Bar = 10 inferred nucleotide substitutions per 100 nucleotides. The root organisms used were *Fervidobacterium nodosum* and *Thermotoga maritima*.

mented and 0.5 to 1.2 mm in diameter after 72 h of growth. Growth occurs at temperatures between 40 and 65°C in *Thermus* medium; the optimum growth temperature for strain VI-R2^T is about 55°C. The optimum pH is between 8.0 and 8.5; growth does not occur at pH 5.0 or 10.0. Yeast extract is required for growth. The major fatty acids are 15:0 iso and 15:0 anteiso; 17:0 iso and 17:0 anteiso are present at low levels. All strains are oxidase positive and catalase negative. Nitrate is reduced to nitrite. α -Galactosidase negative and β -galactosidase positive. Casein, elastin, gelatin, hide powder azure, and starch are degraded. Hydrolysis of fibrin is weak or negative. Strain VI-R2^T utilizes D-glucose, D-fructose, D-mannose, D-galactose, D-xylose, maltose, lactose, D-melibiose, glycerol, D-mannitol, D-sorbitol, ribitol, pyruvate, L-glutamate, L-asparagine, L-serine, L-glutamine, and L-proline. Strain VI-R2^T does not utilize L-arabinose, L-rhamnose, sucrose, D-cellobiose, D-trehalose, D-raffinose, *meso*-erythritol, galactitol, *myo*-inositol, acetate, succinate, citrate, salicin, or acetamide.

The DNA of strain VI-R2^T has a G+C content of 63.6 mol%. This bacterium was isolated from the hot spring at Vizela in northern Portugal. Strain VI-R2^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 9946^T.

Description of *Thermus chliarophilus* sp. nov. *Thermus chliarophilus* (chli. a. ro' phi. lus. Gr. adj. *chliaros*, warm; Gr. adj. *philos*, loving; M. L. adj. *chliarophilus*, warmth loving). *T. chliarophilus* strains form rod-shaped cells that are 0.5 to 0.8 μ m wide in liquid cultures. Long filaments are also present. Gram negative. The cells are nonmotile, and spores are not produced. Colonies are yellow pigmented and 0.8 to 1.2 mm in diameter after 72 h of growth. Growth occurs at temperatures between 40 and 60°C in *Thermus* medium; the optimum growth temperature for strain ALT-8^T is about 50°C. The optimum pH is about 8.0; growth does not occur at pH 5.0 or 10.0. Yeast extract is required for growth. The major fatty acids are 15:0 iso and 17:0 iso; the corresponding anteiso fatty acids are minor components during growth at 50°C. All strains are oxidase positive and catalase negative. Casein, elastin, fibrin, gelatin, hide powder azure, and starch are degraded. Nitrate is reduced to nitrite. α -Galactosidase and β -galactosidase positive. D-Glucose, D-fructose, D-mannose, D-galactose, sucrose, maltose, D-cellobiose, D-trehalose, lactose, D-melibiose, D-mannitol, D-sorbitol, pyruvate, L-glutamate, L-asparagine, L-serine, L-glutamine, and L-proline are utilized by strain ALT-8^T; D-xylose, L-arabinose, L-rhamnose, D-raffinose, glycerol, *meso*-erythritol, galactitol, ribitol, *myo*-inositol, acetate, succinate, citrate, salicin, and acetamide are not utilized.

The DNA of strain ALT-8^T has a G+C content of 69.9 mol%. This bacterium was isolated from the hot spring at Alcafache in central Portugal. Strain ALT-8^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 9957^T.

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