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Thermus aquaticus gen. n. and sp. n., a Nonsporulating Extreme Thermophile

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The isolation of a new thermophilic bacterium, *Thermus aquaticus* gen. n. and sp. n., is described. Successful enrichment requires incubation at 70 to 75 C, and the use of nutrient media relatively dilute with respect to the organic components. Strains of *T. aquaticus* have been isolated from a variety of thermal springs in Yellowstone National Park and from a thermal spring in California. The organism has also been isolated from man-made thermal habitats, such as hot tap water, in geographical locations quite distant from thermal springs. Isolates of *T. aquaticus* are gram-negative nonsporulating nonmotile rods which frequently form long filaments at supraoptimal temperatures or in the stationary phase. All isolates form a yellow cellular pigment, probably a carotenoid. A characteristic structure formed by all isolates is a large sphere, considerably larger than a spheroplast. These large spheres, as well as lysozyme-induced spheroplasts, are resistant to osmotic lysis. Deoxyribonucleic acid base compositions of four strains were determined by CsCl density gradient ultracentrifugation and found to be between 65.4 and 67.4 moles per cent guanine plus cytosine. The growth of all isolates tested is inhibited by fairly low concentrations of cycloserine, streptomycin, penicillin, novobiocin, tetracycline, and chloramphenicol. Nutritional studies on one strain showed that it did not require vitamins or amino acids, although growth was considerably faster in enriched than in synthetic medium. Several sugars and organic acids served as carbon sources, and either NH_4^+ or glutamate could serve as nitrogen source. The organism is an obligate aerobe and has a *p*H optimum of 7.5 to 7.8. The optimum temperature for growth is 70 C, the maximum 79 C, and the minimum about 40 C. The generation time at the optimum is about 50 min. The possible relationships of this new genus to the myxobacteria, flexibacteria, and flavobacteria are discussed.

Although thermophilic bacteria have been known for many years, laboratory studies have been limited primarily to spore-forming species, especially *Bacillus stearothermophilis* (5), and to actinomycetes (7). It is clear from the early literature (9, 12, 13) that a variety of other kinds of thermophilic bacteria exist, although cultural studies have been rare. In the present paper we report some of the characteristics of a nonsporulating thermophile which is widespread in aquatic thermal environments, both natural and man-made. Although this organism is quite easy to isolate, it has apparently not been previously obtained in culture because it is most effectively enriched at temperatures of 70 C or above and in media relatively dilute in organic constituents. This organism is a gram-negative, nonmotile, nonsporulating rod which under certain cultural conditions forms long filaments. Frequently in culture, as well as in nature, the organism forms large spherical structures, probably related to spheroplasts. All isolates so far obtained produce a yellow pigment, probably carotenoid, which in at least one strain has been shown to be associated with the cytoplasmic membrane. The name we have chosen for this organism is *Thermus aquaticus* gen. n. and sp. n.

MATERIALS AND METHODS

Culture media. Initial isolations made use of hot spring water (Mushroom Spring, reference 3) to which 0.1% tryptone (Difco) and 0.1% yeast extract (Difco) were added. Later studies employed a basal salts medium initially designed for the growth of thermophilic blue-green algae (6). This salts medium had the following composition (in micrograms of deionized water per liter): nitrilotriacetic acid, 100; $CaSO_4 \cdot 2H_2O$, 60; $MgSO_4 \cdot 7H_2O$, 100; NaCl, 8; KNO_3, 103; $NaNO_3$, 689; Na_2HPO_4, 111; $FeCl_3$, 2.8; $MnSO_4 \cdot H_2O$, 22; $ZnSO_4 \cdot 7H_2O$, 5; H_3BO_3, 5; $CuSO_4$,

0.16; $Na_2MoO_4 \cdot 2H_2O$, 0.25; $CoCl_2 \cdot 6H_2O$, 0.46; *p*H adjusted to 8.2 with NaOH.

Incubation conditions. Covered water baths were used for most incubations. The temperatures of the water baths were checked frequently with mercury thermometers or with a model 421 thermistor probe and meter (Yellow Springs Instrument Co.). For growth rate studies, a New Brunswick rotary water-bath shaker with cover and electronic water level control was used. Growth was measured turbidimetrically at 640 nm in 125-ml Bellco side arm flasks containing 18 ml of medium by use of a Bausch & Lomb Spectronic 20 colorimeter. At temperatures of 70 C and over, the temperature of the medium in the growth flask itself was measured continuously by using the thermistor probe inserted directly into the bottom of the flask, with the output of the thermistor meter connected to a Cole-Parmer Mark VII recorder. The flask was removed from the water bath for less than 1 min for each turbidimetric reading. During this brief interval, the temperature of the flask dropped a few degrees, but since readings were made about once an hour, it is assumed that this brief drop in temperature was of no consequence.

Large batches of cells were grown in 500-ml Bellco flasks with stainless steel closures in a New Brunswick anhydric rotary incubator shaker.

Agar plates were wrapped in Saran Wrap (Dow Chemical Co.) to prevent drying and incubated either in a dry incubator or just above the surface of the water in a covered water bath.

Nutritional studies were performed by using 5 ml of medium in 16-mm culture tubes with stainless-steel caps. The tubes were incubated unshaken in a water bath at 70 C.

Anaerobic conditions were achieved by use of the Baltimore Biological Laboratory Gas-Pak jar and generator system.

Hot-spring studies. Most of the hot-spring studies were carried out in Yellowstone National Park. The chemical and thermal characteristics of Mushroom Spring and the locations and temperatures of the various stations have been described in detail (3); the other springs studied in the Lower Geyser Basin have similar characteristics. The waters of the springs studied have *p*H values in the range of 8 to 9.

Deoxyribonucleic acid (DNA) base compositions. DNA was isolated and purified by the method of Marmur (11), using 1.5 mg of lysozyme per ml to lyse the cells, followed by sodium lauryl sulfate. Base compositions were calculated from the buoyant density of the DNA as determined in a Beckman model E ultracentrifuge in CsCl, with *Sarcina lutea* and bacteriophage *B. subtilis* 2-C DNA as standards, following the method of Schildkraut et al. (15). The bacteriophage DNA was kindly supplied by Manley Mandel.

Antibotic and inhibitor sensitivity. The antibiotics and inhibitors were dissolved in water or appropriate solvent as stock solutions, and portions were added to the agar-medium just before plates were poured. The inocula were grown overnight in capped tubes of liquid medium, and the plates were inoculated by depositing drops of the undiluted culture on the surface of the plates to give a spot about 1 cm in diameter. Six cultures were inoculated on a single plate. The plates were then incubated at 70 C for 3 days; growth of the spots was estimated visually. Actinomycin D sensitivities were determined by placing filter paper discs containing measured amounts of the antibiotic onto agar plates on which 0.1-ml amounts of an overnight liquid culture had been spread.

Microscopy. A Carl Zeiss Universal phase microscope was used for all microscopic observations. Photomicrography was performed with a Beseler Topcon Super D camera with Kodak Plus-X film developed in Microdol X, diluted 1:3. Electron microscopy of palladium-platinum (80:20) shadowed specimens (shadow angle, 1:3) was done by using an RCA EMU-3C electron microscope. The cells for electron microscopy were grown in basal salts plus 0.1% tryptone + 0.1% yeast extract at 72 C overnight; they were washed twice in distilled water and suspended in distilled water before drying on the Formvar grids.

RESULTS

Enrichment and isolation. Samples of water, soil, or hot-spring microbial mats were added to 10-ml amounts of basal salts containing 0.1% each of tryptone and yeast extract in screw-cap tubes and incubated unshaken at 70 to 75 C. Within 1 to 2 days, visible turbidity was seen in many tubes, often appearing clumpy or as a surface pellicle. When the turbidity had become fairly heavy, the color of the microbial mass was usually yellow or orange. Microscopic examination of these tubes usually revealed short and long filaments (Fig. 2) and small spheroplasts or large spheres (Fig. 6). Occasionally spores were seen and the enrichments were then discarded.

Pure cultures were isolated by streaking samples of the enrichments on plates containing the same medium solidified with 3% agar. Within 1 to 2 days of incubation at 70 C, compact spreading yellow colonies were seen which contained filamentous organisms as revealed by microscopic observation. These colonies were restreaked, and stock cultures were prepared by inoculating agar slants from the second plate. For long-term storage, all isolates have been preserved in the freeze-dried state. In a few cases, isolates were obtained by streaking the inoculum sources directly on agar plates and incubating at 70 C.

Inoculum sources. Most attempts at isolation of *Thermus* from hot-spring sources were successful, provided that incubations were at 70 to 75 C. No isolations were successful with temperatures greater than 80 C. One isolate was obtained by enriching at 80 C, but this organism grew better at 70 to 75 C. Table 1 lists hot-spring inoculum sources which yielded successful

TABLE 1. *Hot-spring sources yielding Thermus isolates*

Location	Inoculum source temp	Strain designation
	C	
Mushroom Spring; Lower Geyser Basin		
Algal-bacterial mat		
Source	72.9	Y-IV-69-3
Station I	71.5	YT-1, Y-IV-69-4
Station II	69.0	Y-IV-69-5
Station III	66.0	Y-IV-69-6
Station V	62.9	
Pool A; Lower Geyser Basin		
Pink bacterial masses	86.5	Y-IV-69-1 Y-VII-70
Perpetual Spouter; Norris Geyser Basin		
Rock with brown deposit	85	Y-IV-69-2
Rock with brown deposit	91–94	
Boulder Spring; Lower Geyser Basin near Fairy Creek		
Grey bacterial masses	75.1	
Flat Cone Spring; Lower Geyser Basin, Sentinel Meadow		
Yellow bacterial masses	79	
Pacheteau's Calistoga, Hot Springs, Calif.		
Pebble	75	Y-VII-51B
Whitish bacterial masses	64	
Greenish rock with bacterial masses	53	Y-VII-51D

cultures. The studies were not always carried to the point of isolating pure cultures, although microscopic examination revealed typical *Thermus*-like organisms in all cases. In many cases, enrichments attempted at 55 C by using the same inocula yielded motile, spore-forming bacilli which were not characterized further.

Isolations from habitats other than hot springs. One experiment was set up to see whether *Thermus* organisms could be isolated from thermal and nonthermal habitats not associated with hot springs. All of the inocula were collected from locations around Bloomington, Indiana, which is over 200 miles from the nearest hot spring. This experiment is summarized in Table 2. Several inocula yielded *Thermus* isolates, but more yielded spore-formers. In another experiment, the enrichments were carried out at 75 instead of 70 C. Under these conditions, five samples of hot tap water each yielded *Thermus* organisms but no spore-formers. Cold tap water and cold spring water yielded no growth at all in enrichments at 75 C, but a cold spring receiving thermal pollution yielded *Thermus* isolates in all cases. Although not exhaustive, these studies permit the conclusion that *Thermus* is widespread in natural and man-influenced environments, and is associated primarily with thermal habitats. The frequent isolation of *Thermus* from hot tap water is noteworthy. The temperature of hot water in most buildings as it issues from the tap is 55 to 58 C, but the water is probably hotter in the heater. It seems possible that *Thermus* organisms may grow within the hot-water heater itself, using organic nutrients derived from the tap water.

DNA base composition. The DNA base compositions of four *Thermus* isolates were determined. The values obtained were: strain YT-1, 67.4% GC (moles per cent guanine plus cytosine); strain Y-VII-51D, 65.4% GC; strain Y-VII 56-14C, 65.4% GC; strain Y-VII 56-3A, 65.4% GC.

Antibiotic and inhibitor sensitivity. Six representative isolates were checked for sensitivity to various antibiotics and inhibitors (Table 3). Although strains from diverse habitats were tested, the antibiotic sensitivities were remarkably similar. Two strains tested were highly sensitive to actinomycin D (Table 4). For a gram-negative organism, *Thermus* is surprisingly sensitive to penicillin, actinomycin D, and novobiocin.

Physiological and nutritional characteristics. Physiological and nutritional studies were done only with strain YT-1. The relationship between growth rate and temperature is shown in Fig. 1. The optimal temperature for growth was 70 C, the maximum was 79 C, and the minimum was about 40 C. The generation time at the optimum

TABLE 2. *Isolation of Thermus from habitats not associated with thermal springs*

Inoculum source[a]	Result	Strain designation
Cold tap water, Jordan Hall, I.U.[b]	*Thermus*, 2-day incubation	Y-VII-56(2C)
Hot tap water, Jordan Hall, I.U.	*Thermus*, 1-day incubation	Y-VII-56-14C Y-VII-56-3A
Duckweed pond, I.U. greenhouse	Spore-former	
Soil in cactus room, I.U. greenhouse	Spore-former	
Cold tap water dripping over ferns, I.U. greenhouse	No growth	
Soil in tropical room, I.U. greenhouse	*Thermus* and spore-former	
Black soil in tropical room containing blue-green algal mat, I.U. greenhouse	No growth	
Spring water receiving thermal pollution, rear of Jordan Hall, Indiana Univ.	*Thermus* and spore-former	
Scrapings from human skin	No growth	
Human saliva	Spore-former	
Scrapings from human teeth and gums	No growth	
Cold spring south of Bloomington, Ind.	No growth	
Monroe Reservoir, Ind.	No growth	

[a] Enrichments at 70 C.
[b] Indiana University.

TABLE 3. *Sensitivity of various Thermus isolates to antibiotics and other inhibitors*[a]

Agent	Y-VII-51B	Y-VII-51D	Y-VII-56(2C)	Y-VII-70	YT-1	Y-IV-69-6
Cycloserine						
100 μg/ml	−	−	−	−	−	−
10 μg/ml	±	+	+	±	+	+
Streptomycin						
10 μg/ml	−	−	−	−	−	−
Penicillin						
10 μg/ml	−	−	−	−	−	−
Novobiocin						
10 μg/ml	−	−	−	−	−	−
Tetracycline						
100 μg/ml	−	−	−	−	−	−
10 μg/ml	+	+	+	+	+	+
Chloramphenicol						
10 μg/ml	−	−	−	−	−	−
Sodium lauryl sulfate						
100 μg/ml	−	−	−	−	−	−
10 μg/ml	+	+	+	−	+	−
Sodium azide						
500 μg/ml	−	−	−	−	−	−
50 μg/ml	+	+	+	+	+	+
Sodium chloride						
2%	−	−	−	−	−	−
Oxgall						
2000 μg/ml	−	−	−	−	−	−
200 μg/ml	−	−	±	−	−	−

[a] Test medium was basal salts + 0.1% tryptone + 0.1% yeast extract + 3% agar. Incubation was for 3 days at 70 C. Concentrations listed are the lowest tested. Symbols: (−) no growth; (±) isolated colonies; (+) good growth.

TABLE 4. *Sensitivity of T. aquaticus and several other bacteria to actinomycin D*[a]

Organism	Zone size (mm) Antibiotic concn (μg per disc)[b]					
	40	8	2	0.8	0.08	0.008
T. aquaticus YT-1	TL	TL	33	28	15	0
T. aquaticus Y-VII-56 (2C)	TL	TL	TL	TL	23	0
Bacillus subtilis 168	39	34	27	23	15	ND
Myxococcus xanthus FB	TL	25	20	13	0	ND
Flexibacter sp.	23	15	0	0	0	ND
Escherichia coli	0	0	0	0	0	ND

[a] Filter-paper discs (13 mm in diameter) were placed on seeded agar plates, and 0.08 ml of antibiotic stock solutions was applied to the discs. Incubation was at 70 C (*Thermus*) or 30 C (other organisms).
[b] Results given are zone sizes, in millimeters. TL = zone sizes too large to read; ND = not done.

was about 50 min. For most cultural work, an incubation temperature of 70 C was used.

Nutritional studies were performed by using the basal salts medium. Good growth was obtained in 0.1 and 0.33% tryptone plus yeast extract, but no growth was obtained at 1% tryptone plus yeast extract. It is not uncommon for aquatic bacteria to be inhibited by high concentrations of organic matter, so that this result is not surprising. Good growth occurred in 0.1% vitamin-free casein hydrolysate or in 0.5% glutamic acid alone as the sole source of carbon, nitrogen, and energy. With NH_4^+ as nitrogen

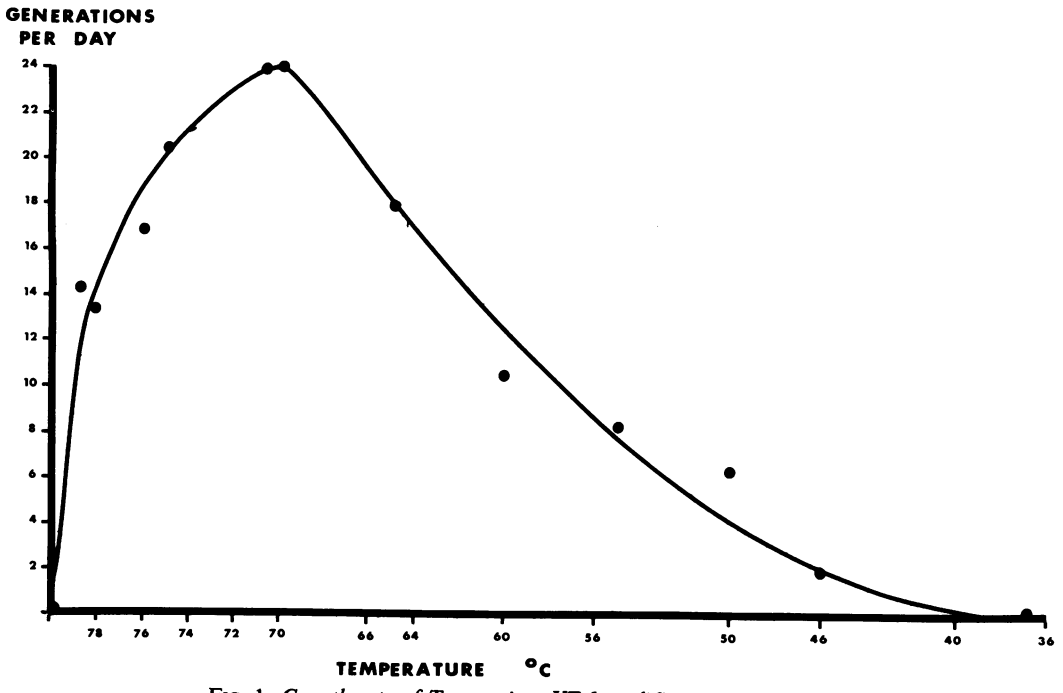


FIG. 1. *Growth rate of T. aquaticus YT-1 at different temperatures.*

source, growth occurred with acetate, sucrose, citrate, succinate, or glucose, although growth was slower than in the complex medium. When one of these carbon sources was present, low concentrations of glutamate could serve as a nitrogen source, as shown by the better growth when one of these carbon sources was added to the low-glutamate medium. No growth occurred with NO_3^- as nitrogen source, and no growth occurred in 0.1% tryptone plus 0.1% yeast extract made up in deionized water instead of basal salts.

In unshaken tubes, the organism frequently grows as a thin pellicle on the surface of the liquid. Both growth rate and growth yield are increased by aeration. Attempts to grow the organism anaerobically have been unsuccessful. From these observations and from the sensitivity of the organism to growth inhibition by sodium azide, we infer that it is an obligate aerobe.

The *p*H optimum for growth of YT-1 is about 7.5 to 7.8. No growth occurred when the initial *p*H was below 6 or above 9.5.

The yellow pigment of YT-1 has been identified by P. Ray and D. C. White as a carotenoid, and studies are in progress to determine its chemical nature.

Morphology. Initial isolates of *Thermus* are usually filamentous, but during continued transfer the filaments become shorter. However, the morphology of the organism is greatly influenced by temperature of growth and by growth stage of the culture. At temperatures of 75 C and above,

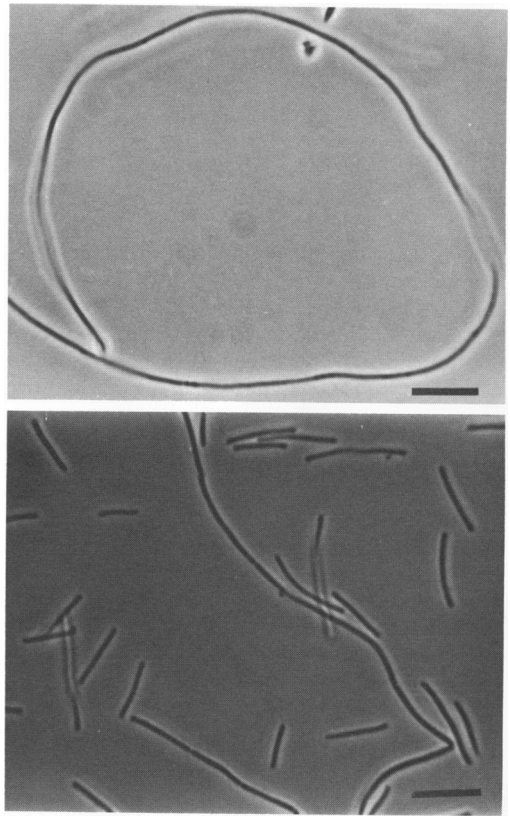


FIG. 2. *Rod and filamentous forms of T. aquaticus. Bar represents 10 μm.*

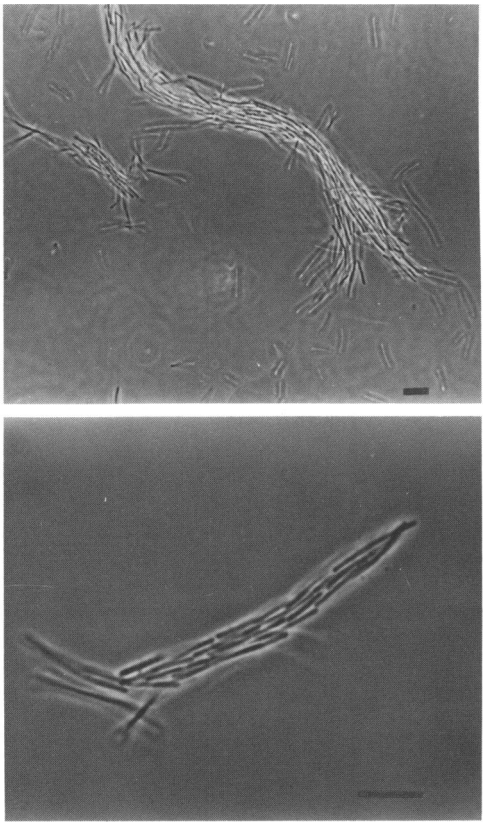


FIG. 3. *T. aquaticus rods aggregating in a linear array. Bar represents 10 μm.*

growth is usually filamentous (Fig. 2, top). Even at temperatures of 65 to 70 C, filaments are common in stationary-phase cultures. A comparison of rod and filamentous forms is shown in Fig. 2 (bottom). Frequently, the rod-shaped forms show a tendency to aggregate, either as linear arrays (Fig. 3) or as rosettes (Fig. 4). We attribute these aggregation phenomena to the presence on the surface of the organism of a slime, which can be seen in Fig. 5. One of the most distinctive characteristics of *Thermus* is the formation of large spherical bodies in older cultures (Fig. 6). These bodies are not simple spheroplasts, since they are much larger than spheroplasts. Spheroplasts as well as large spheres can be induced to form in YT-1 by the use of lysozyme (P. Ray, *unpublished data*). We suggest that the large spheres might be formed by the extrusion of the protoplast of a long rod or filament from one pole. Filaments with swollen ends can be seen frequently in cultures (Fig. 7). Both the large spheres and spheroplasts of YT-1 are quite resistant to osmotic lysis, although they are rapidly destroyed by sodium lauryl sulfate. P. Ray (*unpublished data*) has found that the resistance to osmotic lysis of YT-1 spheroplasts is due to the presence of an unusually stable cell membrane. The spontaneous formation of osmotically stable spheres has been reported in the thermophile *B. stearothermophilus* (1, 10), and we assume that the large spheres of YT-1 form as a result of autolytic phenomena in older cultures. Upon careful microscopic examination, rod-shaped cells of YT-1 can usually be seen wrapped around the large spheres (Fig. 6), and we assume that the large spheres serve as surfaces upon which rod-shaped cells become attached. The manner in which the rods become wrapped around the spheres suggests that the rods possess a degree of flexibility; forms which could be interpreted as flexible are often seen.

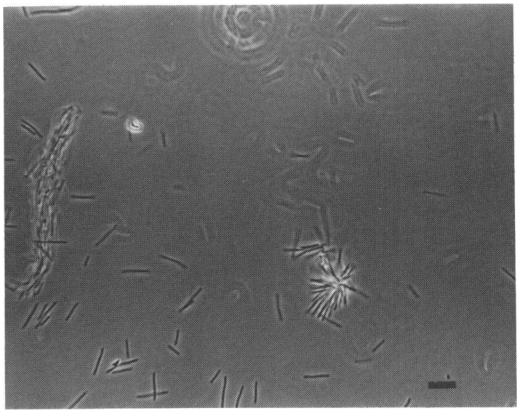


FIG. 4. *T. aquaticus rods aggregating in a rosette. Bar represents 10 μm.*

We have never observed any motility in YT-1, either at room temperature or upon heating the microscope slide to about 70 C. In agar slide cultures incubated at 70 C, slime tracks such as are formed by the gliding myxobacteria were not seen. Colonies on agar plates spread slowly, and the cells at the periphery of the colonies lie parallel to the circumference, so that the edge of the colony is not ragged but quite even, in contrast to the colonies formed by gliding bacteria. Flagella have not been detected either in flagella stains or in shadowed preparations observed with the electron microscope. The organism resembles microscopically many gliding bacteria (M. Dworkin, *personal communication*), and it is possible that our failure to observe gliding is merely a technical difficulty.

The organism is gram-negative. Endospores have never been observed in phase contrast.

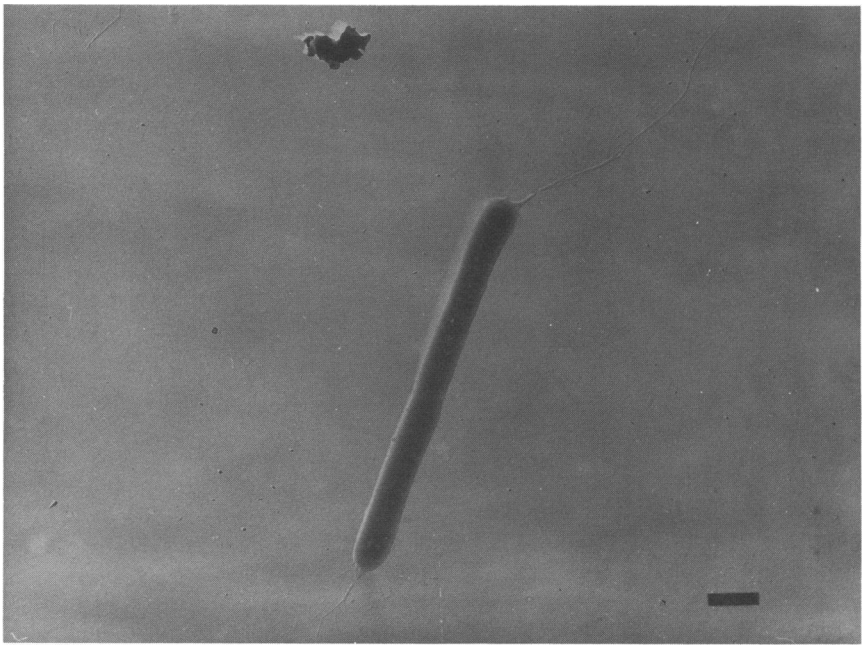


FIG. 5. *Electron micrograph of a T. aquaticus rod, showing presence of slime. Bar represents 1 μm.*

Thermus gen. n.

Morphology: Rods and filaments, 0.5 to 0.8 μm in diameter. Rods 5 to 10 μm in length, filaments of variable length from 20 to greater than 200 μm. Rods occurring singly or in aggregates, the latter either side-to-side or end-to-end (rosette-like). Large spheres 10 to 20 μm in diameter usually formed in old cultures. Gram-negative. Flagella and endospores absent. Gliding motility has not been observed.

DNA base composition: 65.4 to 67.4% GC.

Colony characteristics: Compact slowly spreading colonies. Yellow to bright orange on 0.1% tryptone plus 0.1% yeast extract plus mineral salts-agar.

Liquid culture: Yellow surface pellicle often formed in unshaken liquid cultures.

Temperature relations: Optimum, 70 to 72 C, maximum 79 C, minimum around 40 C.

Nutrition: No growth factor requirements (one strain tested). Nitrogen sources, amino acids and NH_4^+. Carbon sources, sugars, and organic acids. Grows best in complex media such as 0.1 to 0.3% tryptone plus yeast extract.

Relation to oxygen: Obligately aerobic.

Relation to pH: Optimum 7.5 to 7.8. No growth below *p*H 6 or above 9.5.

Inhibitor sensitivity: Inhibited by 10 μg or less of streptomycin, penicillin, novobiocin, actinomycin D, or chloramphenicol per ml. Inhibited by 100 μg or less of cycloserine, tetracycline, and sodium lauryl sulfate per ml. Inhibited by 500 μg or less of sodium azide per ml. Inhibited by 2% NaCl. Inhibited by 200 μg or less of oxgall per ml.

Source: Hot springs, hot tap water.

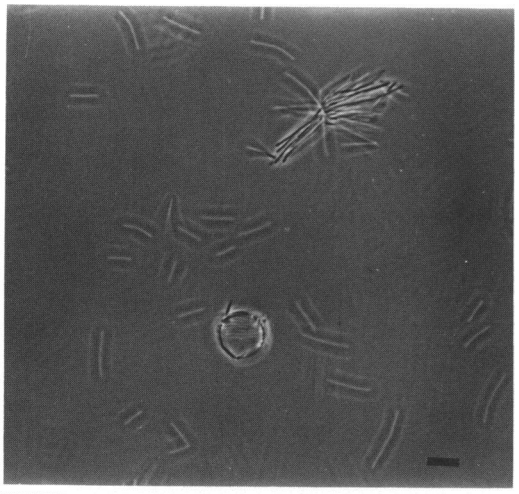
The name given to this organism is derived from the Greek noun, *thermos*, meaning hot. One species.

Thermus aquaticus sp. n.

Definition as for genus. The specific epithet was derived from the Latin noun *aqua*, meaning water. Thus, *T. aquaticus* is an aquatic thermophile. The type strain is YT-1. It is being deposited in the American Type Culture Collection as ATCC 25104. Also being deposited in this collection are strains Y-VII-51B as ATCC 25105 and Y-IV-69-2 as ATCC 25106.

DISCUSSION

For many years, microbiologists have enriched for thermophilic bacteria by incubation at 55 C. It is quite clear, however, that thermophilic bacteria do not all grow optimally at 55 C; they represent a continuum of organisms from those with optima near the mesophilic range to those with optima of 70 C or above (2, 4, 5). At 55 C, *T. aquaticus* grows slowly and hence is probably unable to compete with the spore-



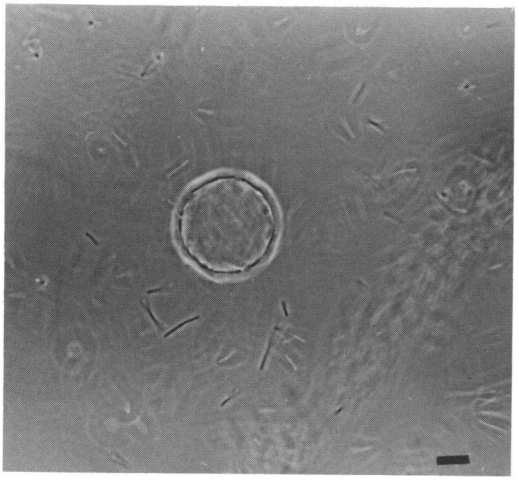


FIG. 6. *Large sphere of the kind frequently seen in T. aquaticus cultures. Bar represents 10 μm.*

forming thermophiles such as *B. stearothermophilus*. This could explain why it has not been seen in the usual thermophilic enrichments. At 70 to 75 C, *Thermus* has a selective advantage and can be easily isolated. It is important to emphasize that the media for *Thermus* enrichments must be fairly dilute in organic constituents, since the organism is inhibited by tryptone and yeast extract at a concentration of approximately 1%. The high content of organic constituents characteristic of most media used for isolation of thermophiles may also explain why *Thermus* has not been seen before. The enrichment conditions prescribed here for *Thermus* are sufficiently selective that the isolation of new strains is extremely easy.

We do not imply that all yellow-pigmented

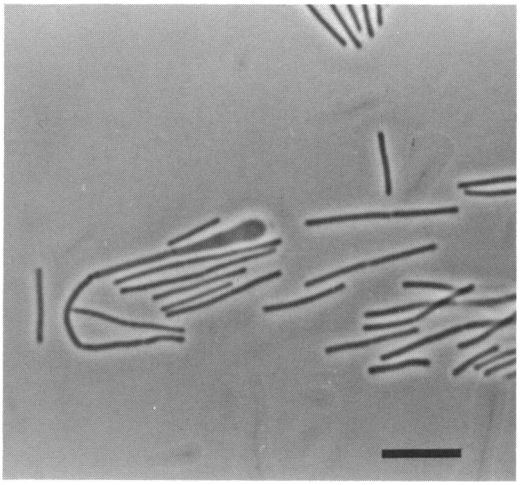


FIG. 7. *T. aquaticus filament with swollen end. Bar represents 10 μm.*

organisms isolated by enrichment with our method will be members of a single species. Detailed study of our strains may reveal differences sufficient to warrant creation of other species or genera. For the moment, however, it seems preferable to classify these organisms in a single species.

The ecological relationships of *Thermus* need further work. Yellow- and orange-pigmented filamentous organisms which resemble *Thermus* morphologically are seen in large numbers in most mildly alkaline hot springs. In the temperature range of 50 to 73 C, these filamentous forms produce extensive gelatinous mats within which the unicellular blue-green alga *Synechococcus* is embedded (T. D. Brock, Phycologia, *in press*). We can routinely isolate *Thermus* from these mats but have not as yet shown that our isolates represent the predominant filamentous organism of the mats, although the physiological and morphological properties of *Thermus* are in agreement with those of the predominant organisms of the mats. At temperatures above 73 C, where blue-green algae do not grow, masses of filamentous bacteria are frequently seen (2; T. D. Brock, Symp. Soc. Gen. Microbiol. 19th, *in press*). Spheroplast-like structures similar to those formed by *Thermus* are often seen in these naturally growing filamentous bacteria (T. D. Brock, Symp. Soc. Gen. Microbiol. 19th, *in press*).

The ease with which *Thermus* can be isolated from hot tap water and other thermal sources suggests that the organism might be a good indicator of thermal pollution.

The determination of the relationship of *Thermus* to other bacteria must wait further studies. As a yellow-pigmented, nonmotile, gram-negative rod, the organism might be considered related to *Flavobacterium*, except that this latter genus is itself poorly defined (M. Mandel, *personal communication*). Also, the formation of long filaments is a property not found in flavobacteria. If the organism could be shown to glide, it might be considered related to the *Flexibacterales* (16), since this group comprises mostly yellow-pigmented, gram-negative organisms, many of which show a rod-filament dimorphism. However, the DNA base composition is considerably higher than that of the flexibacteria (8), and in fact is quite similar to that of the fruiting myxobacteria. In this respect the sensitivity of *T. aquaticus* to actinomycin D is noteworthy. Martin Dworkin has recently informed us that gliding bacteria are considerably more sensitive to actinomycin D than are other gram-negative bacteria. Since *T. aquaticus* shows a sensitivity to actinomycin D as great or greater than the gliding bacteria, this is another feature which prompts a further study of the relationship of *T. aquaticus* to the gliding bacteria. One suggestion is that members of the species *T. aquaticus* represent forms which have lost the ability to glide, yet retain structural features which are responsible for actinomycin D sensitivity.

Filamentous thermophilic bacteria have been described before in hot springs (9, 12, 13) and have usually been given the name *Leptothrix* or *Chlamydothrix* (*see also* 2). Unfortunately, few of these filamentous forms were cultivated or adequately characterized; hence, the relationship of our isolates to these earlier forms is uncertain. Since our isolates do not form either a sheath or motile swarmer cells, it is clear that they bear little relationship to the *Leptothrix-Sphaerotilus* group (14). We have not seen sheathed organisms of the *Leptothrix-Sphaerotilus* group in any of our collections of natural material from hot springs. Further understanding of the taxonomic relationships of *Thermus* to other bacteria will require a better understanding of the taxonomic relationships of the wide variety of filamentous bacteria from nonthermal environments, most of which have been poorly characterized.

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