Thermonema lapsum gen. nov., sp. nov., a Thermophilic Gliding Bacterium

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We previously reported the isolation of five strains of a thermophilic gliding organism. These strains are described here as a new genus and species, *Thermonema lapsum*. The isolates can be readily distinguished from other thermophilic gliding bacteria as they are apparently unicellular aerobic filaments that grow optimally at 60°C. Their cell walls are similar in ultrastructure to those of gram-negative cells, but they are susceptible to penicillin G. Our isolates can be grown on a fully defined medium containing amino acids. Oxidation-versus-fermentation tests indicate that deamination takes place. The type strain, strain 23/9, has been deposited with the American Type Culture Collection as strain ATCC 43542.

A number of nonphotosynthetic gliding bacteria have been isolated from hot spring environments, although none described to date has a temperature optimum above 60°C. Apart from cyanobacteria, two photosynthetic gliding organisms have been isolated from these environments. The first to be described was *Chloroflexus aurantiacus* (7). This organism has a temperature optimum of 52 to 60°C and can grow photoautotrophically or photoheterotrophically under illuminated anaerobic conditions and heterotrophically in both light and dark when it is grown aerobically. *Heliothrix oregonensis* (8) is unique since it contains only one bacteriochlorophyll, bacteriochlorophyll *a*. This organism has not been reported to grow in pure culture, but uptake studies (8) have shown that the temperature optimum is around 40 to 45°C. Sequence comparisons of 5S ribosomal ribonucleic acids indicate that members of the genus *Heliothrix* are most closely related to *Chloroflexus* species (8).

Herpetosiphon geysericola (6) was isolated from a hot spring algal mat, but this organism was grown at 25 to 30°C in subsequent studies (10). Another obligately aerobic heterotroph, *Isosphaera pallida* (2), forms intercalary buds to achieve cell division and can grow at temperatures up to 55°C.

In a previous report (14) we described five new isolates from New Zealand hot springs which exhibit gliding motility. The cells of these strains are long filaments which have terminal swellings, and the organisms have an optimum temperature for growth of 60°C. In the work described in this paper we added to the previous data, and our results support a proposal to place these strains in a new genus, *Thermonema*, with all of the isolates belonging to one species, *Thermonema lapsum*. The type strain has been deposited with the American Type Culture Collection, and one other strain, 19/15, which was isolated from a different hot pool, has also been deposited with the American Type Culture Collection as strain ATCC 43543.

MATERIALS AND METHODS

Strains used. We used four strains whose isolation has been described previously (14); these strains were strains 23/9^T (T = type strain), 23/5, 19/4, and 19/15.

Media and culture conditions. Routine maintenance was on Castenholz medium D under previously described conditions (14).

To test for hydrolysis of insoluble substrates, agar overlay plates were prepared as reported previously for *Thermus* taxonomic tests (4). The upper layer of the overlay contained Sigmacell 50, keratin, or starch at a concentration of 4 g/liter or colloidal chitin, prepared as described by Hsu and Lockwood (3), at a concentration of 3 g/liter.

The fully defined basal medium, which was used to evaluate the substrates utilized, was a modification of American Type Culture Collection medium 284; it contained (per liter) 0.1 g of MgSO₄ · 7H₂O, 0.1 g of KNO₃, 0.1 g of CaCl₂ · 2H₂O, 0.1 g of sodium β-glycerophosphate, and 1 g of tris(hydroxymethyl)aminomethane buffer, as well as a vitamin solution (17), trace elements, and FeCl₃ as described by Ramaley and Hixson (11). The medium was dispensed in 9-ml volumes, and 1-ml additions of sterile stock solutions were made as required.

A synthetic amino acid mixture was prepared as described by Slijkhuis (15). The stock solution which we used contained (per 100 ml) 470 mg of aspartic acid, 210 mg of threonine, 320 mg of serine, 960 mg of glutamic acid, 690 mg of proline, 150 mg of glycine, 200 mg of alanine, 220 mg of valine, 90 mg of methionine, 110 mg of isoleucine, 250 mg of leucine, 70 mg of tyrosine, 140 mg of phenylalanine, 350 mg of lysine, 100 mg of histidine, and 150 mg of arginine. The mixture was filter sterilized and added to the medium in the proportion described above.

Hugh-Leifson oxidation-versus-fermentation tests were carried out as described by Smibert and Krieg (16), using filter-sterilized glucose, maltose, or sucrose at a final concentration of 0.5% (wt/vol).

Ultrastructure. Thin sections and negatively stained grids of strain 23/9^T were prepared for electron microscopy, stained, and examined by using a Philips model EM400 electron microscope as previously described (5).

Antibiotic inhibition. Antibiotic dilutions were prepared to give final concentrations of 100, 10, and 1 µg/ml. Filter-sterilized solutions were added to Castenholz medium D (11) broth, and inoculation was with 0.1 ml of a 24-h broth culture. Growth was recorded after 24 h of incubation at 60°C.

RESULTS AND DISCUSSION

Table 1 shows a comparison of strain 23/9^T with other gliding bacteria which have some of the same characteristics. *Thermonema lapsum* can readily be distinguished from these other organisms by a number of criteria derived from

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TABLE 1. Comparison of *Thermonema lapsum* with other filamentous gliding bacteria^a

Taxon	G+C content (mol%)	Optimum pH	Optimum temp (°C)	Anaerobiosis	Autotrophism	Carotenoids	Cell length (µm)	Cell width (µm)
<i>Thermonema lapsum</i>	47	6.5	60	—	—	+	60+	0.25–0.3
<i>C. aurantiacus</i>	55	7.6–8.4	52–60	F ^b	F	+	2–6	0.6–0.7
<i>Heliothrix oregonensis</i>	NA ^c	NA	40–45	—	+	+	10	1.5
<i>I. pallida</i>	62.2	7.8–8.8	41	—	—	+	2.5–3	2.5–3
<i>Flexibacter</i> spp.	48	NA	Mesophilic	—	—	—	Variable	<1

^a Data from references 2, 7–9, 12, and 14.^b F, Facultative.^c NA, Not available.

the data presented here and by criteria described previously (14).

Both *C. aurantiacus* and *Heliothrix oregonensis* are photosynthetic organisms. *C. aurantiacus* has a higher guanine-plus-cytosine (G+C) content, is a facultative anaerobe, and has a higher optimum pH than the strains described here. *Heliothrix oregonensis* has not been grown in pure culture and has a considerably lower optimum temperature than *Thermonema lapsum*. The obligate aerobe *I. pallida* has a much higher G+C content, has a lower optimum temperature, has a distinctive morphology, and grows at higher pH values than *Thermonema lapsum*.

The members of the genus *Thermus* can also have a filamentous cell morphology, and one species, *Thermus filiformis* (5), consistently produces filaments. However, the isolates of *Thermonema lapsum* differ from *Thermus* species in G+C content, aminopeptidase reaction, motility, and optimum pH and temperature. In addition, while *Thermus* species grow on a range of monosaccharides, amino acids, and tricarboxylic acid cycle intermediates (5), it was noted at an early stage that growth of *Thermonema lapsum* does not occur on carbohydrates and that peptone appears to be a nutritional requirement (14). To confirm this, a range of carbon sources were subsequently tested by adding them to the basal medium. Medium containing Casamino Acids was used as a positive control. Only Casamino Acids and synthetic amino acid mixtures supported growth of *Thermonema lapsum*.

Incubation under both the aerobic and anaerobic conditions of the Hugh-Leifson test resulted in no acid formation. Instead, there was a pronounced alkalization of the medium, suggesting that deamination was occurring. These results are consistent with the nutritional data reported above. Strains of the genus *Thermus* show no reaction in such tests (1).

Figure 1 shows the cell wall structure of strain 23/9^T. The cell wall of this organism is similar in ultrastructure to the cell walls of gram-negative organisms. In negatively stained preparations (Fig. 2) there was evidence of a slime or capsule layer, which is consistent with this organism being a gliding bacterium. No septa were observed in a number of thin sections prepared on different occasions so we concluded that each of the filaments is probably unicellular.

The Gram reaction, cell wall structure, and aminopeptidase test all indicate that *Thermonema lapsum* is a gram-negative organism, whereas members of the genus *Thermus* have many biochemical characteristics similar to those of gram-positive bacteria (5).

All of the *Thermonema lapsum* strains which we tested were susceptible to penicillin G and erythromycin at the lowest concentration tested, while all of the strains were resistant to nalidixic acid at a concentration of 100 µg/ml. The strains grew in the presence of 1 µg of vancomycin per

ml, 10 µg of kanamycin per ml, and 10 µg of D-cycloserine per ml, and all but one strain grew in the presence of 1 µg of neomycin per ml. The susceptibility to penicillin G is shared by *Thermus* species (5), while the newly described strains are more resistant to kanamycin than members of the genus *Thermus* (5). The results for all of the *Thermonema lapsum* strains which we tested were very similar, supporting the conclusion that these organisms are closely related phenotypically.

The strains which we isolated might also represent thermophilic variants of an otherwise mesophilic genus. Of the gliding bacteria, *Thermonema lapsum* most closely resembles the members of the genus *Flexibacter* (12), but it differs in a number of important features which are used to define the mesophilic genus.

Unlike members of the genus *Flexibacter*, the new strains do not produce flexirubin (14) and do not show a change in morphology from filaments to rods as the age of the culture increases. The gliding motility of *Thermonema lapsum* is not "agile" as reported for *Flexibacter* species in young cultures. The cell wall of *Flexibacter polymorphus* is distinctive, with the production of "goblet-like" structures on the outer surface of the cell envelope (13). Thin sections of *Thermonema lapsum* showed no such structures; and the cell walls had a gram-negative appearance and, as such, were similar to the cell walls of another *Flexibacter* sp. strain, strain FS-1 (9). Unlike many members of the order *Cytophagales*, the strains of *Thermonema lapsum* which we tested were unable to hydrolyze the polymers tested. After 72 h of incubation no zones of hydrolysis were noted on cellulose or starch. None of the strains grew on medium containing chitin or keratin.

FIG. 1. Thin-section electron micrograph of *Thermonema lapsum* cell wall showing the gram-negative structure. Bar = 0.1 µm.

FIG. 2. Negatively stained electron micrograph of *Thermanema lapsu* showing possible slime or capsular material (arrow). Bar = 1 μ m.

These differences from known mesophilic and thermophilic gliding bacteria lead us to propose a new genus and species to contain the newly described isolates.

***Thermanema* gen. nov.** *Thermanema* (Ther.mo.ne'ma. Gr. adj. *thermos*, hot; Gr. n. *nema*, a thread; M.L.neut.n. *Thermanema*, a thermophilic thread). Apparently unicellular filaments usually around 60 μ m long and 0.25 to 0.3 μ m in diameter. Aerobic. Gram negative and aminopeptidase positive. Motile by gliding. Cells possess acetone-extractable pigments with an absorbance peak at 450 nm. Oxidase and catalase positive. Thermophilic, growing optimally at 60°C and at temperatures up to 70°C at neutral pH. The type species is *Thermanema lapsu*.

***Thermanema lapsu* sp. nov.** *Thermanema lapsu* (lap'su-m. L. n. *lapsu* glide). The G+C content of type strain 23/9 is 47 mol%. All five strains which we tested are α - and β -galactosidase negative and deoxyribonuclease positive. All strains are proteolytic. No strain hydrolyzes cellulose or starch.

The following basal medium supplements do not support growth: acetate, L-alanine, casein, L-cystine, galactose, gelatin, gluconate, glucose, inositol, lactose, L-malate, L-proline, propan-1-ol, pyruvate, rhamnose, ribose, skim milk, sorbitol, succinate, sucrose, and yeast extract (all at concentrations of 1 g/liter) plus the glutamate amino acid family (glutamate, proline, and arginine) (all at concentrations of 3.3 g/liter).

The following basal medium supplements support growth: Casamino Acids (1, 10, and 25 g/liter), amino acid mixture 1 (as described in Materials and Methods), and amino acid mixture 2 (the same as amino acid mixture 1 except that it lacks methionine, phenylalanine, tyrosine, and leucine).

Type strain 23/9 and one other strain, strain 19/15, were isolated from New Zealand hot springs and have been deposited with the American Type Culture Collection as strains ATCC 43532 and ATCC 43543, respectively.

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LITERATURE CITED

1. Degryse, E., N. Glansdorff, and A. Pierard. 1978. A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch. Microbiol.* 117:189-196.
2. Giovannoni, S. J., E. Schabtach, and R. W. Castenholz. 1987. *Isosphaera pallida*, gen. and comb. nov., a gliding, budding eubacterium from hot springs. *Arch. Microbiol.* 147:276-284.
3. Hsu, S. C., and J. L. Lockwood. 1975. Powdered chitin as a selective medium for enumeration of actinomycetes in water and soil. *Appl. Microbiol.* 29:422-426.
4. Hudson, J. A., H. W. Morgan, and R. M. Daniel. 1986. A numerical classification of some *Thermus* isolates. *J. Gen. Microbiol.* 132:531-540.
5. Hudson, J. A., H. W. Morgan, and R. M. Daniel. 1987. *Thermus filiformis* sp. nov., a filamentous caldophilic bacterium. *Int. J. Syst. Bacteriol.* 37:431-436.
6. Lewin, R. A. 1970. New *Herpetosiphon* species (Flexibacterales). *Can. J. Microbiol.* 16:517-520.
7. Pierson, B. K., and R. W. Castenholz. 1974. A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. *Arch. Microbiol.* 100:5-24.
8. Pierson, B. K., S. J. Giovannoni, D. A. Stahl, and R. W. Castenholz. 1985. *Heliothrix oregonensis* gen. nov., sp. nov., a phototrophic filamentous gliding bacterium containing bacteriochlorophyll a. *Arch. Microbiol.* 142:164-167.
9. Poos, J. C., F. R. Turner, D. White, G. D. Simon, K. Bacon, and C. T. Russel. 1972. Growth, cell division, and fragmentation in a species of *Flexibacter*. *J. Bacteriol.* 112:1387-1395.
10. Quinn, G. R., and V. B. D. Skerman. 1980. Retrofiliation and tumulation by strains of *Herpetosiphon* species. *Curr. Microbiol.* 3:345-348.
11. Ramaley, R. F., and J. Hixson. 1970. Isolation of a nonpigmented, thermophilic bacterium similar to *Thermus aquaticus*. *J. Bacteriol.* 103:527-528.
12. Reichenbach, H., and M. Dworkin. 1981. The order Cytophagales (with addenda on the genera *Herpetosiphon*, *Saprospira* and *Flexithrix*), p. 356-379. In M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes. A handbook on habitats, isolation and identification of bacteria*. Springer-Verlag KG, Berlin.
13. Ridgway, H. F., R. M. Wagner, W. T. Dawsey, and R. A. Lewin. 1975. Fine structure of the cell envelope layers of *Flexibacter polymorphous*. *Can. J. Microbiol.* 21:1733-1750.
14. Schofield, K. M., J. A. Hudson, H. W. Morgan, and R. M. Daniel. 1987. A thermophilic gliding bacterium from New Zealand hot springs. *FEMS Microbiol. Lett.* 40:169-172.
15. Slijkhuys, H. 1983. *Microthrix parvicella*, a filamentous bacterium isolated from activated sludge: cultivation in a chemically defined medium. *Appl. Environ. Microbiol.* 46:832-839.
16. Smibert, R. M., and N. R. Krieg. 1981. General characterization, p. 409-443. In P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), *Manual of methods for general bacteriology*. American Society for Microbiology, Washington, D. C.
17. Wolin, E. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* 238:2882-2886.