

Phylogenetic analysis of the family Thermaceae with an emphasis on signature position and secondary structure of 16S rRNA

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Abstract

The sequences of the 16S rRNA genes from 38 strains of the family Thermaceae were compared by alignment analysis. The genus-specific and species-specific base substitutions or base deletions (signature positions) were found in three hypervariable regions (in the helices 6, 10 and 17). The differentiation of secondary structures of the high variable regions in the 5' end (38–497) containing several signature positions further supported the concept. Based on the comparisons of the secondary structures in the segments of 16S rRNAs, a key to the species of the family Thermaceae was proposed.

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Keywords: Thermaceae; 16S rRNA; Signature position; Secondary structure

1. Introduction

Since Brock et al. isolated and named the extreme thermophile *Thermus aquaticus* from Yellowstone National Park [1], around the world various bacterial strains have been isolated from many hydrothermal areas with water temperatures higher than about 55°C and pH ranging from neutral to alkaline. On the basis of the phylogenetic data of 16S rRNA, the growth temperature, polar lipid pattern and the hydroxy fatty acid composition, Nobre et al. proposed the new genus *Meiothermus* reclassified from the genus *Thermus* [2]. Rainey and da Costa proposed 'Thermales ord. nov.' and 'Thermaceae fam. nov.' [3]. Sako et al. proposed '*Marinothermus hydrothermalis* gen. nov., sp. nov.' [4] and Miroshnichenko et al. pro-

posed '*Oceanothermus profundus* gen. nov., sp. nov.' [5]. Up to now, the order Thermales consists of only one family Thermaceae, which includes four genera: *Thermus*, *Meiothermus*, *Marinothermus* and *Oceanothermus*. The genus *Meiothermus* comprises five validly published species: *M. ruber*, *M. silvanus*, *M. chliarophilus*, *M. cerebereus* [6], and *M. taiwanensis* [7]. Eight species of the genus *Thermus* have been validly published: *T. aquaticus* [1], *T. thermophilus* [8], *T. filiformis* [9], *T. scotoductus* [10], *T. brockianus* [11], *T. oshimai* [12], *T. igniterrae* and *T. antranikianni* [13]. Four species (*Meiothermus rosaceus* [14], *Thermus rehai* [15], *Thermus nonproteolyticus* [16], *Thermus kawarayuenensis* [17]) have not appeared in the validly published list (<http://www.bacterio.net>). The genus *Marinothermus* included a species *M. hydrothermalis* and the genus *Oceanothermus* included a species *O. profundus*. The taxonomy of the Thermaceae remains to be refined. Phylogenetic analysis of 16S rRNA incorporating consideration of secondary structure has been proven to be a powerful tool for the identification and classification of microorganisms [18–20]. In this report, the sequences of the 16S rRNA genes from 38 strains in the family Thermaceae were compared and phylogenetically analyzed. Based on the comparison of secondary structures in hypervariable regions of 16S rRNAs, a key to the species of this family Thermaceae has been proposed.

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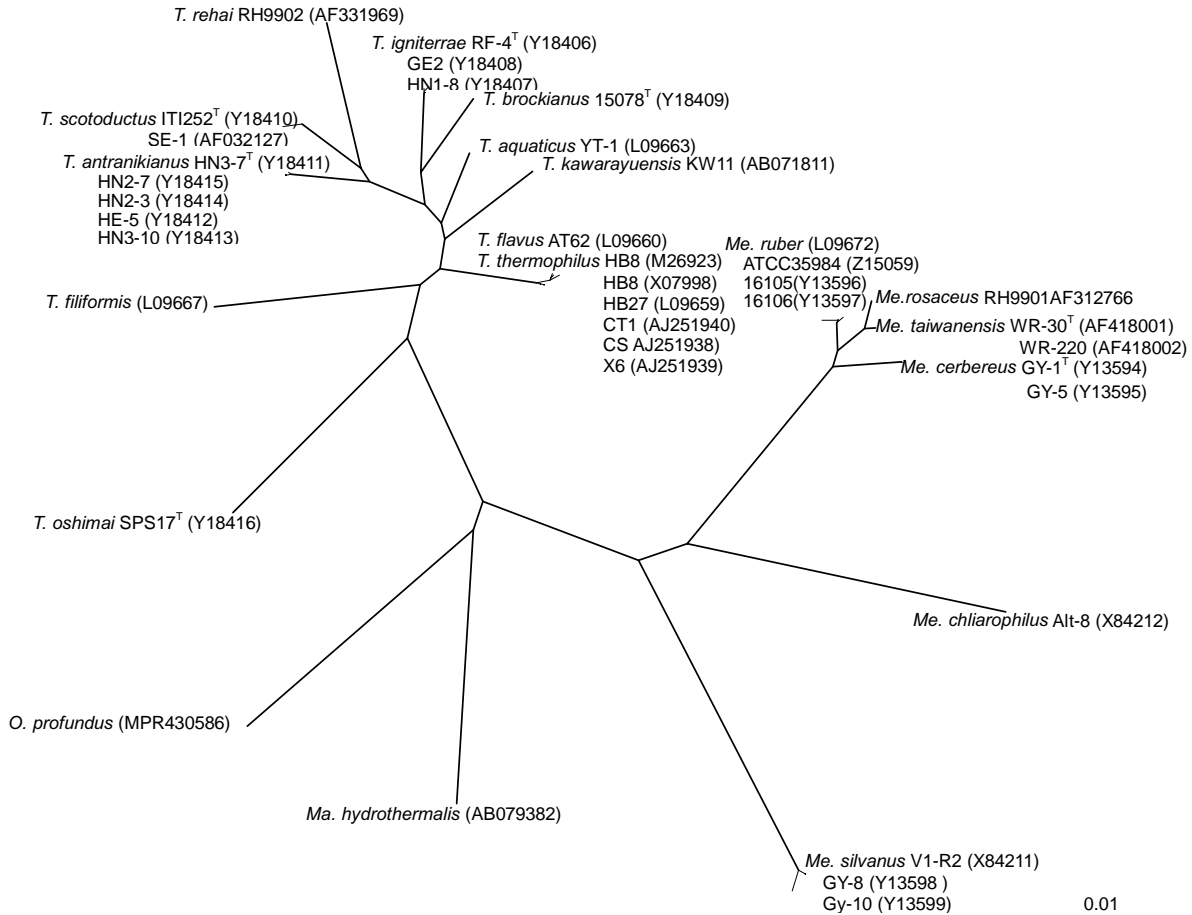


Fig. 1. Unrooted phylogenetic tree for sequences of the 38 published 16S rRNA genes in *Thermaceae*. The phylogenetic tree was constructed by the neighbor-joining method. The scale bar represents one inferred nucleotide change per 100 nucleotides. GenBank accession numbers are in parentheses.

2. Materials and methods

2.1. Sequence alignment

The 16S rDNA sequences of 38 strains in the family Thermaceae available in the public database GenBank were chosen for phylogenetic analysis. Their nucleotide sequence accession numbers were shown in Fig. 1.

The sequences were aligned to the available sequences in GenBank using the software BLASTN. The alignment was then manually refined in variable regions or sign segments using CLUSTALX [21]. A phylogenetic dendrogram was

generated by the parsimony method contained in the PAUP [22] and TreeView package [23].

2.2. Construction of secondary structure

To simplify the construction of secondary structure models, 5' ends of the 16S rRNA of all selected strains were folded by using the free-energy minimization algorithm with the RNAdraw v1.1 program [24]. The base position and helix numbers of *Escherichia coli* were adopted from Maly et al. [25].

Table 1
The genus-specific base substitutes in the 16S rDNAs of the family Thermaceae

Genus	H6	H8	H7	H11	H12	H14	H17	H18	H21	H22	H23	H24	H25	H26	H27	H32	H33	H35	H39	H34	H39	H44	H45																
<i>Thermus</i>	G	G	C	G	C	C	G	C	G	C	A	<u>G</u>	<u>C</u>	<u>G</u>	<u>G</u>	<u>C</u>	C	G	A	<u>A</u>	<u>C</u>	<u>G</u>	<u>G</u>	<u>G</u>	<u>G</u>	<u>G</u>	<u>G</u>	<u>AGC</u>	<u>C</u>	A	<u>G</u>	A	A	A	<u>C</u>	<u>C</u>	C	T	<u>G</u>
<i>Meiothermus</i>	<u>A</u>	<u>G</u>	<u>T</u>	<u>A</u>	<u>G</u>	<u>G</u>	<u>C</u>	<u>T</u>	<u>A</u>	<u>A</u>	C	<u>T</u>	A	<u>T</u>	<u>A</u>	A	G	G	C	T	<u>T</u>	<u>A</u>	<u>C</u>	GAG	T	A	A	<u>C</u>	A	A	T	T	C	T	A				
<i>Marinothermus</i>	G	G	C	G	C	C	G	C	G	C	A	A	C	AA	T	A	T	C	<u>G</u>	<u>G</u>	G	C	C	C	G	G	GAC	T	<u>G</u>	A	A	A	<u>C</u>	T	T	<u>T</u>	<u>C</u>	A	
<i>Oceanothermus</i>	G	<u>A</u>	C	G	C	C	G	C	G	<u>T</u>	A	A	C	AA	T	A	T	C	G	A	G	G	C	<u>C</u>	C	G	G	GAC	T	A	A	A	<u>C</u>	A	T	T	C	T	A

The underlined letters in bold indicate base substitutions that are genus-specific.

3. Results

3.1. Sequence alignment

By comparison of 38 sequences of the family Thermaceae 16S rDNAs deposited in GenBank, 43 genus-specific base substitution events were found (Table 1). Three hypervariable regions were presented in 38 sequences of 16S rDNA in the family Thermaceae. The most drastic differences between the species occurred at positions 70–100, 213–224 and 443–493. The obvious changes in the regions were the deletion events of 7–15 nucleotides (Table 2). Based on the result of the phylogenetic analysis of the 16S rDNA sequences, the 38 strains could be categorized into 18 species: *T. aquaticus*, *T. thermophilus*, *T. filiformis*, *T. scotoductu*, *T. brockianus*, *T. oshimai*, *T. igniterrae*, *T. antranikianni*, *T. rehai*, *T. kawarayuensis*, *M. ruber*, *M. silvanus*, *M. cerebereus*, *M. rosaceus*, *M. taiwanensis*, *O. profundus*, *M. hydrothermalis* (Fig. 1).

3.2. Secondary structure

The secondary structure of the 5' extremities (38–497) containing three hypervariable regions (H6, H10 and H17) of 16S rRNAs from *T. rehai* was predicted using the free-energy minimization algorithm with the RNAdraw program (Fig. 2). The structures of the other species were conducted and not shown in this paper. The significant structural differences in secondary structures of all above species appeared in the helices 6, 9, 10, 12 and 17 (Table 3).

Based on the helices 6, 10 and 12 in the secondary structures of 16S rRNAs, a key to the species of the family Thermaceae was proposed as follows.

1. Helix 6	6(4)	<i>Thermus oshimai</i>
2. Helix 6	8(4)	
a.	Helix 10 4(5)	<i>T. scotoductu</i>
b.	Helix 10 5(4)	<i>T. antranikianni</i>
3. Helix 6	9(4)	
a.	Helix 10 4(3)	<i>T. aquaticus</i>
b.	Helix 10 4(5)	<i>T. igniterrae</i>
c.	Helix 10 5(4)	<i>T. rehai</i>
4. Helix 6	10(3)	<i>T. kawarayuensis</i>
5. Helix 6	11(3)	
a.	Helix 10 4(3)	<i>T. thermophilus</i>
b.	Helix 10 4(4)	<i>T. brockianus</i>
c.	Helix 10 4(5)	<i>T. filiformis</i>
6. Helix 6	2[2]6(4)	
a.	Helix 12 2{1}4(8)	<i>Meiothermus ruber</i>
b.	Helix 12 4{3}4(4)	<i>M. rosaceus</i>
c.	Helix 12 7(9)	<i>M. cerebereus</i>
7. Helix 6	2[2]6(5)	<i>M. taiwanensis</i>
8. Helix 6	2[2]7(4)	<i>M. silvanus</i>
9. Helix 6	7(4)	<i>M. chliarophilus</i>
10. Helix 6	2[2]12[2]3(4)	<i>Marinothermus hydrothermalis</i>
11. Helix 6	13[5]	<i>Oceanothermus profundus</i>

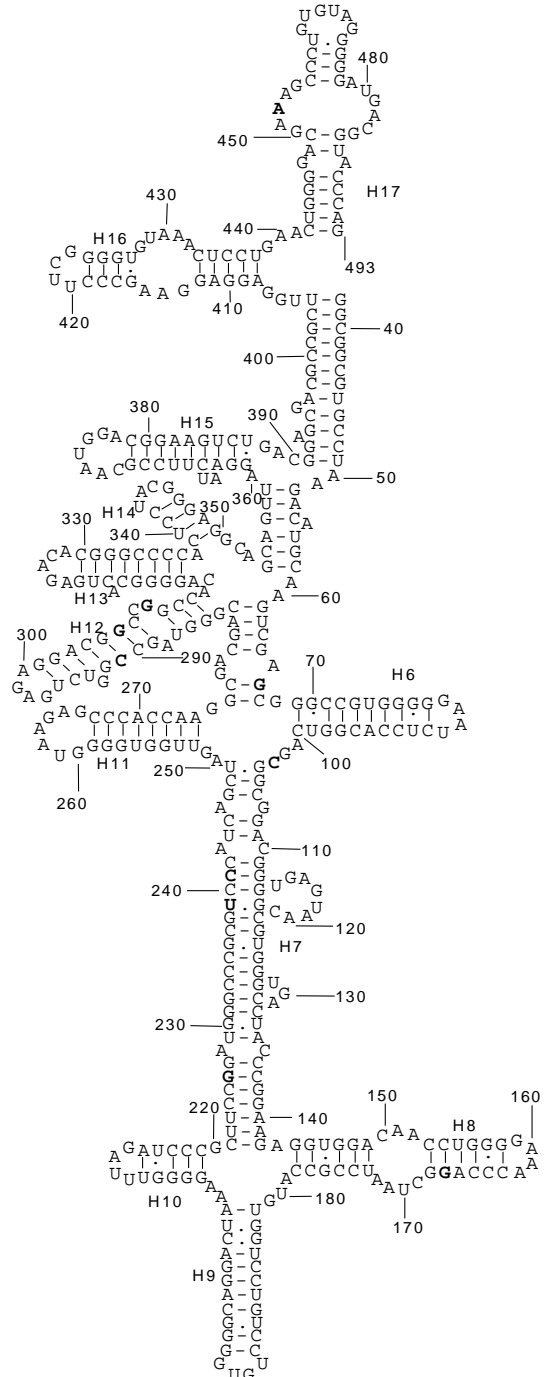


Fig. 2. Secondary structure model of 16S rRNA of *T. rehai* 9902 at positions 38–493 estimated by the free-energy minimization algorithm. Position and helix numbers refer to the equivalent region in the *E. coli* 16S rRNA [25]. Boldface bases refer to the transitional substitutions of bases between *Thermus*, *Meiothermus*, *Marinothermus* and *Oceanothermus*.

In the key, the number without brackets indicates the number of basepairs in the helix. The number in the brackets () indicates the number of bases in the apex loop. The number in the brackets [] indicates the number of bases in the bubble. The number in the brackets { }

Table 2

The genus-specific deletions and species-specific fragments at helices 6, 10 and 17 of the 16S rDNAs in 18 species of four genera in the family Thermaceae

Species	70-100 ^a (in helix 6)	213-224(in helix 10)	443-493(in helix 17)
Genus <i>Thermus</i>			
<i>T. rehai</i>	gccgtg---gggaat-ct-----cacggtc	gggttagatcc- ^b CGCTTc	tggGGACGAaagccctgtga-gggggaTGAcGGTACccaGg
<i>T. aquaticus</i>	gccgtg---gggtat-ct-----cacggtc	gg--tttt-gcc-CGCTTc	ccgGGACGAaacccttgatga-ggggacTGAcGGTACcggGg
<i>T. thermophilus</i>	gccgcg---gggtttact-----ccgtggtc	gg--ctttgcc-CGCTTc	ccgGGACGAaacccttgatga-ggggacTGAcGGTACcggGg
<i>T. igniterrae</i>	gccgtg---gggtttctca-----cggtc	gg-tgacgagcc-CGCTTc	tggGGACGAaagccctgtga-gggggaTGAcGGTACccaGg
<i>T. antranikianii</i>	ggta-----ggttatgc-----ctac--cc	gggtgtagatcccCGCTTc	tggGGACGAaagccctgtga-gggggaTGAcGGTACccaGg
<i>T. filiformis</i>	gctgcg---gggtttact-----ccgtggtc	gg-tgaagagcc-CGCTTc	ccaGGACGAaatccctgatga-gggggaTGAcGGTACtggGg
<i>T. brokianus</i>	gccatg---gggtttact-----ccgtggtc	ggcgagagt-c---CGCTTc	tggGGACGAaagcccttgatga-gggggaTGAcGGTACccaGg
<i>T. scotoductus</i>	ggca-----ggttatgc-----ctgt--tc	gg-tggatagcc-CGCTTc	tggGGACGAaagccctgtga-gggggaTGAcGGTACccaGg
<i>T. kawarayuensis</i>	gccgtg---gggtttcc-----ca-tggtc	gg--ttttacc---CGCTTc	ccgGGACGAaatccctgtga-gggggaTGAcGGTACcggGg
<i>T. oshimai</i>	gggtg-----gttcgcc-----ac--cc	gc--caaaagc---CGCTTc	ctgGGACGAaacccttgatga-gggggaTGAcGGTACcagGg
Genus <i>Meiothermus</i>			
<i>Me. rosaceus</i>	gct-----gttt-at-----gcag--cc	c---ttcgg--CGCTTt	tcaGGACGAta-----aTGAcGGTACccaGg
<i>Me. taiwanensis</i>	gct-----gtttat-----gcag--cc	c---ttcgg--CGCTTt	tcgGGACGAta-----aTGAcGGTACccaGg
<i>Me. ruber</i>	gct-----gttt-at-----gcag--cc	c---ttcgg--CGCTTt	tcgGGACGAta-----aTGAcGGTACccaGg
<i>Me. cerbereus</i>	act-----gtt-cg-----gcag--tt	c---ttcgg--CGCTTt	tcgGGACGAta-----aTGAcGGTACccaGg
<i>Me. chliarophilus</i>	gcc-----ggtttc-----gccgg--cc	c---ctcgg--CGCTTg	tcgGGACGAtg-----gTGAcGGTACccaGg
<i>Me. silvanus</i>	ggct-----gatt-cgg-----tcagg-ct	a---tttat---CGCTTt	aggGGACGAtg-----aTGAcGGTACcgtGc
Genus <i>Marinothermus</i>			
<i>Ma. hydrothermalis</i>	ggggtctttggctttttg-----ctgggggctcct	g--cgcgagc--CGCTTc	ccgGGACGAaagccctgtttgggggaTGAcGGTACcggGg
Genus <i>Oceanothermus</i>			
<i>O. profundus</i>	agcttgcgccgagctctcggacactgggtaagctt	gg--cttcggc--CGCTTc	aggGGACGAaaccctgc--aa-ggggaaTGAcGGTACcctGc

^aNumerals indicate the positions and helix numbers refer to the equivalent region in the *E. coli* 16S rRNA.

^bThe italic and uppercase letters indicate bases that are identical in all species.

Table 3

The main differences in the species-specific region of helices 6, 9, 10, 12 and 17 in the secondary structures of 16S rRNAs in 18 species of Thermaceae

Species	H6	H9	H10	H12	H17
Genus <i>Thermus</i>					
<i>T. rehai</i>	9 ^a (4) ^b	11(4)	5(4)	4{3}4{1}3(4)	5[2]2[1]14(6)
<i>T. aquaticus</i>	9(4)	11(4)	4(3)	3[3]4{1}3(4)	5[2]2[1]24(5)
<i>T. igniterrae</i>	9(4)	11(4)	4(5)	2[2]7(9)	5[2]2[1]14(6)
<i>T. antranikianus</i>	8(4)	11(4)	5(5)	3[3]4{1}3(7)	5[2]2[1]24(5)
<i>T. scotoductus</i>	8(4)	11(4)	4(5)	2[2]7(9)	5[2]2[1]14(6)
<i>T. filiformis</i>	11(3)	11(4)	4(5)	2[2]7(9)	5[2]2[7]6(6)
<i>T. thermophilus</i>	11(3)	5[2]5(4)	4(3)	2[2]7(9)	5[2]2[1]24(5)
<i>T. brokianus</i>	11(3)	11(4)	4(4)	2[2]7(9)	5[2]2[1]24(5)
<i>T. kawarayuensis</i>	10(3)	11(4)	4(4)	2[2]7(9)	5[2]2[10]5(5)
<i>T. oshimai</i>	6(4)	5[2]5(4)	3(4)	2[2]7(9)	5[2]2[1]34(4)
Genus <i>Meiothermus</i>					
<i>M. rosaceus</i>	2[2]6(4)	11(4)	2(4)	4{3}4(4)	5[6]5[6]3(15)
<i>M. ruber</i>	2[2]6(4)	11(4)	2(4)	2{1}4(8)	5[2]2(10)
<i>M. cerbereus</i>	2[2]6(4)	5[2]5(4)	2(4)	7(9)	5[2]2(10)
<i>M. taiwanensis</i>	2[2]6(5)	11(4)	2(4)	2{1}4(8)	5[2]2(10)
<i>M. silvanus</i>	2[2]7(4)	8(6)	(6)	7(9)	5[2]2(10)
<i>M. chliarophilus</i>	7(4)	3(5)	8(8)	4(4)	5[2]2{2}2(4)
Genus <i>Marinothermus</i>					
<i>M. hydrothermalis</i>	2[2]12[2]3(4)	5[2]5(4)	3(4)	2[2]7(9)	5[2]2[1]24(4)
Genus <i>Oceanothermus</i>					
<i>O. profundus</i>	13(5)	11(4)	3(4)	2[2]7(9)	5[2]2[1]15(5)

^aThe number indicates the number of basepairs in the helix.

^bThe number in the brackets () indicates the number of bases in the apex loop.

^cThe number in the brackets [] indicates the number of bases in the bubble.

^dThe number in the brackets {} indicates the number of bases in the bulge.

indicates the number of bases in the bulge. By this key, 38 Thermaceae strains are classified into 18 species.

4. Discussion

In general, phylogenetic analysis using 16S rDNA is based on the comparison of similarity of the 16S rDNA molecules. When comparing 16S rRNA sequence data with those derived from public databases, **the main problem one encounters is how to evaluate sequence differences and how to derive conclusions about the relatedness of organisms.** This has to be accomplished by using an alignment based on secondary structure rather than simply aligning sequences based on sequence similarity. In major taxonomic groups, such as the archaeobacteria and the eubacteria, surprisingly few changes in the secondary structure occur. **Therefore, secondary structure variation suggests considerable phylogenetic diversity [18].** This method was successfully used to identify and classify *Streptomyces*, *Bacillus sivalis* and algae [18–20]. William et al. noted the species-specific regions of 16S rRNA from 18 strains of four *Thermus* species [11,12]. In this paper, the genus- and species-specific regions appeared at same positions of 16S rRNA from 38 strains of three genera in the family Thermaceae, and among closely related species it is common to observe the genus- and species-specific differences in secondary structure. These structures are typically different in terms of the number of pairs in a helix and the number of nucleotides in a loop. **Based on the differences of the helices 6, 10 and 12 in the secondary structures of 16S rRNAs, a key to the species of the family Thermaceae was proposed.** Based on the signatures and secondary structures of 16S rRNAs, 38 Thermaceae strains are classified into 18 species, more than 14 validly published species. It is in accordance with the data listed as taxonomy browser of NCBI (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>).

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