Rapidithrix thailandica gen. nov., sp. nov., a marine gliding bacterium isolated from samples collected from the Andaman sea, along the southern coastline of Thailand

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The taxonomic positions of three strains of marine gliding bacteria, TISTR 1736, TISTR 1741 and TISTR 1750^T, isolated from the southern coastline of Thailand were evaluated by using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the three isolates formed a distinct lineage within the family 'Flammeovirgaceae', phylum *Bacteroidetes*, and were related to the genus *Flexithrix*. The DNA G + C contents of the isolates were in the range 40–43 mol%. The major respiratory quinone was MK-7. The major cellular fatty acids were 16 : 1 ω 5c (*cis*-5-hexadecenoic acid) and 15 : 0 (pentadecanoic acid). The major hydroxyl fatty acids were 3-OH 17 : 0 (3-hydroxyheptadecanoic acid), 3-OH 15 : 0 (3-hydroxypentadecanoic acid) and 3-OH 16 : 0 (3-hydroxyhexadecanoic acid). On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic data, these marine bacteria are considered to represent a novel species of a new genus, for which the name *Rapidithrix thailandica* gen. nov., sp. nov. is proposed. The type strain of *Rapidithrix thailandica* is TISTR 1750^T (=IAM 15448^T).

Gliding bacteria belonging to the *Cytophaga*–*Flavobacterium*–*Bacteroides* (CFB) complex have been poorly investigated in terms of their phylogeny, despite the fact that they are one of the dominant bacterial groups in the marine habitat (Pinhassi *et al.*, 1997). The taxonomic position of the CFB complex remains unclear (Shah & Collins, 1988; Bernardet *et al.*, 1996; Suzuki *et al.*, 2001; Nakagawa *et al.*, 2002). However, this complex has recently been classified as the phylum *Bacteroidetes* (Ludwig & Klenk, 2001). Reclassifications and emended descriptions have since been applied to bacteria belonging to this phylum. New genera and novel species of bacteria from marine coastal ecosystems have been found and described as a result of polyphasic taxonomic studies.

The family 'Flammeovirgaceae', phylum *Bacteroidetes*, consists of five genera: *Flammeovirga*, *Flexithrix*, *Persicobacter*, *Perexilibacter* and *Thermonema*. The genus *Flammeovirga* now has four species, *Flammeovirga kamo-gawensis* (Hosoya & Yokota, 2007) and *Flammeovirga aprica*, *Flammeovirga arenaria* and *Flammeovirga yaeyamensis* (Nakagawa *et al.*, 1997), the genus *Flexithrix* comprises one species, *Flexithrix dorotheae* (Lewin, 1970), the genus *Persicobacter* comprises one species, *Persicobacter diffluens* (Nakagawa *et al.*, 1997), and the genus *Thermonema* comprises two species, *Thermonema lapsum* and *Thermonema rossianum* (Hudson *et al.*, 1989).

The aim of the present study was to establish the taxonomic position of gliding bacteria recovered from the Andaman Sea along the southern coastline of Thailand.

Strains TISTR 1736, TISTR 1741 and TISTR 1750^T were isolated from decayed wood from Phuket Province, seashell material from Trang Province and marine algae from Krabi Province, respectively. The medium used for isolation was

Abbreviation: CFB, *Cytophaga*–*Flavobacterium*–*Bacteroides*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains TISTR 1736, TISTR 1741 and TISTR 1750^T are AB284517, AB265183 and AB265192, respectively.

SAP2 agar (Hosoya *et al.*, 2006). Purification of the isolates was performed by using the Skerman micromanipulation technique as described in Sly & Arunpairojana (1987). These isolates were cultured and maintained at 25 °C on SAP2 agar.

For determination of phylogenetic relationships, the 16S rRNA gene from the novel strains was amplified via PCR by using 16S rRNA gene universal primers. The PCR products were purified by using a GFX PCR DNA and gel band purification kit (Amersham). Sequencing reactions were performed with the ABI PRISM BigDye Terminator cycle sequencing ready kit (Applied Biosystems). Sequences were edited and assembled in the BioEdit program (Hall, 2004) and were compared with sequences by using the BLAST program within the GenBank database (Altschul *et al.*, 1990). Alignments of the 16S rRNA gene sequences of the novel isolates and phylogenetically related genera were carried out by using the CLUSTAL_X program (version 1.83; Thompson *et al.*, 1997). Nucleotide substitution rates (K_{nuc} values; Kimura, 1980) were determined and a distance matrix tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Alignment gaps and unidentified base positions were not taken into consideration in the calculations. The topology of the phylogenetic tree was evaluated by performing a bootstrap analysis with 1000 trials.

Phylogenetic analysis of the 16S rRNA gene sequence data revealed that the novel isolates formed a distinct lineage within the phylum *Bacteroidetes* and belonged to the family 'Flammeovirgaceae' (Fig. 1). The 16S rRNA gene sequence of strain TISTR 1750^T was identical to that of strains TISTR 1736 and TISTR 1741. For analysis of DNA–DNA relatedness, DNA–DNA hybridization experiments were carried out at 40 °C and levels were measured fluorometrically by using the method of Ezaki *et al.* (1989). High levels of DNA–DNA relatedness (87–100 %) were found between these three strains. A BLAST search with the 16S rRNA gene sequence of strain TISTR 1750^T in the GenBank database and subsequent phylogenetic analysis based on 16S rRNA gene sequences showed that strain TISTR 1750^T was most closely related to *Flexithrix dorotheae* IFO 15987^T

(91 % similarity). Strain TISTR 1750^T showed gene sequence similarity levels of 90–91 % to *Persicobacter diffluens* NBRC 15940^T, *Flammeovirga arenaria* NBRC 15982^T and *Reichenbachiella agariperforans* KMM 3525^T.

The G + C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989) whereby DNA was extracted and purified according to the methods of Saitou & Miura (1963). The DNA G + C contents of the novel strains were in the range 40–43 mol%.

Respiratory quinones were identified according to the method of Uchino *et al.* (1998). Quinone fractions were extracted with chloroform/methanol (2:1, v/v) from lyophilized cells. The fractions were then separated with TLC developed with hexane/diethyl ether (85:15, v/v). Quinones were detected under UV light at 275 nm. The quinone spots were scraped off and quinones were extracted with acetone. After concentration, the quinone samples were analysed by reversed-phase HPLC (Komagata & Suzuki, 1987). The major respiratory quinone of the novel strains was MK-7.

Cellular fatty acids of the novel strains were analysed as methyl esters by GC according to the instructions of the Microbial Identification System (MIDI, Inc.). Fatty acid methyl esters were prepared from bacterial cell mass which was separated from trypticase soy agar medium supplemented with artificial seawater and incubated at 25 °C for 3 days. The major cellular fatty acids were 16:1 ω 5c (*cis*-5-hexadecenoic acid) and 15:0 (pentadecanoic acid). Additionally, the major hydroxyl fatty acids were 3-OH 17:0 (3-hydroxyheptadecanoic acid), 3-OH 15:0 (3-hydroxypentadecanoic acid) and 3-OH 16:0 (3-hydroxyhexadecanoic acid) (Table 1).

The phenotypic characteristics of the isolates were determined as follows. Cell morphology and motility were examined under a phase-contrast microscope. Growth at different temperatures and pH values was tested on SAP2 agar. Oxidase activity was determined by the oxidation of 1 % tetramethyl-*p*-phenylenediamine on filter paper.

Fig. 1. Neighbour-joining phylogenetic tree showing the positions of strains TISTR 1736, TISTR 1741 and TISTR 1750^T and related members of the phylum *Bacteroidetes* based on 16S rRNA gene sequence analysis. Bootstrap values of >500 are given at branch points. Bar, 0.02 K_{nuc} .

Table 1. Cellular fatty acid profile of *Rapidithrix thailandica* gen. nov., sp. nov.

The first numerals denote the number of carbon atoms in the aliphatic chain and the second numeral, after the colon, denotes the number of double bonds. 2-OH and 3-OH indicate a hydroxyl group at carbon-2 and carbon-3, respectively; $\omega 5c$, $\omega 6c$, $\omega 7c$ and $\omega 9c$ represent the double bond position from the hydrocarbon end of the carbon chain; *c*, *cis*-isomer; *iso* and *anteiso* indicate an *iso*- or an *anteiso*-methyl branched chain. Components comprising <0.5% of the total are not shown.

Fatty acid	Content (%)
10:0	1.5
11:0 <i>iso</i>	2.0
Unknown 13.565	1.6
14:0	1.2
15:0 <i>iso</i>	6.3
15:0 <i>anteiso</i>	0.8
15:0	0.6
Summed feature 2 (3-OH 14:0/16:1 <i>iso</i> I)	0.8
16:0 <i>iso</i>	1.0
Summed feature 3 (2-OH 15:0 <i>iso</i> /16:1 $\omega 7c$)	1.5
16:1 $\omega 5c$	44.8
16:0	4.0
3-OH 15:0 <i>iso</i>	4.3
Summed feature 4 (17:1 <i>anteiso</i> B/i I)	5.8
17:1 <i>anteiso</i> A	0.7
Unknown 16.582	0.7
17:0 <i>iso</i>	1.7
17:0 <i>anteiso</i>	0.7
3-OH 16:0 <i>iso</i>	0.7
3-OH 16:0	3.8
18:0 <i>iso</i>	0.6
18:0	1.4
3-OH 17:0 <i>iso</i>	10.8

Catalase activity was tested by bubble formation in a 3% H₂O₂ solution. Hydrolysis of casein, starch (Smibert & Krieg, 1994), Tweens 20 and 80, and L-tyrosine (Barrow & Feltham, 1993) were determined by using media in which 0.5 × artificial seawater had been added. Acid production from carbohydrates was studied by using the commercial API 50 CH and API 20 E systems (bioMérieux). Detection of bacterial enzymes was performed by using the commercial API ZYM system (bioMérieux) according to the manufacturer's instructions.

All of the novel strains were Gram-negative, aerobic, non-sporulating, non-fruiting, gliding bacteria. The isolates formed light olive grey colonies. Cells were flexible filaments of 0.7 × 20–100 µm (Fig. 2). The swarm colony spread through the culture plate as a thin film on the agar surface. The typical swarm edge expanded like a sea wave. The phenotypic characteristics of the novel strains are given in the species description below and in Table 2. The novel strains showed many similar characteristics to those of the genera *Flexithrix* and *Persicobacter*, but there were

Fig. 2. Phase-contrast micrograph of filaments of strain TISTR 1750^T. The strain was grown on SAP2 medium at 25 °C for 48 h. Bar, 10 µm.

some differences in cell morphology, colony colour, optimum temperature and pH for growth, oxidase and catalase activity, cystine arylamidase activity, trypsin activity, acid phosphatase activity and DNA G + C content.

On the basis of the results presented here, we propose that the isolates represent a novel species of a new genus within the family 'Flammeovirgaceae', of the phylum *Bacteroidetes*, for which the name *Rapidithrix thailandica* gen. nov., sp. nov. is proposed.

Description of *Rapidithrix* gen. nov.

Rapidithrix (Ra.pid.i.thrix. L. adj. *rapidus* rapid; Gr. n. *thrix* hair; N.L. fem. n. *Rapidithrix* rapidly moving hair).

Cells are unbranched, flexible filaments, 0.7 × 20–100 µm. Motile by gliding. Gram-negative. Resting stages are not known. Chemo-organotrophs. Strict aerobes. Require seawater for growth. The major respiratory quinone is MK-7. The major cellular fatty acids are 16:1 and 15:0. This genus belongs to the family 'Flammeovirgaceae', phylum *Bacteroidetes*. The type species is *Rapidithrix thailandica*.

Description of *Rapidithrix thailandica* sp. nov.

Rapidithrix thailandica (thai.lan.dic.a. N.L. fem. adj. *thailandica* pertaining to Thailand, from where the organisms were isolated).

The main characteristics are as those given in the genus description. In addition, the optimal growth temperature is 25–30 °C. The pH range for growth is 5–10. Colonies are light olive grey. Peptone and ammonium are useful nitrogen sources for growth. Positive in tests for oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl- β -glucosaminidase and α -fucosidase. Negative in tests for catalase, lipase (C14), α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase and α -mannosidase. Decomposes

Table 2. Comparative characteristics of the genera *Rapidithrix* gen. nov., *Flexithrix* and *Persicobacter*

All genera were positive for oxidase and for utilization of peptone and NH_4^+ as a nitrogen source. +, Positive; –, negative; ND, no data available.

Characteristic	<i>Rapidithrix</i> gen. nov.	<i>Flexithrix</i>	<i>Persicobacter</i>
Cell morphology	Non-sheathed filaments	Sheathed filaments	Long rods
Colour of cell mass	Light olive grey	Yellow	Pink to orange
DNA G + C content (mol%)	40–43	37.2	40–42
Catalase	–	+	–
Cystine arylamidase	+	–	ND
Trypsin	+	–	ND
Acid phosphatase	+	–	ND
Optimum temperature (°C)	25–30	35–40	25–30
Optimum pH	5–10	6.5–8	7–9
Cellular fatty acids:			
Non-polar	16:1 ω 5 ζ ; 15:0 iso	16:1 ω 5 ζ ; 15:0 iso	17:1 iso; 16:0; 18:1 iso
3-OH	3-OH 17:0 iso; 3-OH 15:0 iso; 3-OH 16:0	3-OH 17:0 iso; 3-OH 15:0 iso; 3-OH 16:0	3-OH 17:0 iso*
Utilization of NO_3^- as nitrogen source	–	+	–†

*Data from Naganuma & Horikoshi (1994).

†Characteristic variable with different strains: the type strain is positive.

starch, gelatin, L-tyrosine, and Tweens 20 and 80. Acid is produced from glycerol, D-arabinose, D-xylose, galactose, glucose, mannose, amygdalin, aesculin, cellobiose, maltose, lactose, melibiose, trehalose, gentiobiose and L-fucose, but not from erythritol, L-arabinose, ribose, L-xylose, adonitol, fructose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, arbutin, inulin, melezitose, raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol or gluconate. The DNA G + C content is in the range 40–43 mol%. The DNA G + C content of the type strain is 40 mol%.

The type strain, TISTR 1750^T (=IAM 15448^T), was isolated from marine algae from the Andaman Sea, Krabi Province, Thailand. Strains TISTR 1736 (isolated from decayed wood from the Andaman Sea, Phuket Province, Thailand) and TISTR 1741 (isolated from seashell material from the Andaman Sea, Trang Province, Thailand) are reference strains.

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