Myroides pelagicus sp. nov., isolated from seawater in Thailand

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An aerobic, Gram-negative, non-motile, yellow-to-orange pigmented and rod-shaped bacterium, designated strain SM1^T, was isolated from seawater in Thailand and subjected to a polyphasic taxonomic study. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the novel isolate shared 93–95 % sequence similarity with species of the genus *Myroides*. The DNA–DNA relatedness values of strain SM1^T with *Myroides odoratimimus* JCM 7460^T and *Myroides odoratus* JCM 7458^T were below 70 %. The DNA G + C content of strain SM1^T was 33.6 mol%, the major menaquinone was MK-6 and the major fatty acids were C_{15:0} iso, C_{17:1} ω9c iso and C_{17:0} iso 3-OH. Based on its phenotypic and chemotaxonomic characteristics, strain SM1^T was classified as representing a novel species of the genus *Myroides*, for which the name *Myroides pelagicus* sp. nov. is proposed. The type strain is SM1^T (=IAM 15337^T = KCTC 12661^T).

The genus *Myroides* was established by the reclassification of *Flavobacterium odoratum* as *Myroides odoratus*, the type species, on the basis of genomic, chemotaxonomic and phenotypic studies (Vancanneyt *et al.*, 1996). *Myroides odoratimimus* was also described in the report. Currently, the genus *Myroides* comprises only these two species, both of which originated from clinical sources (Holmes *et al.*, 1977). Strain SM1^T was obtained during the isolation of crude oil-utilizing and -emulsifying bacteria from seawater (Maneerat *et al.*, 2005). Strain SM1^T and the two species of the genus *Myroides* were found to produce surface-active compounds in marine broth, which were identified as cholic acid, deoxycholic acid and their conjugates with glycine. These findings led to the first report indicating that bile acids can be produced by prokaryotic cells (Maneerat *et al.*, 2005). In the present study, we attempted to elucidate the phylogenetic position of strain SM1^T using a polyphasic taxonomic approach, including 16S rRNA gene sequence analysis, fatty acid content analysis, quinone analysis and genotypic (G + C content of DNA, DNA–DNA hybridization) and physiological comparisons. Based on these data, it is proposed that strain SM1^T represents a novel species in the genus *Myroides*.

Temperature range and pH range for growth were determined by incubating in Luria–Bertani (LB) medium (Atlas, 2004). Cell morphology was observed by microscopy (BX60; Olympus). Growth under anaerobic conditions was determined after 2 weeks incubation in an AnaeroPack (Mitsubishi

Gas). Catalase activity was determined by bubble formation in 3 % H₂O₂ solution. Oxidase activity was determined by cytochrome oxidase paper (Nissui Pharmaceutical). API 20E and API ZYM strips (bioMérieux) were used to determine physiological and biochemical characteristics. API 20E and API ZYM were read after 48 h incubation at 28 °C and 4 h incubation at 37 °C, respectively. The nutritional features of strain SM1^T and *M. odoratimimus* JCM 7460^T and *M. odoratus* JCM 7458^T were determined using Biolog MicroPlates. The strains were grown on BUG medium (Biolog, supplemented with 5 ml sterile sheep blood l⁻¹) at 28 °C for 24 h and suspended in sterile saline medium within the density range specified by the manufacturer with a photometer (21907; Biolog). Immediately after suspending the cells in saline solution, suspensions were transferred to sterile multichannel pipetter reservoirs (Biolog) and GN2 MicroPlates (Biolog) were inoculated with 150 µl cell suspension per well by using an eight-channel repeating pipetter (Biolog). The inoculated plates were incubated at 28 °C for 6 h and the results were read with a MicroPlate Reader using MICROLOG 3.59 software to perform automated reading.

Determination of the respiratory quinone system and cellular fatty acid content (MIDI) were carried out as described previously (Xie & Yokota, 2003). DNA was prepared according to the method of Marmur (1961) from cells grown on LB medium and DNA G + C content was determined by using the HPLC method of Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989). The hybridization temperature was 40 °C. An approximately

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SM1^T is AB176662.

1500-bp fragment of the 16S rRNA gene was amplified from the extracted DNA using bacterial universal primers specific to the 16S rRNA gene, primer 8F, 5'-AGAGTTT-GATCCTGGCTCAG-3' [positions 8–27, according to the *Escherichia coli* numbering system of Brosius *et al.* (1978)], and primer 1510R, 5'-GGCTACCTTGTTACGTA-3' (positions 1510–1527). PCR and sequencing protocols have been previously described (Xie & Yokota, 2003).

The 16S rRNA gene sequence of strain SM1^T was compared with sequences obtained from GenBank. Sequences were aligned with CLUSTAL W software (Thompson *et al.*, 1994) and evolutionary distances and the *K*_{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when the 1317 bases of 16S rRNA gene nucleotides were compared. A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Similarity values were calculated using PAUP 4.0b1 (Swofford, 1998).

The 16S rRNA gene sequence of strain SM1^T showed the highest similarities to the sequences of *M. odoratimimus* JCM 7460^T (95.8 %) and *M. odoratus* JCM 7458^T (93.8 %), but low sequence similarity (less than 90 %) with other genera. The phylogenetic tree in Fig. 1 shows that strain SM1^T was clustered within the genus *Myroides* with 100 % bootstrap support. DNA–DNA hybridization values between strain SM1^T and *M. odoratimimus* JCM 7460^T and *M. odoratus* JCM 7458^T were 22.0 and 26.0 %, respectively. These results indicate that strain SM1^T represents a separate species of the genus *Myroides* (Stackebrandt & Goebel, 1994).

Chemotaxonomic analysis further supported the phylogenetic results. The major quinone system of strain SM1^T was

Table 1. Cellular fatty acid content (%) of *Myroides pelagicus* sp. nov. and other species of the genus *Myroides*

Values are percentages of total fatty acids. Values < 1 % are not shown. Taxa: 1, *M. pelagicus* sp. nov.; 2, *M. odoratimimus* JCM 7460^T; 3, *M. odoratus* JCM 7458^T. tr, Trace.

Fatty acid	1	2	3
C _{13:0} iso	7.4	1.8	tr
C _{15:0} iso	49.4	43.3	45.5
C _{15:0} anteiso	1.0	1.0	2.0
C _{15:0} iso 3-OH	6.1	5.8	5.8
C _{16:0} 3-OH	2.8	4.0	1.5
C _{16:1} ω7 <i>c</i> /C ₁₅ iso 2-OH	1.9	3.6	tr
C _{17:0} iso 3-OH	10.1	12.5	21.2
C _{17:1} ω9 <i>c</i> iso	13.1	15.1	13.8

menaquinone MK-6. Major fatty acids of strain SM1^T were C_{15:0} iso (49.4 %), C_{17:1}ω9*c* iso (13.1 %) and C_{17:0} iso 3-OH (10.1 %), which is similar to other members of the genus *Myroides* (Table 1). The DNA G + C content of strain SM1^T was 33.6 mol%.

Strain SM1^T could be distinguished from the other species of the genus *Myroides* by various phenotypic characteristics (Table 2). Strain SM1^T was isolated from seawater and could tolerate 9 % NaCl; the other species of the genus *Myroides*, originating from clinical sources, could only tolerate 5–6 % NaCl. Moreover, strain SM1^T could also be distinguished by the following characteristics: negative results for catalase and urease activities, no nitrate reduction and no activities for esterase (C4) and esterase lipase (C8). Some differential characteristics were also obtained from the Biolog results, including positive results for strain SM1^T for the utilization of α-hydroxybutyric and succinamic acids and negative results for the utilization of L-histidine and urocanic acid.

Fig. 1. A neighbour-joining phylogenetic tree of a subset of the genus *Myroides* and representatives of some other taxa based on 16S rRNA gene sequence comparisons. *Sphingobacterium spiritivorum* DSM 2582 was used as the outgroup. Bootstrap percentages above 60 % (from 1000 replicates) are shown at nodes. Bar, 0.01 nucleotide substitutions per site.

Table 2. Differential characteristics of *Myroides pelagicus* sp. nov. and related *Myroides* species

Taxa: 1, *M. pelagicus* sp. nov.; 2, *M. odoratimimus* JCM 7460^T; 3, *M. odoratus* JCM 7458^T. Data are from this and earlier studies (Holmes *et al.*, 1977; Vancanneyt *et al.*, 1996; Maneerat *et al.*, 2005). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Source	Seawater	Wound swab	Faeces
Cell morphology	Short rods	Rods	Long rods
Cell diameter (µm)	0.5–1	3.5–4	11–12
Cell width (µm)	0.2–0.3	0.2–0.3	0.2–0.3
Pigmentation	Yellow-to-orange	Pale-yellow	Yellow
NaCl range (% w/v)	0–9	0–6	0–5
pH range	5–9	6–9	6–9
Catalase	w	+	+
Urease	–	+	+
Nitrate reduction	–	+	+
Activity of:			
Esterase (C4)	–	+	+
Esterase lipase (C8)	–	+	+
Utilization of:			
L-Histidine	–	+	+
α-Hydroxybutyric acid	+	–	+
Succinamic acid	+	w	+
Urocanic acid	–	+	+
DNA G + C content (mol%)	33.6	31.7	34.7

On the basis of phenotypic and phylogenetic characteristics, strain SM1^T could be assigned as representing a separate species of the genus *Myroides*. We therefore propose the name *Myroides pelagicus* sp. nov. for strain SM1^T.

Description of *Myroides pelagicus* sp. nov.

Myroides pelagicus (pe.la'gi.cus. L. masc. adj. *pelagicus* belonging to the sea).

Cells are Gram-negative, aerobic, straight, short and single rods (0.5–1 × 0.2–0.3 µm). Cells lack flagella. Neither cellular gliding movement nor swarming growth are observed. Colonies grown on LB agar medium are circular, convex and yellow-to-orange. The temperature range for growth is 10–37 °C; no growth occurs at 4 or 45 °C. The pH range for growth is 5.0–9.0. NaCl is not required for growth, but is able to tolerate up to 9.0 % NaCl (w/v). Catalase reaction is weakly positive, whereas oxidase reaction is positive. Gelatin hydrolysis, Voges–Proskauer test and citrate utilization results are positive, but activities of ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease, production of hydrogen sulfide and indole and reduction of nitrate and nitrite are negative. Positive results for activities of alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative results for esterase (C4), esterase lipase (C8), lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and tryptophan deaminase. Glycogen,

methyl pyruvate, monomethyl succinate, acetic acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, inosine, uridine and thymidine are utilized, but cyclodextrin, dextrin, Tween 40, Tween 80, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, cellobiose, *i*-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, furanose, xylitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, alaninamide, D-alanine, L-histidine, hydroxyl-L-proline, L-phenylalanine, L-pyrogutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol DL-α-glycerol phosphate, glucose 1-phosphate and D-glucose 6-phosphate are not utilized. The major quinone system is menaquinone MK-6. The major cellular fatty acids are C_{15:0} iso, C_{17:1} ω9c iso and C_{17:0} iso 3-OH. The DNA G + C content of the type strain is 33.6 mol%.

The type strain, SM1^T (=IAM 15337^T=KCTC 12661^T), was isolated from seawater, off the coast of Thailand.

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