Myroides phaeus sp. nov., isolated from human saliva, and emended descriptions of the genus *Myroides* and the species *Myroides profundus* Zhang *et al.* 2009 and *Myroides marinus* Cho *et al.* 2011

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A novel bacterial strain, designated MY15^T, was isolated from a saliva sample taken from a student during a teaching experiment in China. Phylogenetic analyses based on 16S rRNA gene sequences showed that the novel strain was most closely related to *Myroides marinus* JS-08^T, *Myroides odoratimimus* LMG 4029^T and *Myroides profundus* D25^T with 96.5%, 96.3% and 96.1% gene sequence similarities, respectively, demonstrating that the novel strain belonged to the genus *Myroides*. Strain MY15^T formed pale yellow colonies that turned to brown on Luria–Bertani (LB) agar and that gave off a characteristic fruity odour. Cells were Gram-staining-negative, rod-shaped and non-motile. The new isolate contained menaquinone 6 (MK-6) as the major respiratory quinone and C_{15:0} iso (51.2%), C_{17:0} iso 3-OH (12.9%) and C_{13:0} iso (10.5%) as the dominant fatty acids. The G + C content of the DNA was 34.3 mol%. On the basis of this study, based on a polyphasic taxonomic approach, strain MY15^T (=DSM 23313^T=LMG 25566^T) represents a novel species of the genus *Myroides*, for which the name *Myroides phaeus* sp. nov. is proposed. Emended descriptions of the genus *Myroides* and of the species *M. profundus* and *M. marinus* are also given.

The genus *Myroides* (family *Flavobacteriaceae*, phylum *Bacteroidetes*; Hugo *et al.*, 2006) was established by Vancanneyt *et al.* (1996). It was created when strains originally classified as *Flavobacterium odoratum*, derived from clinical sources (Holmes *et al.*, 1977), were reclassified as representing two novel species, *M. odoratus* (type species) and *M. odoratimimus*. Three further species of the genus *Myroides*, *M. pelagicus* (Yoon *et al.*, 2006), *M. profundus* (Zhang *et al.*, 2008) and *M. marinus* (Cho *et al.*, 2011), have since been described and were isolated from seawater and deep-sea sediment. Although the two original species of the genus were repeatedly isolated from clinical sources (Davis *et al.*, 1979; Macfarlane *et al.*, 1985; Ferrer *et al.*, 1995; Hsueh *et al.*, 1995; Green *et al.*, 2001; Yağcı *et al.*, 2000), recent studies have indicated that they are also common inhabitants of soil and water and behave as low-grade opportunistic pathogens (Hugo *et al.*, 2006). A novel bacterial strain, designated MY15^T, was isolated from a saliva sample obtained from an asymptomatic student during a teaching experiment at Weifang Medical University, China. A further attempt to isolate the bacterium from the same student a

week later failed. The aim of the present study was to determine the exact taxonomic position of strain MY15^T by using a polyphasic taxonomic approach.

The saliva sample was diluted with sterile saline (0.9% NaCl; w/v) and spread onto trypticase soy agar (TSA; Difco) plates that were incubated at 37 °C for 48 h. Strain MY15^T, which produced a yellow to brown diffusible pigment, was purified by streaking three times on TSA. Cultures were maintained on Luria–Bertani (LB) agar [1.0% (w/v) tryptone, 0.5% (w/v) yeast extract, 1.0% (w/v) NaCl, 2.0% (w/v) agar] at 28 °C and preserved at –80 °C in sterile saline supplemented with 15% (v/v) glycerol.

For 16S rRNA gene sequencing, cells grown in LB broth at 28 °C for 24 h were harvested by centrifugation. Extraction and purification of total genomic DNA from the cells and PCR amplification of the 16S rRNA gene were performed as described by Jin *et al.* (2010). The nearly complete 16S rRNA gene sequence (1356 nt) of strain MY15^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Multiple sequence alignment was performed by using CLUSTAL_X 1.8

The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of *Myroides phaeus* sp. nov. MY15^T is GU253339.

A supplementary figure is available with the online version of this paper.

(Thompson *et al.*, 1997). The phylogenetic trees were constructed using the neighbour-joining, maximum-likelihood and maximum-parsimony methods with Kimura 2-state parameter model analyses implemented with the MEGA version 5 program (Tamura *et al.*, 2007). In each case, bootstrap values were calculated based on 1000 replicates. *Sphingobacterium spiritivorum* DSM 2582 (GenBank no. AJ459411) was used as an outgroup. The 16S rRNA gene sequence of strain MY15^T showed the highest similarities to *M. marinus* JS-08^T (96.5%), *M. odoratimimus* LMG 4029^T (96.3%), *M. profundus* D25^T (96.1%), *M. pelagicus* KCTC 12661^T (96.1%) and *M. odoratus* ATCC 4651^T (95.5%). Sequence similarity was <92% with other members of the family *Flavobacteriaceae*. The neighbour-joining tree, showing the phylogenetic relationships between strain MY15^T and closely related species, is presented in Fig. 1. The topology of the maximum-parsimony and maximum-likelihood trees was essentially the same (data not shown). Strain MY15^T clustered within the genus *Myroides* with 99–100% bootstrap values in the three phylogenetic trees.

Colony morphology was determined after incubation for 24 h at 28 °C on LB agar. Cell morphology was examined using light microscopy (CX; Olympus) and transmission electron microscopy (JEM-1200EX; JEOL) after negative staining with 1% (w/v) phosphotungstic acid. Flagellar motility was assessed using the hanging-drop method (Hu & Li, 2007). The presence of gliding motility and the

production of flexirubin-type pigments were investigated using the methods recommended by Bernardet *et al.* (2002). Growth under anaerobic conditions was tested on LB agar and TSA that was either supplemented with 0.25% (w/v) NaNO₃ or not supplemented and prepared under a nitrogen atmosphere and incubated in an anaerobic jar (HP015, HiTech) filled with nitrogen. The temperature range for growth was determined on LB agar by incubating cultures at 6, 10, 18, 28, 37, 42, and 45 °C for 5 days and at 0 and 4 °C for 30 days. The pH range for growth was tested in LB broth adjusted to pH 3.5–12 (at intervals of 0.5 pH unit) with citrate/phosphate, Tris/HCl or Na₂CO₃/NaOH buffers (Breznak & Costilow, 1994). Tolerance to NaCl was tested in LB broth supplemented with 0–15% NaCl (w/v, at 1% intervals) at 28 °C. The optimal pH and the NaCl concentration for growth were determined spectrophotometrically (OD₆₀₀) with three replicates. Haemolysis was tested by growing cells on blood-agar plates (LB agar supplemented with 6.5% defibrinated sheep blood) according to the method described by Tindall *et al.* (2007). DNA hydrolysis was examined using DNase test agar (Oxoid). Activities of constitutive enzymes and biochemical properties were assessed by using the API 20E, API 20NE and API ZYM strips (bioMérieux) and Gram-negative (GN2) MicroPlates (Biolog) according to the manufacturers' instructions. Standard protocols (Beveridge *et al.*, 2007; Tindall *et al.*, 2007) were used to perform Gram,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain MY15^T, other species of the genus *Myroides* and representatives of some other related members of the family *Flavobacteriaceae*. *Sphingobacterium spiritivorum* DSM 2582 (GenBank no. AJ459411) was used as an outgroup. Bootstrap values >70% (1000 replicates) are indicated at nodes. Bar, 0.02 substitutions per nucleotide position.

endospore and flagellum staining, to test catalase and oxidase activities and examine nitrite reduction and the degradation of casein, gelatin, Tween 80, starch, agar, CM-cellulose and egg yolk. The following methods (Tindall *et al.*, 2007) were used in parallel to assess the reliability of the corresponding tests in the API system: nitrate reduction from KNO₃, H₂S production from thiosulphate and indole production from L-tryptophan. For the tests mentioned above, *M. marinus* JS-08^T, *M. odoratimimus* LMG 4029^T and *M. profundus* D25^T

were used as reference strains and grown under the same conditions as strain MY15^T. The morphological, physiological and biochemical characteristics of strain MY15^T are given in the species description and in Table 1, and the cellular morphology of strain MY15^T is shown in Fig. S1 (available in IJSEM Online). Strain MY15^T mainly differed from recognized species of the genus *Myroides* by the limited number of carbon sources it was able to oxidize in the GN2 MicroPlate.

Table 1. Differential characteristics of *Myroides phaeus* sp. nov. compared with other *Myroides* species

Strains: 1, *Myroides phaeus* sp. nov. MY15^T; 2, *M. marinus* JS-08^T; 3, *M. odoratimimus* LMG 4029^T; 4, *M. profundus* D25^T; 5, *M. odoratus* ATCC 4651^T (Vancanneyt *et al.*, 1996; Yoon *et al.*, 2006); 6, *M. pelagicus* KCTC 12661^T (Yoon *et al.*, 2006). Data for strains 1–4 are from this study, except when otherwise stated. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6
Colony pigmentation	Yellow to brown	Yellow to orange	Pale yellow	White to pale yellow	Yellow	Yellow to orange
Diffusible pigment on LB agar	+	+	+	+	ND	ND
Flexirubin-type pigments	–	+	+	–	+	ND
Cell size (µm)	0.4–0.5 × 0.7–1.2	0.2–0.3 × 0.6–1†	0.2–0.3 × 3.5–4†	0.7–0.9 × 1.6–2.4†	0.2–0.3 × 11–12	0.2–0.3 × 0.5–1
Gliding motility	–	+	–	+	–	–
NaCl range (% w/v)	0–6	0–5	0–6	0–6§	0–5	0–9
Temperature range (°C)	6–37	10–37	18–37	6–42§	18–37	10–37
Hydrolysis of urea	+	+	+	–	+	–
Nitrate reduction	–	–	–	–	–	–
Nitrite reduction	+	+	+	+	+	–
Enzyme activity (API ZYM):						
Esterase (C4)	+	+	w	w	+	–
Esterase lipase (C8)	+	+	w	w	+	–
Oxidation of (GN2 MicroPlate):						
Glycogen	–	–	–	–	+	+
L-Arabinose	–	–	–	+	+	–
α-Ketoglutaric acid	–	–	w	+	+	+
L-Alaninamide	–	–	+	–	+	–
L-Glutamic acid	–	+	+	+	+	+
Glycyl L-aspartic acid	–	+	+	+	+	+
Glycyl L-glutamic acid	–	+	+	+	+	+
L-Proline	–	+	+	+	+	+
L-Serine	–	+	+	–	+	+
L-Threonine	–	+	+	–	+	+
Inosine	–	+	+	–	+	+
L-Leucine	–	+	+	–	+	+
L-Ornithine	–	–	+	–	+	+
L-Alanine	–	+	+	–	+	+
Succinamic acid	–	–	+	–	+	+
DL-Lactic acid	–	+	+	–	+	+
Methyl pyruvate	–	–	+	–	+	+
Acetic acid	–	+	+	–	+	+
DNA G + C content (mol%)	34.3	34.2¶	33.8¶	33.9¶	36.7¶	35.8¶

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†Data from Cho *et al.* (2011); Zhang *et al.* (2008) and Vancanneyt *et al.* (1996), respectively.

‡Contradictory data were reported by Zhang *et al.* (2008) and Cho *et al.* (2011).

§Zhang *et al.* (2008) reported NaCl and temperature ranges for growth of *M. profundus* D25^T of 0–8 % and 8–42 °C, respectively.

||Method 1 of Tindall *et al.* (2007) and the corresponding API 20NE test both gave negative results, confirming the original reports by Cho *et al.* (2011), Zhang *et al.* (2008) and Vancanneyt *et al.* (1996).

¶Data from Cho *et al.* (2011).

Chemotaxonomic characteristics were determined from cells grown at 28 °C for 2 days in trypticase soy broth (TSB; Difco). Fatty acid methyl esters of strain MY15^T and the three reference strains were prepared and analysed according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990) with Sherlock version 6.0 and the peaks were identified using the TSBA40 database. Strain MY15^T and the three reference strains contained C_{15:0} iso and C_{17:0} iso 3-OH as the predominant fatty acids (Table 2). According to published data obtained using unspecified growth conditions, *M. odoratus* and *M. pelagicus* also contained the same dominant fatty acids (Yoon *et al.*, 2006). Strain MY15^T differed from the reference strains by containing a significant amount of C_{15:0} anteiso and only traces of summed feature 3. The analysis of respiratory quinones was carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. In accordance with other members of the family *Flavobacteriaceae*, strain MY15^T contained menaquinone 6 (MK-6) as the major respiratory quinone (>99%). Strain MY15^T also contained <1% of menaquinone 7 (MK-7), which was not reported in other species of the genus *Myroides*. The G+C content of the DNA of strain MY15^T was 34.3 mol%

Table 2. Cellular fatty acid content (%) of *Myroides phaeus* sp. nov. and other species of the genus *Myroides*

Strains: 1, *Myroides phaeus* sp. nov. MY15^T; 2, *M. marinus* JS-08^T; 3, *M. odoratimimus* LMG 4029^T; 4, *M. profundus* D25^T; 5, *M. odoratus* ATCC 4651^T (Yoon *et al.*, 2006; growth conditions unspecified); 6, *M. pelagicus* KCTC 12661^T (Yoon *et al.*, 2006; growth conditions unspecified). Data for 1–4 are from this study. –, Not detected; tr, trace (<1%). Fatty acids amounting to <1% of the total fatty acids in all strains listed are not shown.

Fatty acid	1	2	3	4	5	6
C _{14:0}	tr	1.2	1.4	1.0	–	–
C _{16:0}	1.6	2.4	3.3	3.2	–	–
C _{18:0}	–	–	2.0	–	–	–
C _{13:0} iso	10.5	8.9	9.0	4.5	tr	7.4
C _{15:0} iso	51.2	52.9	46.7	49.5	45.5	49.4
C _{16:0} iso	tr	1.4	1.6	tr	–	–
C _{15:0} anteiso	4.4	tr	1.6	tr	2.0	1.0
C _{16:0} 3-OH	6.7	6.7	7.7	7.8	1.5	2.8
C _{15:0} iso 3-OH	4.2	4.0	3.9	4.4	5.8	6.1
C _{16:0} iso 3-OH	tr	tr	1.1	tr	–	–
C _{17:0} iso 3-OH	12.9	9.8	10.1	12.5	21.2	10.1
Summed features*						
3	tr	3.8	2.1	3.9	tr	1.9
8	–	–	1.7	–	–	–
9	2.6	5.3	3.3	8.6	13.8	13.1

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 contained C_{16:1}ω7c and/or C_{15:0} iso 2-OH; summed feature 8 contained C_{18:1}ω6d/C_{18:1}ω7c; summed feature 9 contained C_{17:1}ω9c iso and/or C_{16:0} 10-methyl.

as determined by the method of Mesbah & Whitman (1989) using reverse-phase HPLC. This value falls within the range (33.8–36.7%) reported for strains of the genus *Myroides* (Cho *et al.*, 2011).

On the basis of phylogenetic inference and phenotypic characteristics, strain MY15^T is assigned to the genus *Myroides* as a novel species for which the name *Myroides phaeus* sp. nov. is proposed. The additional data obtained in this study also make emended descriptions of the genus *Myroides* and the species *M. profundus* and *M. marinus* necessary.

Emended description of the genus *Myroides* Vancanney *et al.* 1996

Myroides (My.roi'des. Gr. n. *myron* perfume; Gr. adj. suffix *oides* similar to; N.L. masc. n. *Myroides* resembling perfume).

The following description includes characteristics taken from the original description as well as corrections and additions resulting from this study. Cells are strictly aerobic, Gram-staining-negative rods that may form chains of 4–10 cells in broth culture. Spores are not formed. Cells lack flagella and flagellar motility. Some species display gliding motility. A characteristic fruity odour is produced by most strains. Non-haemolytic on blood agar. All strains grow at 18–22 °C and at 37 °C, and some strains grow at 6 and 42 °C. Oxidase- and catalase-positive. Most species reduce nitrite, but do not reduce nitrate. Some strains produce flexirubin-type and diffusible pigments on LB agar. Menaquinone 6 is the major isoprenoid quinone. The dominant fatty acids are C_{15:0} iso and C_{17:0} iso 3-OH. The DNA G+C content ranges from 33 to 37 mol%. The type species is *M. odoratus*.

Emended description of *Myroides profundus* Zhang *et al.* 2009

The description is the same as that given by Zhang *et al.* (2008) except that gliding motility is present and that the NaCl and temperature ranges for growth are 0–6% (w/v) and 6–42 °C, respectively.

Emended description of *Myroides marinus* Cho *et al.* 2011

The description is the same as that given by Cho *et al.* (2011) except that nitrite is reduced.

Description of *Myroides phaeus* sp. nov.

Myroides phaeus (pha'e.us. Gr. adj. *phaios* brown; N.L. masc. adj. *phaeus* brown, referring to the colour of the colonies).

Displays the following characteristics in addition to those listed in the genus description. Cells are straight rods that occur singly or in pairs and are 0.4–0.5 µm in diameter and

0.7–1.2 µm in length. Gliding motility is not observed. Colonies grown on LB agar are circular with regular margins, convex and yellow to brown. The pigments are diffusible and non-flexirubin type. Growth occurs at 6–37 °C (optimum, 28 °C; no growth occurs at 4 or 42 °C) and at pH 5.0–10.0 (optimum, pH 8). NaCl is not required for growth. Tolerates up to 6.0 % NaCl (w/v) (optimum, 1.0 %). Gelatin and DNA are hydrolysed, while casein, Tween 80, agar, starch, CM-cellulose and egg yolk are not. Positive in tests for arginine dihydrolase and urease activities, citrate utilization and nitrite reduction and for the Voges–Proskauer reaction. Negative result in tests for β -galactosidase (ONPG), lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and β -glucosidase activities, production of hydrogen sulfide and indole, and reduction of nitrate. In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but leucine arylamidase, valine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are absent. Acid is produced from inositol, D-sorbitol, sucrose and amygdalin, but not from D-glucose, D-mannitol, L-rhamnose, melibiose or L-arabinose. In the GN2 MicroPlate, Tween 80, monomethyl succinate, succinic acid, L-asparagine and L-aspartic acid are oxidized; all other substrates in the GN2 MicroPlate are not oxidized. The main cellular fatty acids (>10 % of the total fatty acids) are C_{15:0} iso, C_{17:0} iso 3-OH and C_{13:0} iso.

The type strain, MY15^T (=DSM 23313^T=LMG 25566^T), was isolated from human saliva in China. The DNA G+C content of the type strain is 34.3 mol%.

Acknowledgements

This work was supported by the National High Technology R&D Program of China (no. 2007AA09Z434) and the National Natural Science Foundation of China (no. 40876067). Professor Seung Bum Kim (Chungnam National University, Korea) and Professor Yuzhong Zhang (Shandong University, China) kindly provided *Myroides marinus* JS-08^T and *Myroides profundus* D25^T, respectively.

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