Myroides marinus sp. nov., a member of the family Flavobacteriaceae, isolated from seawater

Sung-Heun Cho,¹ Song-Hee Chae,¹ Wan-Taek Im² and Seung Bum Kim¹

Correspondence
Seung Bum Kim
sbk01@cnu.ac.kr

¹Department of Microbiology and Molecular Biology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

²Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

A Gram-negative, aerobic, non-motile, yellow-pigmented, rod-shaped bacterium (strain JS-08^T) isolated from seawater was subjected to a polyphasic taxonomic study. 16S rRNA gene sequence analysis indicated that strain JS-08^T belongs to the genus *Myroides*, a member of the phylum *Bacteroidetes*. Its closest phylogenetic relative was *Myroides odoratimimus* JCM 7460^T, with which it shared 97.0 % 16S RNA gene sequence similarity. Strain JS-08^T contained menaquinone-6 (MK-6) as the predominant menaquinone, and the dominant fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH and a summed feature consisting of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. The DNA G + C content of strain JS-08^T was 34.2 mol%. Based on phenotypic, genotypic and phylogenetic evidence, it is suggested that strain JS-08^T represents a novel species of the genus *Myroides*, for which the name *Myroides marinus* sp. nov. is proposed. The type strain is JS-08^T (=KCTC 23023^T =JCM 16529^T).

The genus *Myroides*, belonging to the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), was first described with the reclassification of *Flavobacterium odoratum* strains as *Myroides odoratus* and *Myroides odoratimimus* based on a polyphasic approach (Vancanneyt *et al.*, 1996). At the time of writing, the genus *Myroides* comprised four recognized species, namely *M. odoratus*, *M. odoratimimus* (Vancanneyt *et al.*, 1996), *M. pelagicus* (Yoon *et al.*, 2006) and *M. profundus* (Zhang *et al.*, 2008).

Strains of *Myroides* have been found in various terrestrial and aquatic environments. Most strains of *M. odoratus* and *M. odoratimimus* have been isolated from clinical specimens (Holmes *et al.*, 1977), whereas those of the other species have been isolated from the marine environment (Yoon *et al.*, 2006; Zhang *et al.*, 2008), freshwater fish (González *et al.*, 2000) and the insect gut (Spiteller *et al.*, 2000).

A number of studies have focused on *Myroides* strains as causative agents in various infections (Bachmeyer *et al.*, 2008; Douce *et al.*, 2008; Green *et al.*, 2001; Källman *et al.*, 2006; Thomas *et al.*, 2007; Yağci *et al.*, 2000). Attention has also been given to strains belonging to *Myroides* because novel types of metalloenzymes have been reported from various species of the genus (Chen *et al.*, 2009; Mammeri *et al.*, 2002) and biosurfactant compounds have been isolated from strains of *M. pelagicus* (Maneerat *et al.*, 2005,

2006). These reports clearly indicate the wide distribution of members of the genus *Myroides* in nature and their potential as a source of industrially useful compounds, yet the diversity within the genus remains to be fully unravelled. In the present study, we report the description of a novel *Myroides* species isolated during a culture-dependent study of halophilic bacterial diversity associated with marine environments. The taxonomic status of the organism was determined by using a polyphasic approach.

Strain JS-08^T was originally isolated by using the standard dilution plating technique on marine agar 2216 (MA; Difco) from seawater collected off Boryeong Province, Chungnam, Republic of Korea. Examination of the morphological and biochemical properties of strain JS-08^T followed previously described procedures (Yoon *et al.*, 2006; Zhang *et al.*, 2008). The following phenotypic tests were performed: Gram stain, cell morphology, catalase and oxidase activities and hydrolysis of casein, cellulose, DNA, starch, Tweens 20, 40, 60 and 80 and urea. Growth at 4, 10, 20, 25, 30, 37 and 42 °C and at pH 4–12 (at intervals of 1.0 pH unit, adjusted by using 1 M HCl or NaOH) was examined in marine broth (Difco). Growth in the presence of 0, 3, 5, 10, 15 and 20 % NaCl was investigated by using a basal medium containing (per litre) 10 g peptone and 5 g yeast extract. Other biochemical and physiological properties were examined by using API 20E and API ZYM strips (bioMérieux) according to the manufacturer's instructions. Utilization of 95 selected carbon sources was examined by using Biolog GN2 microplates according to the manufacturer's instructions.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JS-08^T is GQ857652.

The isoprenoid quinone content was analysed via reversed-phase HPLC as described by Cho *et al.* (2008). Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID) (Kämpfer & Kroppenstedt, 1996). The DNA G + C content was determined by using the methods outlined by Cui *et al.* (2007).

The 16S rRNA gene of strain JS-08^T was amplified by PCR from genomic DNA and sequenced as described previously (Cho *et al.*, 2008). The resultant 16S rRNA gene sequence was aligned with corresponding sequences of the type strains of recognized *Myroides* species by using the PHYDIT program, version 3.1 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by using the Fitch–Margoliash, maximum-likelihood, maximum-parsimony and neighbour-joining methods following procedures described previously (Kim *et al.*, 2003).

Strain JS-08^T formed yellow-to-orange, circular, convex colonies with entire margins on MA at 30 °C within 48 h. Growth occurred at 10–37 °C. Cells were aerobic, Gram-negative, non-spore-forming, non-motile rods. Other phenotypic characteristics are given in the species description

and in Table 1. Strain JS-08^T could be readily distinguished phenotypically from recognized species of the genus *Myroides*. Notably, the novel strain was in general positive for more enzyme activities and had a wider carbon source utilization spectrum, implying that strain JS-08^T had a wider metabolic capability than the representative *Myroides* strains investigated.

Menaquinone-6 (MK-6) was the predominant quinone isoprenologue. Strain JS-08^T contained iso-C_{15:0} (24.2 % of the total fatty acid composition), iso-C_{17:0} 3-OH (18.1 %) and a summed feature consisting of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (16.8 %) as the predominant fatty acids. Table 2 shows the detailed fatty acid profile of strain JS-08^T in comparison with those of recognized *Myroides* species. The presence of C_{18:1}ω6c (1.8 %) and C_{17:0} 3-OH (1.3 %) was characteristic of strain JS-08^T. The DNA G + C content of strain JS-08^T was 34.2 mol%, within the range reported for the genus (33.8–36.7 mol%).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JS-08^T is a member of the family *Flavobacteriaceae* and forms a coherent cluster with species of the genus *Myroides* (Fig. 1). Strain JS-08^T was related

Table 1. Differential phenotypic characteristics between strain JS-08^T and the type strains of recognized *Myroides* species

Strains: 1, JS-08^T; 2, *M. odoratimimus* JCM 7460^T; 3, *M. profundus* DSM 19823^T; 4, *M. pelagicus* KCTC 12661^T; 5, *M. odoratus* JCM 7458^T. Data for source of isolation and DNA G + C content values in parentheses were taken from previous studies (Vancanneyt *et al.*, 1996; Yoon *et al.*, 2006; Zhang *et al.*, 2008); other data were obtained in this study.

Characteristic	1	2	3	4	5
Isolation source	Seawater	Wound swab	Deep-sea sediment	Seawater	Faeces of patients
Cell morphology	Short rods	Rods	Spindle-shaped rods	Short rods	Rods
Pigment	Yellow to orange	Pale yellow	Pale yellow, diffusible	Yellow to orange	Yellow
Growth temperature (°C)	10–37	10–37	10–42	20–37	20–37
Hydrolysis of:					
Casein	–	–	+	–	–
Tween 20	–	+	+	–	–
Tween 80	–	–	+	–	–
Activity of:					
Urease	+	+	+	–	–
Leucine arylamidase	+	+	+	+	–
Valine arylamidase	+	–	–	–	–
Cystine arylamidase	+	–	–	–	–
Trypsin	+	–	–	–	–
α-Chymotrypsin	+	–	–	–	–
Utilization of (Biolog):					
L-Alanine	+	–	+	–	–
α-Hydroxybutyric acid	+	–	+	–	–
γ-Hydroxybutyric acid	+	–	–	–	–
α-Ketoglutaric acid	–	+	+	+	–
L-Leucine	+	–	+	–	–
Propionic acid	+	–	–	–	–
Pyruvic acid methyl ester	–	+	+	–	–
Succinamic acid	–	+	+	+	–
Thymidine	–	+	+	+	+
DNA G + C content (mol%)	34.2	33.8 (31.7)	33.9 (33)	35.8 (33.6)	36.7 (34.7)

Table 2. Fatty acid contents of strain JS-08^T and the type strains of recognized *Myroides* species

Strains: 1, JS-08^T; 2, *M. odoratimimus* JCM 7460^T; 3, *M. profundus* DSM 19823^T; 4, *M. pelagicus* KCTC 12661^T; 5, *M. odoratus* JCM7458^T. Data were obtained in this study and are percentages of total fatty acids. —, Less than 1.0 % of the total or not detected.

Fatty acid	1	2	3	4	5
Straight-chain saturated					
C _{16:0}	1.3	1.4	1.3	—	—
Branched saturated					
iso-C _{13:0}	—	2.0	1.0	1.0	—
iso-C _{14:0}	2.6	1.5	1.7	3.3	—
iso-C _{15:0}	24.2	44.7	27.6	29.3	43.7
anteiso-C _{15:0}	1.1	3.8	—	—	8.1
iso-C _{16:0}	3.4	2.7	4.3	8.7	1.2
iso-C _{17:0}	—	—	2.2	—	—
anteiso-C _{17:0}	—	—	3.8	—	—
Unsaturated					
C _{17:1} ω8c	—	—	2.3	—	—
C _{18:1} ω6c	1.8	—	—	—	—
iso-C _{17:1} ω9c	2.4	2.8	3.3	5.7	13.2
Hydroxy					
C _{15:0} 2-OH	2.8	—	1.5	—	—
C _{15:0} 3-OH	2.3	—	—	1.0	—
iso-C _{15:0} 3-OH	4.7	3.6	4.0	5.3	4.4
C _{16:0} 3-OH	6.9	6.5	5.2	4.5	3.0
iso-C _{16:0} 3-OH	3.0	2.2	2.2	4.3	1.3
C _{17:0} 2-OH	—	—	—	—	1.7
C _{17:0} 3-OH	1.3	—	—	—	—
iso-C _{17:0} 3-OH	18.1	16.9	14.5	16.5	18.8
Summed feature*	16.8	9.2	15.4	8.0	1.3

*Comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

most closely to *M. odoratimimus* and *M. profundus*, but formed an independent phylogenetic line. Levels of 16S rRNA gene sequence similarity between strain JS-08^T and the type strains of *M. odoratimimus* and *M. profundus* were 97.0 % (40 differences out of 1349 bp) and 96.8 % (43 differences out of 1349 bp), respectively, values that can be considered low enough for distinction at the species level.

Thus, the phylogenetic and phenotypic evidence presented indicates clearly that strain JS-08^T represents a novel species of the genus *Myroides*, for which the name *Myroides marinus* sp. nov. is proposed.

Description of *Myroides marinus* sp. nov.

Myroides marinus (ma.ri'nus. L. masc. adj. *marinus* of the sea, marine).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile, short rods (0.6–1.0 µm long and 0.2–0.3 µm wide). Colonies are yellowish orange, circular and convex with entire margins on MA. The optimal temperature for

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the relationship between strain JS-08^T and the type strains of recognized *Myroides* species and other related species. Asterisks indicate branches that were also recovered by using the Fitch–Margoliash, maximum-likelihood and maximum-parsimony algorithms. Numbers at nodes are levels of bootstrap support (percentages) based on a neighbour-joining analysis of 1000 resampled datasets by using Jukes–Cantor distances; only values >50 % are given. Bar, 0.01 substitutions per nucleotide position.

growth is 30 °C. Growth occurs at 10–37 °C but not at 4 or 42 °C. The pH range for growth is pH 5–9, and optimal growth occurs at pH 6–7. Growth occurs in the presence of 0–5 % (w/v) NaCl within 7 days. The predominant menaquinone is MK-6. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH and a summed feature comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Catalase- and oxidase-positive. Voges–Proskauer test is positive. Citrate is utilized. Indole is produced weakly. Nitrate and nitrite are not reduced, and hydrogen sulphide is not produced. Hydrolyses DNA and Tweens 40 and 60, but not casein, cellulose, starch or Tweens 20 or 80. Acid is not produced from amygdalin, arabinose, glucose, inositol, mannitol, melibiose, rhamnose, sorbitol or sucrose. As determined from API ZYM and API 20E strips, positive for alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, gelatinase and urease activities, but negative for lipase (C14), α-galactosidase, β-glucuronidase, β-galactosidase, β-glucosidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. In Biolog GN2 microplates, the following compounds exhibit positive reactions: Tweens 40 and 80, succinic acid monomethyl ester, acetic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic 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-proline, L-serine, L-threonine, inosine, uridine and

2,3-butanediol. The DNA G + C content of the type strain is 34.2 mol%.

The type strain, JS-08^T (=KCTC 23023^T =JCM 16529^T), was isolated from seawater collected off Boryeong Province, Chungnam, Republic of Korea.

Acknowledgements

This research was supported by the Project on Survey and Excavation of Korean Indigenous Species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Korea, and also by a National Research Foundation of Korea grant funded by the Korean government (R01-2007-000-21120-0).

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