RESEARCH LETTER

Myroides profundi sp. nov., isolated from deep-sea sediment of the southern Okinawa Trough

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Abstract

A Gram-negative, nonmotile, aerobic and oxidase- and catalase-positive bacterium, designated D25^T, was isolated from the deep-sea sediments of the southern Okinawa Trough area. Phylogenetic analyses of 16S rRNA gene sequences showed that strain D25^T fell within the genus *Myroides*, with 99.2%, 96.0% and 93.4% sequence similarities to the only three recognized species of *Myroides*. However, the DNA–DNA similarity value between strain D25^T and its nearest neighbour *Myroides odoratimimus* JCM 7460^T was only 49.9% ($<$ 70%). Several phenotypic properties could be used to distinguish strain D25^T from other *Myroides* species. The main cellular fatty acids of strain D25^T were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{17:0}$3-OH and Summed Feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH). The major respiratory quinone was MK-6. The DNA G+C content was 33.0 mol%. The results of the polyphasic taxonomy analysis suggested that strain D25^T represents a novel species of the genus *Myroides*, for which the name *Myroides profundi* sp. nov. is proposed. The type strain is D25^T (= CCTCC M 208030^T = DSM 19823^T).

Introduction

The genus *Myroides*, belonging to the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), was proposed in 1996 to reclassify *Flavobacterium odoratum* strains (Vancanneyt *et al.*, 1996). At the time of writing, only three species of the genus *Myroides* have been delineated, i.e. *Myroides odoratus* (Vancanneyt *et al.*, 1996), *Myroides odoratimimus* (Vancanneyt *et al.*, 1996) and *Myroides pelagicus* (Yoon *et al.*, 2006). Most strains of *M. odoratus* and *M. odoratimimus* originated from clinical specimens (Holmes *et al.*, 1977; Vancanneyt *et al.*, 1996) while that of *M. pelagicus*, which was reported to be able to produce biosurfactant compounds (Maneerat *et al.*, 2005, 2006), originated from a marine environment (Yoon *et al.*, 2006). Strain D25^T was one of over 300 marine bacterial strains isolated from deep-sea sediments of the southern Okinawa Trough (Qin *et al.*, 2007), and was regarded as a marine protease-producing bacterium on the basis of its ability to form clear hydrolysis halos around colonies on a skimmed-milk agar plate. Preliminary analysis of the 16S rRNA gene sequence indicated that strain D25^T belongs to the genus *Myroides*. In this paper, a novel *Myroides* species is proposed for strain D25^T based on the results of a polyphasic taxonomy study.

Materials and methods

Sample collection

The deep-sea sediment samples were collected from the site MD05-2907 (24°47.19′N,122°29.30′E) near the southern Okinawa Trough at a water depth of 1245 m using a core sampler during the IMAGES XII, MD-147-Marco Polo Leg 2 cruise of the R/V Marion Dufresne of the French Polar Institute in May–June 2005. Samples were kept in a freezer at -20 °C till use.

Isolation and culture condition

Strain D25^T was isolated from the subseafloor sediments at 4 m below seafloor using the serial dilution method. Briefly, *c.* 1 g (wet weight) of subseafloor sediment was diluted in 10 mL artificial seawater (Qin *et al.*, 2007) and further serially 10-fold diluted to 10^{-6} dilution in the same

medium. Aliquots of 100 μL diluted deep-sea sediment samples (10^{-3}–10^{-6} dilution) were spread on marine agar 2216 (Difco) plates. The plates were then incubated at 25 °C for 3–5 days. Morphologically different colonies growing on the plates were isolated by repeated streaking on marine agar 2216 (Difco) plates. The isolated strain D25^T was routinely cultivated at 30 °C on marine agar medium containing 10 g L^{-1} peptone (Oxoid), 5 g L^{-1} yeast extract (Oxoid), 15 g L^{-1} agar and artificial seawater (hereafter referred to as marine agar), and stored at −70 °C in marine broth (10 g L^{-1} peptone, 5 g L^{-1} yeast extract and artificial seawater, hereafter referred to as marine broth) with 20% (v/v) glycerol (Qin *et al.*, 2007).

Genotypic characterization

The 16S rRNA gene of strain D25^T was amplified by PCR from genomic DNA and sequenced as described by Hu & Li (2007). The obtained 16S rRNA gene sequence of strain D25^T (1479 nucleotides, GenBank accession number: EU204978) was aligned manually with those of its closely related neighbours retrieved from GenBank using the MEGA software package (version 4.0). Phylogenetic trees with 1000 bootstrap replicates were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971) using the same software. Phylogenetic distances were calculated using the MEGA software package and the Jukes & Cantor (1969) model. The DNA G+C content of strain D25^T was determined by HPLC (Mesbah *et al.*, 1989). DNA–DNA hybridization experiment, between strain D25^T and *M. odoratimimus* JCM 7460^T obtained from the Japan Collection of Microorganisms, was conducted at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DSMZ) according to the methods of Huss *et al.* (1983).

Phenotypic characterization

Cellular morphology and the presence of flagella were examined using transmission electron microscopy (JEM-100CXII) as described previously (Yoon *et al.*, 2005). Flagellar motility was assessed using the hanging-drop method (Hu & Li, 2007) and gliding motility was examined as described by Bowman (2000). The temperature (4–45 °C) and pH (4–12, adjusted with 1 M HCl or 1 M NaOH solution) ranges for growth were tested in marine broth. The requirement for and tolerance to NaCl were determined in the medium containing 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, different amounts of NaCl (0–15%) and distilled water, pH 7.0 (Qin *et al.*, 2007). The presence of flexirubin-type pigments was detected using 20% KOH (w/v) as described by Bernardet *et al.* (2002). An antibiotic susceptibility test was performed using the disc-diffusion method (Kobayashi *et al.*, 2003) on marine agar. The discs were placed on the marine agar plate, on which 0.1 mL of the marine broth culture of strain D25^T was spread. The growth inhibition zones were observed after 48 h of incubation at 30 °C. The following physiological and biochemical characteristics were examined using standard procedures (Gerhardt *et al.*, 1994): Gram-staining, glucose fermentation, catalase and oxidase activities, nitrate and nitrite reduction, hydrolysis of starch, casein, DNA and carboxymethyl cellulose. Different enzymatic activities and production of H_2S, indole and acetoin were detected using API 20E and API ZYM strips (bioMérieux) (Yoon *et al.*, 2006). The profile of carbon substrate oxidation was obtained using a Biolog GN2 microplate (Biolog) with artificial seawater as the cell suspension medium as described by Qin *et al.* (2007).

Chemotaxonomic analyses

Chemotaxonomic analyses were carried out by Dr Brian Tindall of DSMZ. The analysis of cellular fatty acids, for cells grown on the trypticase soy broth agar for 24 h at 20 °C, was carried out according to the instructions of the Sherlock Microbial Identification System (MIDI). Analysis of quinones was performed by HPLC.

Results and discussion

Genotypic characteristics

The phylogenetic tree showed that strain D25^T was grouped within the genus *Myroides* with a high bootstrap value of 100% (Fig. 1). Strain D25^T had 99.2% 16S rRNA gene sequence similarity to its closest phylogenetic neighbour *M. odoratimimus* JCM 7460^T but only 96.0% and 93.4% sequence similarities to *M. pelagicus* IAM15337^T and *M. odoratus* JCM 7458^T, respectively. DNA–DNA hybridization experiments showed that the DNA–DNA similarity value between strain D25^T and *M. odoratimimus* JCM 7460^T was 49.9%. This value was below the threshold value of 70% proposed for the delineation of bacterial species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Hence, strain D25^T represents a new species in the genus *Myroides*. The DNA G+C content of strain D25^T was 33.0 mol%, a value within the range reported for *Myroides* strains (Yoon *et al.*, 2006).

Chemotaxonomic characteristics

The predominant cellular fatty acids of strain D25^T were iso-$C_{15:0}$ (42.8%), iso-$C_{17:1}\omega9c$ (20.1%), iso-$C_{17:0}$3-OH (10.5%) and Summed Feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH) (6.7%). The fatty acid pattern of strain D25^T was similar to those of the other *Myroides* species although

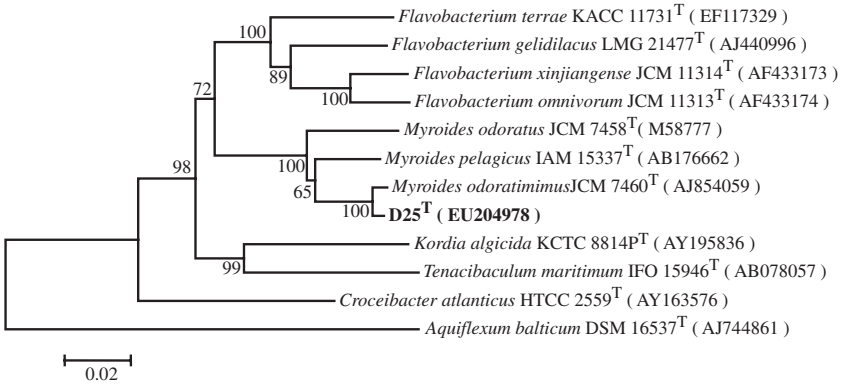

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain D25^T, other *Myroides* species and representatives of some other related members of the family *Flavobacteriaceae*. *Aquiflexum balticum* DSM 16537 was used as an outgroup. Percent bootstrap values above 50 (1000 replicates) are indicated at nodes. Scale bar = 0.02 substitutions per nucleotide position.

the contents of iso-$C_{17:1}\omega9c$ and Summed Feature 3 in strain D25^T were slightly higher than those in other species (Table 1). The major respiratory quinone of strain D25^T was MK-6, in accordance with other members of the family *Flavobacteriaceae*.

Phenotypic characteristics

Colonies of strain D25^T were white after 48 h of cultivation on marine agar at 30 °C, but gradually turned pale yellow and produced diffusible pale-yellow pigments with the extension of the incubation time. Cells were rods (0.7–0.9 × 1.6–2.4 μm) and resembled elongated spindles (Fig. 2). Strain D25^T had a particular carbon substrate oxidation profile (using Biolog GN2 microplate) in which only a few carbon sources could be oxidized compared with other *Myroides* species (Vancanneyt *et al*., 1996; Yoon *et al*., 2006). Other phenotypic characteristics of strain D25^T are shown in Table 2 and in the species description below.

Besides the difference in the isolation source, strain D25^T could be differentiated from other *Myroides* species by several key phenotypic features, including (1) the characteristic cell shape, (2) the tolerance to 8% NaCl, (3) the negative result for urease activity, (4) the resistances to various antibiotics and (5) the unique carbon substrate oxidation profile (Biolog GN2 microplate). Based on phenotypic, genotypic and chemotaxonomic characteristics, strain D25^T should be classified as a novel species of the genus *Myroides*, for which the name *Myroides profundi* sp. nov. is proposed.

Description of *M. profundi* sp. nov.

Myroides profundi (pro.fun'di. L. gen. n. *profundi* of the depths of the sea, of the deep sea).

Cells are spindle-shaped Gram-negative rods (0.7–0.9 μm in diameter and 1.6–2.4 μm in length), aerobic. Cells lack flagella and are nonmotile. Colonies grown on marine agar are pale yellow, circular, convex with entire margins and

Table 1. Fatty acid compositions (%) of strain D25^T and other *Myroides* species

Fatty acid	1	2	3	4
Iso-$C_{13:0}$	2.6	1.8	7.4	tr
$C_{15:0}$	2.5	tr	tr	tr
Iso-$C_{15:0}$	42.8	43.3	49.4	45.5
Anteiso-$C_{15:0}$	tr	1.0	1.0	2.0
Iso-$C_{15:0}$3-OH	4.6	5.8	6.1	5.8
$C_{16:0}$	1.0	tr	tr	tr
$C_{16:0}$3-OH	3.6	4.0	2.8	1.5
Iso-$C_{17:0}$3-OH	10.5	12.5	10.1	21.2
Iso-$C_{17:1}\omega9c$	20.1	15.1	13.1	13.8
Summed Feature 3*	6.7	3.6	1.9	tr
Unknown ECL 16.582	1.4	tr	tr	tr

Fatty acids amounting to < 1% of the total fatty acids in all strains listed are not shown.
Strains: 1, D25^T; 2, *Myroides odoratimimus* JCM 7460^T; 3, *Myroides pelagicus* IAM 15337^T; 4, *Myroides odoratus* JCM 7458^T.
tr, trace amount (< 1%); ECL, equivalent chain length.
Data from Yoon *et al*. (2006) and this study.
*Summed Feature 3, comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH.

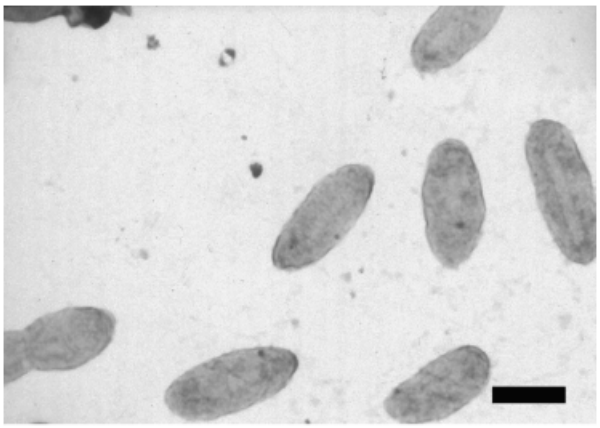

Fig. 2. Transmission electron micrograph of negatively stained cells of strain D25^T cultured in marine broth at 30 °C for 18 h. Scale bar = 1 μm.

Table 2. Differential characteristics of strain D25^T and other *Myroides* species

Characteristic	1	2	3	4
Source	Deep-sea sediment	Wound swab	Sea water	Faeces of patients
Pigment	Pale yellow, diffusible	Pale yellow	Yellow-to-orange	Yellow
Cell morphology	Spindle-shaped rod	Rod	Short rod	Rod
Range for growth				
NaCl (%)	0–8	0–6	0–9	0–5
pH	5–9	6–9	5–9	6–9
Reduction of nitrite	+	+	−	+
Activity of				
Catalase	+	+	w	+
Esterase (C4)	+	+	−	+
Esterase lipase (C8)	+	+	−	+
Urease	−	+	−	+
Oxidation of (Biolog)				
Acetic acid	−	+	+	+
L-Histidine	−	+	−	+
Methylpyruvate	−	+	+	+
Tween 80	−	+	−	+
DNA G+C content (mol%)	33.0	31.7	33.6	34.7

Strains: 1, D25^T; 2, *Myroides odoratimimus* JCM 7460^T; 3, *Myroides pelagicus* IAM 15337^T; 4, *Myroides odoratus* JCM 7458^T.
Data from Holmes *et al*. (1977), Vancanneyt *et al*. (1996), Yoon *et al*. (2006) and this study.
+, positive; −, negative; w, weakly positive.

smooth surfaces. The pigments are diffusible and nonflexirubin type. Oxidase and catalase positive. Growth occurs in the presence of 0–8% NaCl (optimum, 1%), at 8–42 °C (optimum, 30–35 °C) and at pH 5.0–9.0 (optimum, pH 6–7). No growth is observed at 4 or 45 °C. Hydrolyses casein, gelatin and DNA but not starch and carboxymethyl cellulose. Nitrite is reduced, nitrate is not reduced. Result of the Voges–Proskauer test is positive. Citrate is not utilized. Indole and H_2S are not produced. As determined by API ZYM and API 20E strips, alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and gelatinase activities are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities are absent. Acids are not produced from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose (API 20E). Using the Biolog GN2 microplate, the type strain oxidizes L-arabinose, succinic acid monomethyl ester, α-ketoglutaric acid, bromosuccinic acid, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline and thymidine. All other substrates in the GN2 microplate are not oxidized. The type strain is sensitive to chloramphenicol (30 μg), erythromycin (15 μg) and vancomycin (30 μg), but resistant to ampicillin (10 μg), cefalexin (30 μg), cefazolin (30 μg), gentamycin (10 μg), kanamycin (30 μg), norfloxacin (10 μg) and streptomycin (10 μg). The major respiratory quinone is MK-6. The main cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{17:0}$3-OH and Summed Feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH). The DNA G+C content of the type strain is 33.0 mol%.

The type strain is D25^T (=CCTCC M 208030^T=DSM 19823^T), isolated from the deep-sea sediments of the southern Okinawa Trough.

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Authors' contribution

X.-Y.Z. and Y.-J.Z. contributed equally to this work.

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