Mesonía mobilis sp. nov., isolated from seawater, and emended description of the genus *Mesonía*

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The taxonomic position of a novel marine, heterotrophic, strictly aerobic, gliding and yellow-pigmented bacterium, designated strain KMM 6059^T, was determined. 16S rRNA gene sequence analysis revealed that this strain represents a member of the genus *Mesonía*. Phenotypic and chemotaxonomic data showed that the isolate represents a novel species of the genus *Mesonía*, for which the name *Mesonía mobilis* sp. nov. is proposed. The type strain is KMM 6059^T (=KCTC 12708^T=LMG 23670^T). An emended description of the genus *Mesonía* based on the new data is also given.

The genus *Mesonía*, comprising the single species *Mesonía algae* (Nedashkovskaya *et al.*, 2003), was erected to accommodate Gram-negative, strictly aerobic, heterotrophic, yellow-pigmented and non-motile marine bacteria belonging to the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). Strains of *M. algae* were isolated from the common Pacific green alga *Acrosiphonia sonderi*. The genus *Mesonía* forms a phylogenetic cluster with the genera *Salegentibacter* and *Gramella*.

During June 2000 we isolated an unknown bacterial strain, designated KMM 6059^T, from a seawater sample collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan. A polyphasic taxonomic study of this strain indicated that it represents a novel species of the genus *Mesonía*.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed the procedures given in Kim *et al.* (1998). To establish the precise taxonomic position of strain KMM 6059^T, 1434 nt of its 16S rRNA gene sequence was determined, 1405 bp of which were used for comparative phylogenetic analysis. The sequence data obtained were aligned with sequences of representative members of the family *Flavobacteriaceae* retrieved from EMBL using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>).

Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from Kimura's two-parameter model (Kimura, 1980), and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets, using the SEQBOOT and CONSENSE programs of the PHYLIP package.

16S rRNA gene sequence analysis indicated that strain KMM 6059^T was a member of the family *Flavobacteriaceae* and formed a distinct branch within the genus *Mesonía* (Fig. 1). The level of 16S rRNA gene sequence similarity between KMM 6059^T and *M. algae* KMM 3909^T was 95.8%.

Genomic DNA was isolated according to the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA base composition of KMM 6059^T was 36.1 mol% G+C.

In order to determine whole-cell fatty acid and polar lipid profiles, strains KMM 6059^T and *M. algae* KMM 3909^T were grown at 28 °C for 48 h on marine agar 2216 (Difco). Lipids were extracted by a method modified from that of Bligh & Dyer (1959). Polar lipids were separated by two-dimensional micro-TLC in solvent systems as described by Vaskovsky & Terekhova (1979). Lipids were detected by TLC using 10% H₂SO₄ in methanol with subsequent

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Mesonía mobilis* KMM 6059^T is DQ367409.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences of KMM 6059^T and members of related genera of the family *Flavobacteriaceae*. The topology was not changed in trees constructed with the least-squares or maximum-likelihood methods. Numbers at nodes are bootstrap values (percentages of 1000 resampled datasets). Bar, 0.01 substitutions per nucleotide position.

heating to 180 °C, and using specific reagents for phospholipids (Vaskovsky *et al.*, 1975) and amino group-containing lipids (2% ninhydrin in acetone). The lipids were treated with 5% HCl in methanol at 80 °C for 180 min to produce fatty acid methyl esters (FAMES) (Christie, 1982). FAMES were analysed in a flame ionization detector gas chromatograph (Shimadzu GC-17) with a fused silica capillary column (30 m × 0.25 mm) coated with Supelcowax 10 at 210 °C. Helium was used as carrier gas. FAMES were identified by comparing the retention times with those of authentic standards and using equivalent chain length

measurements. To ensure correct identification, FAMES were also analysed by GC-MS (Shimadzu QP5050A) with an MDN-5S capillary column (30 m × 0.25 mm). The column temperature was programmed as follows: 1 min hold at 170 °C, followed by an increase to 240 °C at 2 °C min⁻¹, and a hold at 240 °C for 20 min. The temperature of the injector and detector was 250 °C.

Phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids of KMM 6059^T and *M. algae* KMM 3909^T were straight-chain unsaturated, branched-chain unsaturated and saturated, namely iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0}, iso-C_{15:1}, C_{16:1}ω7, iso-C_{17:1} and iso-C_{15:0} 2-OH (Table 1).

The physiological, morphological and biochemical characteristics of strain KMM 6059^T were tested as described previously (Nedashkovskaya *et al.*, 2003, 2004); they are given in the species description below and in Table 2. Similarities in phenotypic characteristics and cellular fatty acid composition support the inclusion of strain KMM 6059^T within the genus *Mesononia*. However, strain KMM 6059^T differed from *M. algae* based on several phenotypic features, including its ability to move by gliding, to grow at 39 °C, to produce acid from D-glucose and D-maltose and to utilize L-arabinose and D-mannose (Table 2). Whereas *M. algae* strains were able to degrade casein and Tween 40, KMM 6059^T could not hydrolyse these substrates. Susceptibility to benzylpenicillin, resistance to carbenicillin and oleandomycin and higher G+C content of the DNA

Table 1. Fatty acid compositions of *Mesononia* type strains

Values are percentages of the total fatty acids. Only those accounting for 1.0% or more in one of the strains are given. —, Not detected.

Fatty acid	<i>M. mobilis</i> KMM 6059 ^T	<i>M. algae</i> KMM 3909 ^T
iso-C _{15:1}	38.3	36.7
anteiso-C _{15:0}	4.0	11.4
iso-C _{15:0}	9.7	9.8
C _{15:0}	7.6	6.0
C _{15:1} ω6	2.3	2.9
iso-C _{16:0}	1.0	3.4
anteiso-C _{16:1}	0.6	1.0
C _{16:0}	2.2	0.9
C _{16:1} ω7	8.1	3.6
iso-C _{17:1}	6.2	6.5
C _{17:0}	0.4	1.0
C _{17:1} ω8	1.0	1.0
C _{17:1} ω6	2.6	1.9
C _{18:1} ω7	1.0	0.5
iso-C _{15:0} 2-OH	8.3	2.9
iso-C _{16:0} 2-OH	0.7	1.0
iso-C _{16:0} 3-OH	0.5	1.4
iso-C _{17:0} 3-OH	2.3	3.7
anteiso-C _{17:0} 3-OH	—	1.0

Table 2. Differential phenotypic characteristics of *Mesonia* species

All strains were positive for the following tests: presence of oxidase, catalase and alkaline phosphatase, Na⁺ requirement for growth, growth with 1–12% NaCl and at 4–34 °C, hydrolysis of gelatin and Tween 20, susceptibility to lincomycin and resistance to gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. All of the strains were negative for the following tests: requirement for organic growth factors, β -galactosidase activity, nitrate reduction, flexirubin pigments, indole and acetoin production, degradation of agar, DNA, starch, cellulose (carboxymethylcellulose and filter paper), chitin and urea, acid production from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-lactose, L-raffinose, D-melibiose, L-rhamnose, sucrose, L-sorbose, adonitol, dulcitol, glycerol, inositol, mannitol, malate, fumarate and citrate and utilization of D-lactose, sucrose, adonitol, dulcitol, inositol, mannitol, sorbitol, malonate and citrate. Data are taken from Nedashkovskaya *et al.* (2003) (for four strains of *M. algae*) and this study.

Characteristic	<i>M. mobilis</i> KMM 6059 ^T	<i>M. algae</i>
Gliding motility	+	–
Growth at 39 °C	+	–
H ₂ S production	–	+
Degradation of casein and Tween 40	–	+
Acid production from D-glucose and D-maltose	+	–
Utilization of L-arabinose, D-glucose and D-mannose	+	–
Susceptibility to:		
Benzylpenicillin	+	–
Carbenicillin and oleandomycin	–	+
DNA G+C content (mol%)	36.1	32–34

may be also used to differentiate the new isolate from *M. algae*.

Thus, on basis of the phenotypic, genotypic and molecular distinctiveness of strain KMM 6059^T, we suggest that it represents a novel species in the genus *Mesonia*, for which the name *Mesonia mobilis* sp. nov. is proposed.

Although bacteria belonging to the genus *Mesonia* were described as non-motile organisms, gliding motility was observed for cells of strain KMM 6059^T. For this reason, and because data on phospholipid composition are now available, we provide an emended description of the genus *Mesonia*.

Description of *Mesonia mobilis* sp. nov.

Mesonia mobilis (mo.bi'lis. L. fem. adj. *mobilis* movable, mobile, referring to the ability to move by gliding).

Cells are Gram-negative, strictly aerobic with respiratory metabolism, chemo-organotrophic, motile by gliding, asporogenic and rod-shaped, ranging from 0.4 to 0.5 μ m in width and from 1.0 to 2.1 μ m in length. Oxidase-, catalase- and alkaline phosphatase-positive and β -galactosidase-negative. Colonies are circular, convex and shiny with entire edges. Colonies are 1–3 mm in diameter when grown on marine agar. Produces non-diffusible yellow pigments. Flexirubin-type pigments are absent. Grows in the presence of 1–12% NaCl, at 4–39 °C and at pH 6.0–9.5. Optimal growth is observed with 3–4% NaCl, at 28–30 °C and at pH 7.5. Degrades gelatin and Tween 20. Does not hydrolyse

agar, casein, starch, cellulose (carboxymethylcellulose and filter paper), chitin, DNA, urea or Tweens 40 or 80. Forms acid from D-glucose and D-maltose, but not from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-lactose, D-melibiose, sucrose, L-raffinose, L-rhamnose, L-sorbose, DL-xylose, N-acetylglucosamine, adonitol, dulcitol, glycerol, inositol, mannitol, malate, fumarate or citrate. Nitrate is not reduced. H₂S, indole and acetoin (Voges–Proskauer reaction) are not produced. Susceptible to ampicillin, benzylpenicillin and lincomycin. Resistant to carbenicillin, gentamicin, kanamycin, neomycin, oleandomycin, polymyxin B, streptomycin and tetracycline. The predominant cellular fatty acids are straight-chain unsaturated, branched-chain unsaturated and saturated, namely iso-C_{15:0} (9.7%), anteiso-C_{15:0} (4%), C_{15:0} (7.6%), iso-C_{15:1} (38.3%), C_{16:1} ω 7 (8.1%), iso-C_{17:1} (6.2%) and iso-C_{15:0} 2-OH (8.3%). The G+C content of the DNA is 36.1 mol%.

The type strain, KMM 6059^T (=KCTC 12708^T=LMG 23670^T), was isolated from seawater collected in Troitsa Bay, Gulf of Peter the Great, East Sea (also known as the Sea of Japan).

Emended description of the genus *Mesonia* Nedashkovskaya *et al.* 2003

The description is as given by Nedashkovskaya *et al.* (2003), with the following changes. Cells may be motile by means of gliding. Phosphatidylethanolamine is the only phospholipid identified. The G+C content of the DNA is in the range 32–37 mol%. The type species is *Mesonia algae*.

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