Mesonía phycicola sp. nov., isolated from seaweed, and emended description of the genus *Mesonía*

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An aerobic, Gram-reaction-negative, non-motile, catalase- and oxidase-positive bacterium, designated strain MDSW-25^T, was isolated from seaweed collected in the vicinity of Mara Island in Jeju province, Republic of Korea. Colonies were smooth, circular and convex with entire edges and yellow in colour. Growth occurred at 10–30 °C, at pH 6.1–9.1 and in the presence of 1–12 % (w/v) NaCl. The major fatty acids were iso-C_{15:0} (25.6 %) and iso-C_{15:1} G (11.3 %), and the major menaquinone was MK-6. The DNA G + C content was 30.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain MDSW-25^T belonged to the genus *Mesonía*, family *Flavobacteriaceae*. Sequence similarity with *Mesonía mobilis* and *Mesonía algae* was 97.5 and 95.4 %, respectively, but DNA relatedness between strain MDSW-25^T and *M. mobilis* KCTC 12708^T was only 47 %. A battery of phenotypic data, phylogenetic inference and DNA–DNA hybridization analyses supports the conclusion that strain MDSW-25^T (=KCTC 22373^T =DSM 21425^T) represents a novel species of the genus *Mesonía*, for which the name *Mesonía phycicola* sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of the genus *Mesonía* is also proposed.

The genus *Mesonía* and the species *Mesonía algae* were described by Nedashkovskaya *et al.* (2003) to accommodate a Gram-reaction-negative, strictly aerobic, heterotrophic, yellow-pigmented, non-motile strain isolated from green algae. In contrast, *Mesonía mobilis*, subsequently isolated from seawater, is motile by means of gliding (Nedashkovskaya *et al.*, 2006). The genus *Mesonía* is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes* (Nedashkovskaya *et al.*, 2003). In this study, a seaweed isolate was subjected to a polyphasic taxonomic characterization. The results justify the description of a new species of the genus *Mesonía*.

Strain MDSW-25^T was isolated from a seaweed sample collected in the vicinity of Mara Island in Jeju province, Republic of Korea. The procedure and agar medium used for bacterial isolation was described previously (Lee, 2006). A colony was transferred onto marine agar (MA; Difco) at 30 °C. The pure culture was preserved as a suspension in natural seawater, distilled water and glycerol (60/20/20, by vol.). *Mesonía algae* KCTC 12908^T and *M. mobilis* KCTC 12708^T were grown on MA at 30 °C, studied in parallel with strain MDSW-25^T for phenotypic investigations and used as references in DNA–DNA hybridization experiments.

Cell morphology was observed by using phase-contrast and transmission electron microscopy, using cells grown on

MA for 5 days at 30 °C. The cells were negatively stained with 1 % phosphotungstic acid and the grid, after air-drying, was observed with a model 1200EXII transmission electron microscope (JEOL). Gliding motility was assessed by phase-contrast microscopy using the hanging drop technique (Bernardet *et al.*, 2002) after growing cells in 0.1 × marine broth (MB; Difco) for 24 h at 30 °C, with *M. mobilis* KCTC 12708^T as a positive control and *M. algae* KCTC 12908^T as a negative control. Colony morphology and pigmentation were observed on MA after 5 days at 30 °C. Unless specified otherwise, physiological characteristics were examined at 30 °C using MA as the basal medium. The temperature and pH for growth were tested at 4, 10, 20, 30, 37, 42 and 45 °C and at pH 4.1–12.1 (at 1 pH unit intervals), respectively. The medium pH was adjusted with 6 M HCl or 10 M NaOH and verified after autoclaving. NaCl tolerance was examined on NaCl-free MB supplemented with 1.5 % (w/v) agar as a basal medium to which NaCl was added at the final concentrations of 0–15 % (at 1 % intervals) and 20 % (w/v) NaCl. Plates were incubated for 5 days at 30 °C. Oxidase and catalase activities were determined with N, N, N', N'-tetramethyl-*p*-phenylenediamine dihydrochloride and 3 % H₂O₂ solutions, respectively. Gram staining was carried out using the Gram 2 kit (bioMérieux) following the instructions of the manufacturer. Hydrolysis of casein and starch were tested as described previously (Lee, 2007). Degradation of agar, chitin, cellulose, Tween 40 and Tween 80 was determined on MA supplemented with 1.0 % (w/v) Bacto-agar (Difco),

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MDSW-25^T is FM882228.

0.5 % (w/v) colloidal chitin, 0.5 % (w/v) CM-cellulose (Sigma), 1.0 % (v/v) Tween 40 or 1.0 % (v/v) Tween 80, respectively. DNA hydrolysis was tested on DNase test agar (Difco) prepared with MB instead of distilled water. Anaerobic growth was tested by using the GasPak EZ Anaerobe Pouch System (BD) according to the manufacturer's instructions. H₂S production was tested on trypticase soy broth (Difco) prepared with MB instead of distilled water using a lead acetate strip. Production of acid from D-glucose and maltose was determined on Bacto OF basal medium (Difco) supplemented with 2 % (w/v) sea salts (Sigma) to which the filter-sterilized carbon source was added at a final concentration of 1 % (w/v). Physiological and biochemical features were investigated using API 20NE and API ZYM strips (bioMérieux), following the manufacturer's instructions. For these tests, cells were grown on MA for 3 days at 30 °C and suspended in a 2 % sea salts (Sigma) solution. Results were recorded after 4 h of incubation at 37 °C for the API ZYM strip and after 2 days of incubation at 30 °C for the API 20NE strip. The phenotypic characteristics of strain MDSW-25^T are given in the species description and in Table 1.

Chromosomal DNA was isolated and purified by using the Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's instructions. PCR amplification of the 16S rRNA gene and sequencing were performed as described previously (Lee *et al.*, 2000; Lee, 2007). Multiple alignments of the sequences were performed by using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. The neighbour-joining tree was drawn by using evolutionary distances calculated by the model of Jukes & Cantor (1969). The reliability of tree topology was assessed by bootstrap analysis (Felsenstein, 1985), based on 1000 resampled datasets.

The nearly complete 16S rRNA gene sequence (1437 nt) of strain MDSW-25^T determined in this study was aligned against sequences of representatives of the family *Flavobacteriaceae*. The neighbour-joining tree based on 16S rRNA gene sequence (Fig. 1) showed that strain MDSW-25^T belonged to the genus *Mesononia* and formed a tight cluster with *M. mobilis*, with a high bootstrap value (98 %). A similar topology was also found in the maximum-parsimony and maximum-likelihood trees. 16S rRNA gene sequence similarities of strain MDSW-25^T with *M. mobilis* and *M. algae* were 97.5 and 95.4 %, respectively.

DNA–DNA hybridization experiments were performed as described by Weon *et al.* (2008). The probe consisted of DNA from strain MDSW-25^T labelled with the non-radioactive DIG High Prime System (Roche). DNA relatedness of strain MDSW-25^T and *M. mobilis* KCTC 12708^T was 47 %, well below the threshold value of 70 % accepted for the definition of a bacterial genospecies (Wayne *et al.*, 1987).

Table 1. Differential characteristics of strain MDSW-25^T and the type strains of species of the genus *Mesononia*

Strains: 1, *Mesononia phycicola* sp. nov. MDSW-25^T; 2, *Mesononia algae* KCTC 12098^T; 3, *Mesononia mobilis* KCTC 12708^T. All data from this study, except for temperature and pH ranges for growth and DNA G+C content of *M. algae* KCTC 12098^T and *M. mobilis* KCTC 12708^T that are taken from Nedashkovskaya *et al.* (2003, 2006). All strains are positive or weakly positive for the following characteristics: presence of alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities and gelatin hydrolysis. All strains are negative for the following characteristics: production of flexirubin pigments, presence of esterase (C4), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosamidase, α -mannosidase, α -fucosidase, arginine dihydrolase and urease activities, nitrate reduction, indole production, glucose fermentation, H₂S production, degradation of CM-cellulose, starch, chitin and agar and assimilation of D-arabinose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Gliding motility	–	–	+
Temperature range (°C) for growth	10–30	4–34	4–39
pH range for growth	6.1–9.1	6.1–11.1	6.0–9.5
Tolerance to NaCl (%)	1–12	1–15	1–12
Enzyme activities (API ZYM)			
β -Galactosidase	–	w	–
Esterase lipase (C8)	+	+	–
α -Glucosidase	+	–	+
Hydrolysis of:			
DNA	+	–	–
Casein	–	+	–
Aesculin	+	–	+
Tween 40	–	+	–
Tween 80	–	+	+
Acid production from:			
D-Glucose	–	–	+
Maltose	–	–	+
Utilization of:			
D-Glucose	–	–	+
D-Mannose	–	–	+
DNA G+C content (mol%)	30.0	32.7–34.0	36.1

For chemotaxonomic analyses, cells were harvested from a MB culture grown for 3 days at 30 °C in a shaking incubator. Respiratory quinones were extracted according to Minnikin *et al.* (1984) and analysed by HPLC (Kroppenstedt, 1985). Polar lipids were determined by TLC as described by Minnikin *et al.* (1977). For fatty acid analysis, strain MDSW-25^T, *M. algae* KCTC 12098^T and *M. mobilis* KCTC 12708^T were grown on MA at 30 °C for 5 days. The cellular fatty acids were determined using the Sherlock Microbial Identification System (version 6.0;

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship of strain MDSW-25^T and representatives of the main branches of the family Flavobacteriaceae and related taxa. *Rhodothermus marinus* OKD7 (X95071) was used as an out-group (not shown). Asterisks indicate the branches also found in the maximum-likelihood and maximum-parsimony trees. Bootstrap values >50% are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

MIDI) according to the manufacturer's instructions. The G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989).

The major respiratory quinone was menaquinone 6 (MK-6), as reported for *M. algae* (Nedashkovskaya *et al.*, 2003). The predominant polar lipid of strain MDSW-25^T was phosphatidylethanolamine, in line with *M. mobilis* (Nedashkovskaya *et al.*, 2006). Cells of strain MDSW-25^T, *M. algae* KCTC 12098^T and *M. mobilis* KCTC 12708^T showed similar fatty acid profiles including large amounts of saturated, unsaturated and branched acids together with hydroxy acids. The major fatty acids of strain MDSW-25^T were iso-C_{15:0} (25.6%) and iso-C_{15:1} G (11.3%). Strain MDSW-25^T differed from the type strains of other species of the genus *Mesonia* by the presence of C_{15:0} 2-OH (1.1%), C_{15:0} 3-OH (1.5%) and an unknown fatty acid with an equivalent chain-length of 13.566 (5.3%). The complete fatty acid composition of strain MDSW-25^T is given in Table 2. The G+C content of the DNA was 30.0 mol%, a value distinctly lower than the range reported for species of the genus *Mesonia* (Nedashkovskaya *et al.*, 2003, 2006).

Characteristics distinguishing strain MDSW-25^T from the type strains of recognized species of the genus *Mesonia* are given in Table 1.

A battery of phenotypic data, DNA relatedness and phylogenetic inference showed that strain MDSW-25^T

represents a novel species of the genus *Mesonia*, for which the name *Mesonia phycicola* sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of the genus *Mesonia* is also proposed.

Emended description of the genus *Mesonia* Nedashkovskaya *et al.* 2003 emend. Nedashkovskaya *et al.* 2006

The description is as given by Nedashkovskaya *et al.* (2003, 2006), with the following modifications. Cellular fatty acids are characterized by saturated, unsaturated, methyl-branched and hydroxy fatty acids. The predominant cellular fatty acid is iso-C_{15:0}. The other major fatty acids are species-dependent. The G+C content of the DNA is 30.0–36.1%. The type species is *Mesonia algae*.

Table 2. Cellular fatty acid composition (%) of strain MDSW-25^T and the type strains of species of the genus *Mesonia*

Strains: 1, *Mesonia phycicola* sp. nov. MDSW-25^T; 2, *Mesonia algae* KCTC 12098^T; 3, *Mesonia mobilis* KCTC 12708^T. All data from this study. Fatty acids amounting to <1% of the total fatty acids in all strains were omitted. —, Not detected; tr, trace (<1%).

Fatty acid	1	2	3
Saturated			
C _{15:0}	4.2	4.3	3.8
C _{16:0}	5.9	3.8	7.4
C _{18:0}	9.8	4.2	12.6
Unsaturated			
C _{15:1} ω6c	3.6	1.5	1.0
C _{17:1} ω6c	tr	1.6	tr
iso-C _{15:1} G	11.3	9.0	9.7
iso-C _{16:1} H	1.4	3.8	tr
iso-C _{17:1} ω9c	4.3	7.1	2.8
anteiso-C _{17:1} ω9c	—	3.8	—
Branched			
iso-C _{15:0}	25.6	19.8	31.0
iso-C _{16:0}	1.0	5.5	1.1
anteiso-C _{15:0}	2.3	9.1	2.4
Hydroxy			
C _{15:0} 2-OH	1.1	—	—
C _{15:0} 3-OH	1.5	—	—
iso-C _{15:0} 3-OH	5.4	3.9	6.7
iso-C _{16:0} 3-OH	1.6	2.1	—
iso-C _{17:0} 3-OH	5.5	6.0	7.3
Summed feature:*			
4	6.0	5.8	5.1
5	—	—	1.1
Unknown 13.566†	5.3	—	—

*Summed features are combinations of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed features 4 and 5 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and iso-C_{17:1} I and/or anteiso-C_{17:1} B, respectively.

†Unknown fatty acid with an equivalent chain-length of 13.566.

Description of *Mesonia phycicola* sp. nov.

Mesonia phycicola [phy.ci.co'la. L. n. *phycos* -i (from Gr. n. *phukos*) seaweed; L. suff. -cola (from L. masc. n. *incola*) an inhabitant of a place, a resident; N.L. n. *phycicola* an inhabitant of seaweed].

Cells are Gram-reaction-negative, aerobic rods, 0.6–1.6 µm in diameter and 2.1–2.5 µm in length. Catalase- and oxidase-positive. Devoid of flagellar and gliding motility. On marine agar 2216, colonies are circular, convex with entire edges and yellow in colour. Flexirubin pigments are not produced. Growth occurs at 10–30 °C (optimum, 25–30 °C), at pH 6.1–9.1 (optimum, pH 7.1) and in the presence of 1–12 % (w/v) NaCl. Aesculin, gelatin and DNA are hydrolysed but agar, CM-cellulose, chitin, starch, Tween 40 and Tween 80 are not. H₂S is not produced. Acid is not produced from D-glucose or maltose. Negative for nitrate reduction, indole production, glucose fermentation, and for arginine dihydrolase, urease and β-galactosidase activities. D-Glucose, D-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are not assimilated (API 20NE). Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present; esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase activities are absent. The major respiratory quinone is menaquinone 6 (MK-6). The major cellular fatty acids are iso-C_{15:0} and iso-C_{15:1}. G. The DNA G+C content of the type strain is 30.0 mol%.

The type strain is MDSW-25^T (=KCTC 22373^T =DSM 21425^T), isolated from seaweed collected in the vicinity of Mara Island in Jeju province, Republic of Korea.

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