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Mesonia sediminis sp. nov., isolated from a sea cucumber culture pond

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Abstract A yellow-pigmented, Gram-stain negative and facultatively anaerobic bacterium, designated MF326^T, was isolated from a sample of sediment collected from a sea cucumber culture pond in Rongcheng, China (122°14′34″E 36°54′36″N). Cells of strain MF326^T were found to be catalase negative and oxidase positive. Optimal growth was found to occur at 30 °C and pH 7.0–7.5 in the presence of 2.0–3.0 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MF326^T is a member of the genus *Mesonia* and exhibits the high sequence similarity (94.3 %) with the type strain of *Mesonia ostreae*, followed by *Mesonia algae* (93.9 %). The dominant fatty acids of strain MF326^T were identified as iso-$C_{15:0}$, an unidentified fatty acid with an equivalent chain-length of 13.565 and anteiso-$C_{15:0}$. The major polar lipids were found to be two unidentified lipids and phosphatidylethanolamine. The major respiratory quinone was found to be MK-6 and the genomic DNA G+C content was determined to be 40.7 mol%. On the basis of the phylogenetic analysis and differential phenotypic characteristics, it is concluded that strain MF326^T (=KCTC 42255^T =MCCC 1H00125^T) should be assigned to the genus *Mesonia* as the type strain of a novel species, for which the name *Mesonia sediminis* sp. nov. is proposed.

Keywords *Mesonia sediminis* sp. nov. · 16S rRNA gene · Polyphasic taxonomy · Sediment

The GenBank accession number for the 16S rRNA gene sequence of *Mesonia sediminis* MF326^T is KR061432.

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Introduction

Bacteria that belong to the *Cytophaga–Flavobacterium–Bacteroides* group are often isolated from natural microbial communities of marine environments (Nedashkovskaya et al. 2003; Glöckner et al. 1999; Bano and Hollibaugh 2002; Kirchman 2002). The genus *Mesonia* is a member of the family *Flavobacteriaceae* within the phylum *Bacteroidetes* and was first proposed by Nedashkovskaya et al. (2003) with the description of *M. algae* KMM 3909^T. Subsequently, five further species have been described: *M. mobilis* (Nedashkovskaya et al. 2006),

M. phycicola (Kang and Lee 2010), *M. ostreae* (Lee et al. 2012), *M. aquimarina* (Choi et al. 2015) and *M. hippocampi* (Kolberg et al. 2015). Members of the genus *Mesonia* have been isolated from marine related environments and are described as Gram-stain negative, thin-rod shaped bacteria forming yellow colonies and do not for spores. Seawater or Na^+ ions are reported to be a prerequisite for growth (Kolberg et al. 2015). Some species of the genus *Mesonia* can produce flexirubin pigments (Kolberg et al. 2015). MK-6 is the major respiratory quinone and straight- and branched-chain unsaturated fatty acids are the predominant fatty acids (Nedashkovskaya and Kim 2011). The genomic DNA G+C content varies between 30 and 42.1 mol% (Lee et al. 2012).

In this study, we report a novel facultatively anaerobic species of the genus *Mesonia* that originated from a sample of sediment collected from a sea cucumber culture pond in Rongcheng, China, for which the name *Mesonia sediminis* sp. nov. is proposed.

Materials and methods

Organism, maintenance and cultural conditions

Strain MF326^T was isolated from a sample of sediment collected from a sea cucumber culture pond in Rongcheng, China (122°14′34″E 36°54′36″N) in May 2014. The sample was processed with the enrichment culture technique using an enrichment medium which consisted of the following ingredients in seawater: 1 g NH_4Cl l^{-1}, 2 g CH_3COONa l^{-1}, 0.2 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 0.2 g yeast extract l^{-1} and 0.2 g peptone l^{-1}. The pH of the medium was adjusted to pH 7.5 and then autoclaved. A 10 % (w/v) $NaHCO_3$ solution was filtered and a 2 % (w/v) KH_2PO_4 solution was autoclaved separately and added into the final medium to give final concentrations of 0.1 and 0.02 % respectively (Du et al. 2014). After 2 weeks of incubation at 28 °C in a 500 mL sealed glass bottle (filled with the enrichment medium), 1 mL of liquid medium from the sealed glass bottle was diluted with 9 mL of sterilized saline solution and serially diluted 10-fold for inoculum preparation. The plates were incubated at 28 °C for 3–7 days after spreading the diluted inoculum (100 μL) on 2216E agar (Hopebio). A yellow colony (MF326^T) that arose after 5 days was transferred to 2216E agar for purification and routinely incubated at 30 °C. Strain MF326^T was stored at −80 °C in sterile distilled water supplemented with 1 % (w/v) NaCl and 15 % (v/v) glycerol.

The type strains of the closely related species *M. ostreae* KCTC 23500^T and *M. algae* KCTC 12089^T were obtained from the Korean Collection for Type Cultures (KCTC), Daejeon, South Korea and used as reference strains in parallel for physiological tests and chemotaxonomic characteristics (except polar lipids analysis). *M. ostreae* KCTC 23500^T and *M. algae* KCTC 12089^T were grown on 2216E agar at 30 °C. Unless otherwise specified, all characteristics described hereafter were based on cultures grown on 2216E agar under the same conditions.

Morphological, physiological and biochemical analysis

Cell morphology, size, flagellum and motility were investigated under a light microscope (Ci-L, Nikon) and by transmission electron microscopy (Jem-1200, Jeol), using cells grown on 2216E agar at 30 °C for 48 h. Gliding motility was determined as described by Bowman (2000). The procedures for Gram reaction and hydrolysis of starch, gelatin, CM-cellulose, Tween 20, 40, 60 and 80 were performed as described by Smibert and Krieg (1994). Anaerobic growth was tested for 7 days at 30 °C on 2216E agar with or without 0.1 % (w/v) $NaNO_3$ in an anaerobic jar. The procedure to test the reduction of nitrate was performed as described by Dong and Cai (2001). The temperature range for growth of strain MF326^T was examined at 4, 10, 15, 20, 25, 28, 30, 33, 37, 42 and 45 °C on 2216E agar and in 2216E liquid medium (Hopebio). NaCl requirements were examined in 2216E liquid medium (seawater in 2216E liquid medium was replaced by artificial seawater: 3.2 g $MgSO_4$ l^{-1}, 2.2 g $MgCl_2$ l^{-1}, 1.2 g $CaCl_2$ l^{-1}, 0.7 g KCl l^{-1}, 0.2 g $NaHCO_3$ l^{-1}) containing NaCl at concentrations from 0 to 15 % (w/v, in 1 % intervals). The effect of pH on growth was investigated with shaking in 2216E liquid medium from pH 5.5 to 11.0 adding at a concentration of 20 mM during pH adjustment: MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0, 9.5, 10.0, 10.5 and 11.0). Bacterial growth was followed by the increase in turbidity at 660 nm determined with a spectrophotometer. Oxidase activity was tested using the oxidase

reagent kit (bioMérieux) according to the manufacturer's instructions. Catalase activity was determined by pouring 3 % H_2O_2 solution on bacterial colonies and observing bubble production.

As strain MF326^T showed poor growth on isosensitest agar (Oxoid) and Mueller-Hinton agar (Hopebio), susceptibility to antibiotics was assessed on 2216E agar using streptomycin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), ofloxacin (5 μg), neomycin (30 μg), sulfamethoxydiazine (5 μg), kanamycin (30 μg), tobramycin (10 μg), vancomycin (30 μg), gentamycin (10 μg) and clindamycin (30 μg) according to the Kirby-Bauer disc diffusion method described by Du et al. (2014), followed by the protocols of the clinical and laboratory standards institute (CLSI 2012).

Acid production from carbohydrates was investigated using API 50CHB test strips (bioMérieux) according to the manufacturer's instructions except that the salinity of the 50CHB medium was adjusted to 2 % (w/v) before inoculation. Classical tests of acid production from carbohydrates were performed as described previously (Leifson 1963). The MOF medium described by Leifson (1963) has the following composition with some modifications: 1 g tryptone l^{-1}, 0.1 g yeast extract l^{-1}, 0.5 g ammonium sulfate l^{-1}, 0.5 g tris buffer l^{-1}, 3 g agar l^{-1}, phenol red, 1.0 ml of 0.1 % aqueous solution per 100 ml of medium; HCl to pH 7.5 (about 0.3 ml of 1 N HCl per 100 ml of medium); 10 g carbohydrate l^{-1}, artificial sea water. Three milli litre medium was placed aseptically in tubes (13 × 100 mm) and the solid medium was stab-inoculated. Two tubes were inoculated with each culture, one of which was covered with paraffin oil to form a layer about 1 cm thick. Carbon source utilisation was tested using GEN III MicroPlates from the Microlog system (Biolog) according to the manufacturer's recommendations, with 3.0 % (w/v) NaCl added to the relevant media. In addition, various biochemical and additional enzyme activities were assayed using API 20E, 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions with the single modification of adjusting the NaCl concentration to 3.0 % (w/v). All the tests were performed in duplicate.

Molecular studies and phylogenetic analysis

Chromosomal DNA was extracted using a commercial gene extraction kit (TaKaRa MiniBEST Bcteria Genomic DNA Extraction Kit Ver. 3.0) and used to determine the DNA G+C content via HPLC as described by Tamaoka and Komagata (1984) and Mesbah et al. (1989). λDNA (Takara) was used as a standard. The nearly complete 16S rRNA gene sequence of strain MF326^T was obtained bidirectionally as described by Liu et al. (2014) and sequenced using an ABI BigDye 3.1 Sequencing Kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The almost full-length 16S rRNA gene sequence of strain MF326^T was determined and subjected to comparative analysis with available sequences of validly named species from NCBI BLASTN. The EzTaxon server was used to obtain the sequences of the related type strains (http://eztaxone.ezbiocloud.net/, Kim et al. 2012). A multiple sequence alignment of representative sequences was created using CLUSTAL_X program (version 1.81) (Thompson et al. 1997). A phylogenetic tree was constructed using the maximum likelihood method and general time reversible model in the computer program MEGA (version 6.0) (Felsenstein, 1981). The neighbour-joining and maximum parsimony algorithm (Tamura et al. 2013) were included to estimate and verify the taxonomic positions of the novel isolate and reference strains in the tree.

Chemotaxonomic characterisation

Cells of strain MF326^T were harvested at the late-exponential growth phase in 2216E liquid medium at 30 °C for characterisation of isoprenoid quinones, cellular fatty acids and polar lipids. The major menaquinones were detected according to the method described by Minnikin et al. (1984). The microbial identification system (MIDI, Microbial ID) was used to analyse fatty acid compositions, according to procedures described by Sasser (1990). Polar lipids were extracted from 100 mg of freeze dried cell material using a chloroform: methanol: 0.3 % (w/v) aqueous NaCl mixture 1:2:0.8 (v/v/v), modified after Bligh and Dyer (1959), recovered into the chloroform phase by adjusting the chloroform: methanol: 0.3 % (w/v) aqueous NaCl mixture to a ratio of 1:1:0.9 (v/v/v), and separated by two dimensional silica gel thin layer chromatography (Macherey-Nagel art. no. 818 135). Total lipid material was detected using molybdophosphoric acid and specific functional groups detected using spray reagents specific for defined functional groups (Tindall et al. 2007). The polar

lipids analysis was performed by the identification service of the DSMZ, Braunschweig, Germany.

Results and discussion

Morphological, physiological and biochemical analysis

Strain MF326^T was observed to be Gram-stain negative, non-motile and rod-shaped with cells of 0.4–0.6 μm in width and 1.0–3.0 μm in length (see Fig. 1). Colonies are yellow, 0.5–1.0 mm in diameter, circular and shiny with a smooth surface and an entire edge when cultivated on 2216E agar at 30 °C for 48 h. Growth was found to occur in 0.5–7.0 % (w/v) NaCl (optimum, 2.0–3.0 %), at 4–42 °C (optimum, 30 °C) and pH 6.0–10.5 (optimum, pH 7.0–7.5). There were no visible colonies formed under anaerobic conditions on 2216E agar. However, acid was found to be produced from trehalose, D-xylose, mannitol, arabinose, glucose, sucrose and starch according to classical tests where oxygen was excluded with an oil layer. These results suggest that strain MF326^T should be regarded as a facultatively anaerobic strain. Unlike other members of the genus *Mesonia*, cells of strain MF326^T were found to be susceptible to erythromycin (15 μg), streptomycin (10 μg), acetylspiramycin (30 μg), rifampin (5 μg), clarithromycin (15 μg), clindamycin (30 μg), cefotaxime sodium (30 μg), penicillin (10 μg), chloramphenicol (30 μg), norfloxacin (10 μg) and ofloxacin (5 μg); and resistant to tetracycline (30 μg), gentamycin (10 μg), streptomycin (10 μg), tobramycin (10 μg), sulfamethoxydiazine (5 μg), kanamycin (30 μg) and neomycin (30 μg). Like other members of the genus *Mesonia*, strain MF326^T is Gram-stain negative and forms yellow-pigmented colonies. Seawater or Na^+ ions are a prerequisite for growth. Nitrate was not reduced and indole was not produced, which is consistent with the properties of the genus *Mesonia*. More detailed data about morphological, cultural, physiological and biochemical characteristics of strain MF326^T are given in the species description and Table 1. Strain MF326^T can be distinguished clearly in Table 1 which contains a comparison to the other type strains of *Mesonia* species.

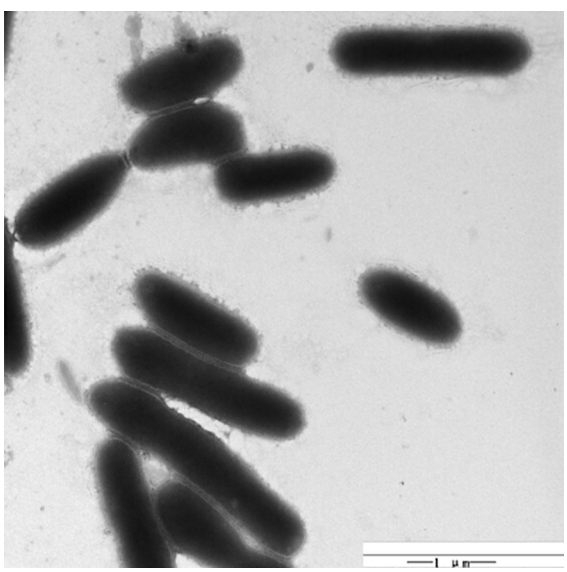

Fig. 1 Transmission electron micrograph of strain MF326^T. Cells grown on 2216E agar at 30 °C for 48 h. *Bar* = 1.0 μm

Molecular studies and phylogenetic analysis

The almost complete 16S rRNA gene sequence of strain MF326^T determined in this study comprised 1451 nucleotides (GenBank accession number KR061432). The highest 16S rRNA gene sequence similarity was obtained with *M. ostreae* (94.3 %). Strain MF326^T exhibited 16S rRNA gene sequence similarity values of 93.3–94.3 % to the type strains of *Mesonia* species, values which are lower than the borderline used for delineation of bacterial species (i.e. 97 %). The maximum likelihood tree showed that strain MF326^T formed a cluster with six species of the genus *Mesonia*, and this cluster was distantly related to another cluster comprising the members of other genera of the family *Flavobacteriaceae* (see Fig. 2). Thus the new strain can be placed within the monophyletic genus *Mesonia*. This close phylogenetic relationship was also revealed in the neighbour-joining (see Supplementary Fig. S1) and maximum parsimony trees (see Supplementary Fig. S2). This analysis indicated clearly that strain MF326^T should be considered to represent a novel species of the genus *Mesonia*.

The DNA G+C content of strain MF326^T is 40.7 mol% which is within range of the DNA G+C content (30.0–42.1 mol%) of the genus *Mesonia* (Lee et al. 2012).

Table 1 Differential phenotypic characteristics of strain MF326^T and the closely related species of the genus *Mesonia*

	1	2	3	4	5	6	7
Gliding motility	−	−	−	−	−	−	+
Temperature range (°C) for growth	4–42	4–31	4–34	4–36	10–37	10–30	4–39
pH range for growth	6.0–10.5	5.5–8.5	6.0–9.5	6.5–12.5	5.5–9.5	6.1–9.1	6.0–9.5
Tolerance to NaCl (%)	0.5–7	0–12	1–15	1–10	0.5–15	1–12	1–12
H_2S production	−	−	+	ND	−	−	−
Catalase	−	+	+	+	+	+	+
Hydrolysis of							
Tween 40	+	+	+	ND	ND	−	−
Tween 80	+	−	+	ND	+	−	−
CM-cellulose	−	−	−	ND	+	−	−
Gelatin	+	+	+	−	+	+	+
Enzyme activity							
Esterase (C4)	+	+	W	+	+	−	−
Lipase (C14)	W	−	W	−	−	−	−
Valine arylamidase	+	+	+	−	+	+	−
Trypsin	W	−	W	+	−	−	+
β-Glucosidase	−	−	−	−	+	+	−
α-Glucosidase	−	−	−	−	−	+	+
DNA G+C content (mol%)	40.7	42.1[a]	32.7–34.0[b]	30.0	41.4	30.0	36.1

Strains *1* strain MF326^T (data from this study), *2 M. ostreae* KCTC23500^T (data from this study except DNA G+C content), *3 M. algae* KCTC 12089^T (data from this study except DNA G+C content), *4 M. hippocampi* 96_Hippo_TS_3/13^T (Kolberg et al. 2015), *5 M. aquimarina* IMCC 1021^T (Choi et al. 2015), *6 M. phycicola* MDSW-25^T (Kang and Lee 2010), *7 M. mobilis* KMM 6059^T (Nedashkovskaya et al. 2006)

All strains were positive for oxidase and enzyme activities for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase; negative for indole production, reduction of nitrate, hydrolysis of starch, enzyme activities for lipase (C14), cysteine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase

+ Positive, − negative, *W* weakly positive, *ND* no data available

[a] Data from Lee et al. (2012)

[b] Data from Nedashkovskaya et al. (2003)

Chemotaxonomic characterisation

The predominant isoprenoid quinone present in strain MF326^T was identified as MK-6, which is compatible with the genus *Mesonia* (Nedashkovskaya et al. 2003). The major fatty acids (>5 % of the total fatty acids) identified in strain MF326^T were iso-$C_{15:0}$ (53.8 %), an unidentified fatty acid with an equivalent chain-length of 13.565 (13.0 %), anteiso-$C_{15:0}$ (9.6 %) and iso-$C_{17:0}$ 3-OH (6.5 %), which is similar to the profile of the type strains of *M. algae* KCTC 12089^T and *M. ostreae* KCTC 23500^T (see Supplementary Table S1) (Nedashkovskaya et al. 2003; Lee et al. 2012). The major polar lipids were found to be two unidentified lipids and phosphatidylethanolamine. Two aminolipids, an aminoglycolipid, a phosphoaminolipid, two unidentified lipids and a glycolipid are present in moderate to minor amounts in the polar lipid profile (see Supplementary Fig. S3). The polar lipids of strain MF326^T are similar to those of the type strains of *M. algae* KCTC 12089^T and *M. ostreae* KCTC 23500^T which contained phosphatidylethanolamine as the major polar lipid (Nedashkovskaya et al. 2003; Lee et al. 2012). The composition of the major fatty acids and polar lipids is the typical pattern which is described for the genus (Kolberg et al. 2015), although there were differences in the proportions.

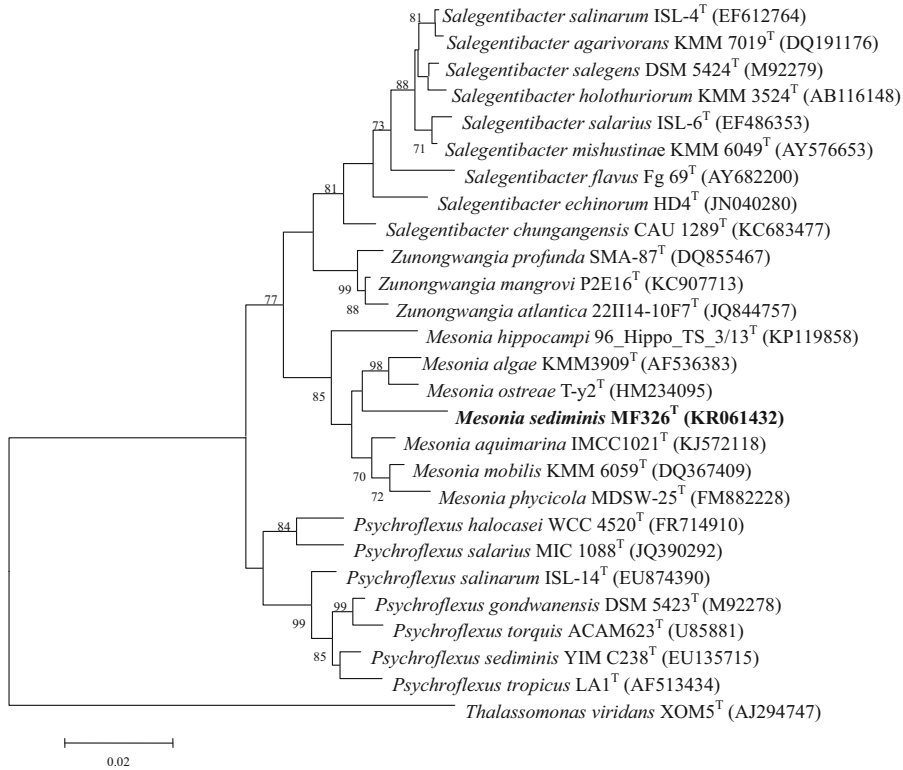

Fig. 2 Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic status of strain MF326^T, the type strains of other *Mesonia* species and some related taxa. *Thalassomonas viridans* XOM5^T was used as outgroup. Bootstrap values (expressed as percentages of 1000 replications) of ≥70 % are shown at branching nodes. *Bar* 0.02 substitutions per nucleotide position

Conclusion

The results obtained from phylogenetic analysis based on 16S rRNA gene sequences and chemotaxonomic analyses are sufficient to prove that strain MF326^T is a member of the genus *Mesonia*. Furthermore, the novel isolate can be clearly distinguished from the other *Mesonia* species by a combination of phenotypic, genotypic and phylogenetic features, e.g., antibiotic sensitivity, enzyme activities and range of growth conditions (see Table 1). Strain MF326^T can be distinguished from the other six species of the genus *Mesonia* by its ability to grow at 42 °C and low tolerance to NaCl. H_2S is not produced, which distinguishes the novel isolate from *M. algae* KCTC 12089^T. Strain MF326^T could be distinguished from *M. ostreae* KCTC 23500^T by hydrolysis of Tween 80. Therefore, on the basis of the phenotypic, chemotaxonomic, phylogenetic and genetic data, strain MF326^T is considered to represent a novel species of the genus *Mesonia*, for which the name *Mesonia sediminis* sp. nov. is proposed.

Description of *Mesonia sediminis* sp. nov

Mesonia sediminis (se.di.mi'nis. L. gen. n. *sediminis,* of sediment)

Cells are Gram-stain negative, facultatively anaerobic, non-motile and rod-shaped (0.4–0.6 × 1.0–3.0 μm). Colonies are yellow, 0.5–1.0 mm in diameter, circular and shiny with a smooth surface and an entire edge when cultivated on 2216E agar at 30 °C for 48 h. Growth occurs at 4–42 °C (optimum 30 °C), with 0.5–7.0 % (w/v) NaCl (optimum 2.0–3.0 %) and at pH 6.0–10.5 (optimum pH 7.0–7.5). H_2S and indole production are negative. Nitrate is not reduced. Positive for hydrolysis of Tween 20, 40, 60 and 80, negative for hydrolysis of starch, agar, gelatin and CM-cellulose. Alkaline phosphatase, acid phosphatase, valine

arylamidase, leucine arylamidase, cystine arylamidase, esterase lipase (C8), esterase (C4), lipase (C14), trypsin, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase are detected but α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-glucosaminidase and α-mannosidase are not. Acid is produced from esculin, 5-ketone potassium gluconate, trehalose, D-xylose, mannitol, arabinose, glucose, sucrose and starch. The following substrates are utilised as sole carbon and energy sources for growth: dextrin, D-raffinose, α-D-glucose, D-fucose, gelatin, glycyl-L-proline, L-arginine, L-aspartic acid, L-glutamic acid, tween-40, sucrose, L-fucose, L-rhamnose, inosine, D-fructose-6-PO_4, α-D-lactose, D-melibiose, D-fructose, D-galactose and D-sorbitol. The predominant fatty acids are iso-$C_{15:0}$, an unidentified fatty acid with an equivalent chain-length of 13.565 and anteiso-$C_{15:0}$. The major polar lipids are two unidentified lipids and phosphatidylethanolamine and the major respiratory quinone is MK-6. The DNA G+C content of the type strain is 40.7 mol%.

The type strain, MF326^T (=KCTC 42255^T =MCCC 1H00125^T), was isolated from a sample of sediment collected from sea cucumber culture pond in Rongcheng, China. The GenBank accession number for the 16S rRNA gene sequence of *M. sediminis* MF326^T is KR061432.

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