Mesonia aestuariivivens sp. nov., Isolated From a Tidal Flat

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Abstract

A Gram-negative, aerobic, non-flagellated and ovoid or rod-shaped bacterial strain (JHPTF-M18^T), which was isolated from a tidal flat sediment in Republic of Korea, was taxonomically characterized. 16S rRNA gene sequence analysis showed that strain JHPTF-M18^T forms phylogenetic lineage within the radiation comprising type strains of *Mesonia* species. The 16S rRNA gene of strain JHPTF-M18^T shared sequence similarities of 97.7% with that of type strain of *M. mobilis* and 92.9-96.8% with those of type strains of eight other *Mesonia* species. The DNA G+C content was 33.1% based on its genomic sequence. ANI and dDDH values between strain JHPTF-M18^T and the type strains of *M. mobilis*, *M. oceanica*, *M. phycicola* and *M. algae* were 73.1-79.7% and 18.5-22.8%, respectively. Strain JHPTF-M18^T contained MK-6 as the predominant menaquinone and iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) as its major fatty acids. Major polar lipids of strain JHPTF-M18^T were phosphatidylethanolamine and two unidentified lipids. Strain JHPTF-M18^T was separated from recognized *Mesonia* species by its phenotypic properties together with the phylogenetic and genetic distinctiveness. Based on data presented in this study, strain JHPTF-M18^T is considered to represent a novel species of the genus *Mesonia*. The name *Mesonia aestuariivivens* sp. nov. is proposed for JHPTF-M18^T (= KACC 22185^T = NBRC 115119^T).

Introduction

The genus *Mesonia* was created by Nedashkovskaya et al. (2003) with the assignment of *Mesonia algae* as the type species and belonged to the family *Flavobacteriaceae* of the phylum *Bacteroidetes* (Bernardet 2011). The genus *Mesonia* currently consists of ten species with validly published names (https://lpsn.dsmz.de/genus/mesonia; Parte 2018). Members of the genus *Mesonia* are known to be Gram-stain-negative, aerobic, catalase- and oxidase-positive and rod-shaped and to contain menaquinone-6 as predominant isoprenoid quinone, phosphatidylethanolamine as only major phospholipid identified and DNA G+C contents of 31.4-42.1 mol% (Kang and Lee 2010; Kolberg et al. 2015; Lee et al. 2012; Lucena et al. 2020; Nedashkovskaya et al. 2003, 2006). Isolation sources of *Mesonia* species described so far include green alga, seaweed, seawater, diseased Barbour's Seahorse and sea cucumber culture pond (Kang and Lee 2010; Kolberg et al. 2015; Lee et al. 2012; Lucena et al. 2020; Nedashkovskaya et al. 2003, 2006; Wang et al. 2015). Recently, in the course of screening novel bacteria from a tidal flat at Seocheon on the Yellow Sea of Korean peninsula, many bacterial isolates have been obtained followed by identified by 16S rRNA sequence analysis. Of these bacterial isolates, one strain (designated as JHPTF-M18^T) which showed the closest affiliation to members of the genus *Mesonia* was selected for further taxonomic study. In this study, strain JHPTF-M18^T is characterized further using a polyphasic characterization.

Materials And Methods

Bacterial strains and culture conditions

A tidal flat sediment was collected from Seocheon (36°01'44.6"N, 126°39'56.8"E) close to the Yellow Sea of Republic of Korea. The sample (about 1-2 g) was serially diluted with 0.85% (w/v) saline solution and spread on marine agar 2216 (MA; BD Difco). After incubation for 7 days at 25°C, strain JHPTF-M18^T was isolated from the MA plates and speak onto fresh MA. Strain JHPTF-M18^T was cultivated routinely on MA at 25°C, and its cells suspended in a sterile solution containing 20% (w/v) glycerol were stored at –80°C for long-term preservation. *Mesonia mobilis* KCTC 12708^T and *Mesonia algae* KCTC 12809^T, which were used as experimental control strains, were obtained from the Korean Collection for Type Cultures (KCTC; South Korea). Cells of strain JHPTF-M18^T were obtained from culture grown for 3 days in marine broth 2216 (MB; BD Difco) at 25°C and they were used to extract DNA and to analyze isoprenoid quinones and polar lipids. Cell masses for cellular fatty acid analysis were obtained under the following conditions: 2, 3 and 5 days at 25 ºC for strain JHPTF-M18^T and 3 days at 25 ºC for *M. mobilis* KCTC 12708^T and *M. algae* KCTC 12809^T

Sequencing and phylogenetic analysis of 16S rRNA gene

Chromosomal DNA extraction was performed using a Wizard Genomic DNA isolation kit (Promega) according to the manufacturer's instruction. The 16S rRNA gene amplification was performed as described previously (Yoon et al. 1997) using PCR in which 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1512R (5'-ACGGTTACCTTGTTACGACTT-3') were used. Sequencing of the 16S rRNA gene followed by phylogenetic analysis were carried out as described by Yoon et al. (2003). Similarity between 16S rRNA gene sequences was calculated using alignment obtained using Clustal W program.

Genomic analysis

A TruSeq DNA LT Sample Prep kit (Illumina) was used to prepare a library for genomic sequencing. The library was sequenced using Illumina MiSeq platform. Sequencing data were assembled with SPAdes (Bankevich et al. 2012). Contamination of genome sequence was assessed using ContEst16S (Lee et al. 2017). Library construction and sequencing were performed by Chunlab Inc. (Republic of Korea). The ANI value based on BLAST+ was calculated using JSpecies WS (http://jspecies.ribohost.com/jspeciesws/; Richter et al. 2015). The dDDH value was estimated using TYGS (https://tygs.dsmz.de/user_requests/new) with BLAST+ in which the recommended formula 2 (Meier-Kolthoff et al. 2013) was used. Phylogenetic tree was constructed based on genomic sequences using previous methods (Lefort et al. 2015; Meier-Kolthoff et al. 2013) described in the TYGS. Intergenomic distances inferred under the algorithm 'trimming' and distance formula d_5 (Meier-Kolthoff et al. 2013) and 100 distance replicates were calculated each. The resulting distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1 including SPR postprocessing (Lefort et al. 2015).

Chemotaxonomic characterization

Extraction and HPLC analysis of isoprenoid quinones were performed as described by Komagata and Suzuki (1987) and Park et al. (2014), respectively. Fatty acid analysis was performed as described by

Park et al. (2014) using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.2B), GC (Hewlett Packard 6890) and TSBA6 database of the Microbial Identification System (Sasser 1990). Extraction of polar lipids were carried out according to procedures described by Minnikin et al. (1984). They were separated by two-dimensional TLC using the solvent systems as described by Embley and Wait (1994). The TLC plates were sprayed with various reagents as described by Park et al. (2014) and individual polar lipids were visualized followed by identified with heating at 150 ºC for 3 min.

Morphological, cultural, physiological and biochemical characterization

Cell shape, Gram reaction, pH range for growth, anaerobic growth, growth at various concentrations of NaCl, requirement for Mg^{2+} ions, hydrolysis of gelatin and urea, susceptibility to antibiotics were investigated as described by Park et al. (2014). Growth at 4, 10, 20, 25, 28, 30, 35, 37 and 40°C was measured on MA to estimate the optimal temperature and temperature range for growth. Nitrate reduction and hydrolysis of aesculin and Tween 80 were investigated as described previously (Lányí 1987) using artificial seawater (Bruns et al. 2001) for the preparation of the media. Hydrolysis of other substrates was tested as described by Barrow and Feltham (1993) with the modification that MA was used. Activity of catalase and oxidase was determined as described by Lányí (1987). Other enzyme activities were determined using the API ZYM system (bioMe´rieux); the results were checked after incubation for 8 h at 25°C. Acid production from carbohydrates was tested as described by Leifson (1963).

Results And Discussion

Phylogenetic analysis based on 16S rRNA gene sequence

The almost complete 16S rRNA gene sequence of strain JHPTF-M18^T determined in this study had a continuous stretch of 1448 nucleotides, corresponding to positions 28-1491 (95%) of the *Escherichia coli* 16S rRNA sequence. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain JHPTF-M18^T fell within the clade comprising the type strains of *Mesonia* species, particularly joining the type strain of *M. mobilis* by a bootstrap resampling value of 64.9% (Fig. 1). Strain JHPTF-M18^T shared the highest 16S rRNA gene sequence similarity value (97.7%) to *M. mobilis* KCTC 12708^T. It also shared 92.9-96.8% 16S rRNA gene sequence similarities with the type strains of the other *Mesonia* species. These sequence similarities indicated that strain JHPTF-M18^T might be a member of species different from recognized *Mesonia* species according to the values (97% or 98.7%) recommended for delineation of a bacterial species by Stackebrandt and Goebel (1994) and Kim et al. (2014).

Genomic features

The genome size of strain JHPTF-M18^T obtained from the assembly of sequencing reads was 3,328,752 bp with a sequencing depth of coverage of 482.93X. The genomic sequence of strain JHPTF-M18^T contained 73 contigs with N50 length of 192,421 bp. The complete 16S rRNA gene sequence extracted from the genomic data using ContEst16S (Lee et al. 2017) was found to be identical to respective 16S rRNA gene information previously obtained by Sanger sequencing. This indicated that strain JHPTF-

M18^T and its genomic data were not mislabeled and did not originate from any source of contamination (Chun et al. 2018). Based on its genomic sequence data, the DNA G+C content of strain JHPTF-M18^T was 33.1%, a value in the range reported for *Mesonia* species (Lucena et al. 2020). The genome of strain JHPTF-M18^T had 2,967 protein-coding genes, within the range reported for *Mesonia mobilis* DSM 19841^T (2,897), *Mesonia oceanica* ISS653^T (3,789), *Mesonia phycicola* DSM 21425^T (2,939) and *Mesonia algae* DSM 15361^T (2,828). The genomic sequence of strain JHPTF-M18^T was shown to have 4 rRNA-encoding genes with one 5S, one 16S and two 23S rRNAs, whereas those of the type strains of *M. mobilis*, *M. oceanica*, *M. phycicola* and *M. algae* have rRNA-encoding genes of 6-10. The phylogenetic trees based on genomic sequences showed that strain JHPTF-M18^T form a cluster with the type strain of *Mesonia mobilis* (Fig. S1). The genomic sequence data of strain JHPTF-M18^T had ANI values of 79.7, 78.5, 78.0 and 73.1% to those of *M. mobilis* DSM 19841^T, *M. oceanica* ISS653^T, *M. phycicola* DSM 21425^T and *M. algae* DSM 15361^T, respectively. Strain JHPTF-M18^T had dDDH values of 22.8, 21.5, 21.2 and 18.5% to *M. mobilis* DSM 19841^T, *M. oceanica* ISS653^T, *M. phycicola* DSM 21425^T and *M. algae* DSM 15361^T, respectively. The ANI and dDDH values of the genomic sequences between strain JHPTF-M18^T and the type strains of the four *Mesonia* species were lower than the values (95-96 and 70%, respectively) recommended for delineation of a bacterial species (Goris et al. 2007; Konstantinidis and Tiedje 2005; Richter and Rosselló-Móra 2009).

Chemotaxonomic characteristics

The predominant isoprenoid quinone detected in strain JHPTF-M18^T was menaquinone-6 (MK-6), at a peak area ratio of approximately 95%, consistent with the results shown in the genus *Mesonia* (Lucena et al. 2020; Nedashkovskaya et al. 2003). The major fatty acids (> 10% of the total fatty acids in all growth phases) found in strain JHPTF-M18^T were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$) (Table S1). The fatty acid profiles of strain JHPTF-M18^T were similar to those of the type strains of *M. mobilis* and *M. algae* in that iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH are major fatty acids, even though there were differences in the proportions of some fatty acids, e.g. iso-$C_{16:0}$, anteiso-$C_{17:1}$ $\omega 9c$ and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$) (Table S1). The major polar lipids detected in stain JHPTF-M18^T were phosphatidylethanolamine and two unidentified lipids; minor amounts of eight other unidentified lipids, two unidentified aminolipids and one unidentified aminophospholipid were also present (Fig. S2). The polar lipid profile of strain JHPTF-M18^T was similar to that of the type strain of *M. algae* in that phosphatidylethanolamine is the only major phospholipid identified and one unidentified lipid is major component, but distinguished from that of the type strain of *M. algae* by the absence of one unidentified glycolipid as a major component (Fig. S2).

Morphological, cultural, physiological and biochemical characteristics

Strain JHPTF-M18^T showed non-flagellated property and did not reduce nitrate, as inferred by the absence of genes involved in flagella biosynthesis and nitrate reduction retrieved from “NCBI Prokaryotic

Genome Annotation Pipeline". Strain JHPTF-M18^T also showed catalase and oxidase activities which could be confirmed by the presence of relevant genes retrieved from "NCBI Prokaryotic Genome Annotation Pipeline". Strain JHPTF-M18^T could not hydrolyze casein and susceptible to tetracycline, whereas the type strains of *M. mobilis* and *M. algae* hydrolyzed casein and resistant to tetracycline (Table 1). Strain JHPTF-M18^T was susceptible to carbenicillin (100 µg), chloramphenicol (100 µg), lincomycin (15 µg), oleandomycin (15 µg) and tetracycline (30 µg), but resistant to ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), kanamycin (30 µg), neomycin (30 µg), novobiocin (5 µg), penicillin G (20 IU), polymyxin B (100 IU) and streptomycin (50 µg). Phenotypic characteristics of strain JHPTF-M18^T are given in the species description, Table 1 or Fig. S3.

Table 1

Differential characteristics of strain JHPTF-M18^T and the type strains of *Mesonia mobilis* and *Mesonia algae*.

Characteristic	**1**	**2**	**3**
Hydrolysis of			
Aesculin	+	+	–
Casein	–	+	+
Acid production form			
D-Glucose	–	+	–
Maltose	–	+	–
D-Mannose	–	+	–
Susceptibility to			
Ampicillin	–	w	+
Cephalothin	–	+	+
Tetracycline	+	–	–
Enzyme activity (API ZYM)			
α-Glucosidase	–	+	–
β-Glucosidase	+	–	–
DNA G+C content (mol%)[a]	33.1	35.1	33.1

Strains: 1, JHPTF-M18^T; 2, *M. mobilis* KCTC 12708^T; 3, *M. algae* KCTC 12089^T. Data of column 1 obtained from this study and data of columns 2 and 3 obtained from Lee *et al*. [6]. +, positive reaction; –, negative reaction; w, weakly positive reaction. All strains are rod-shaped and positive for activity of catalase and oxidase; hydrolysis of gelatin and Tween 80; susceptibility to carbenicillin, chloramphenicol, lincomycin and oleandomycin; and activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase. All strains are negative for Gram-staining; production of flexirubin-type pigments; nitrate reduction; hydrolysis of hypoxanthine, xanthine, starch and urea; acid production from L-arabinose, D-cellobiose, D-fructose, D-galactose, lactose, D-melezitose, melibiose, D-raffinose L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose, *myo*-inositol, D-mannitol and D-sorbitol; susceptibility to gentamicin, kanamycin, neomycin, novobiocin, penicillin G, polymyxin B and streptomycin; and activity of lipase (C14), trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase.

[a]Data obtained from genomic sequences.

Conclusion

Combined results obtained from the phylogenetic, genomic and chemotaxonomic analyses made it reasonable to assign strain JHPTF-M18^T as a member of the genus *Mesonia* (Fig. 1; Figs. S1 & S2; Table S1). Strain JHPTF-M18^T was distinguished from the type strains of *M. mobilis* and *M. algae* by differences in several phenotypic characteristics, including hydrolysis and acid production from some substrates, activity of some enzymes and susceptibility to some antibiotics (Table 1). Distinguished phenotypic properties, 16S rRNA gene sequence similarities and genetic distinctiveness based on ANI and dDDH values suggest that strain JHPTF-M18^T is separated from recognized species of the genus *Mesonia* (Chun et al. 2018; Goris et al. 2007; Konstantinidis and Tiedje 2005; Richter and Rosselló-Móra 2009; Stackebrandt and Goebel 1994). Based on the polyphasic taxonomic data presented, strain JHPTF-M18^T is considered to represent a novel species of the genus *Mesonia*, for which we propose the name *Mesonia aestuariivivens* sp. nov.

Description of Mesonia aestuariivivens **sp. nov.**

Mesonia aestuariivivens (aes.tu.a.ri.i.vi'vens. L. neut. n. *aestuarium -i* tidal flat; L. part. *vivens* living; N.L. part. adj. *aestuariivivens* living in a tidal flat).

Cells are ovoid or rod-shaped measuring approximately 0.2-0.4 μm in diameter and 0.3-3.0 μm in length. Gram-staining reaction is negative. Spore is not formed. No flagellum is found. Colonies on MA are circular, slightly convex, smooth, glistening, vivid yellow in colour and 0.5-1.0 mm after incubation for 3 days at 25°C. Grows optimally at 25°C and pH 7.0-7.5. Growth occurs at 4 and 37°C but not at 40°C, and occurs at pH 5.5 but not at pH 5.0. Growth occurs in the presence of 0.5-14.0% (w/v) NaCl with an optimum of approximately 2.0-3.0% (w/v) NaCl. Mg^{2+} ions are not required for growth. Anaerobic growth does not occur on MA and on MA supplemented with nitrate. It is catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Aesculin, gelatin, Tween 80 and L-tyrosine are hydrolysed, but casein, hypoxanthine,starch, urea and xanthine are not. Acid is not produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, L-rhamnose, D-raffinose, D-ribose, sucrose, D-trehalose, D-xylose, *myo*-inositol, D-mannitol and D-sorbitol. In assays with the API ZYM system, activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *β*-glucosidase are present, but activities of other enzymes are absent. The predominant menaquinone is MK-6. The major fatty acids (> 10% of total fatty acids) are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylethanolamine and two unidentified lipids. The DNA G+C content of the type strain is 33.1% (from genome sequence data).

The type strain, JHPTF-M18^T (= KACC 22185^T = NBRC 115119^T), was isolated from a tidal flat sediment collected from Seocheon on the Yellow Sea, South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and GenBank accession number for the whole genome shotgun sequence of strain JHPTF-M18^T are MW364546 and JAHWDF000000000, respectively.

Abbreviation

ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; GC, Gas chromatograph

Declarations

Acknowledgements

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Supplementary Table

Table S1 is not available with this version.

Figures

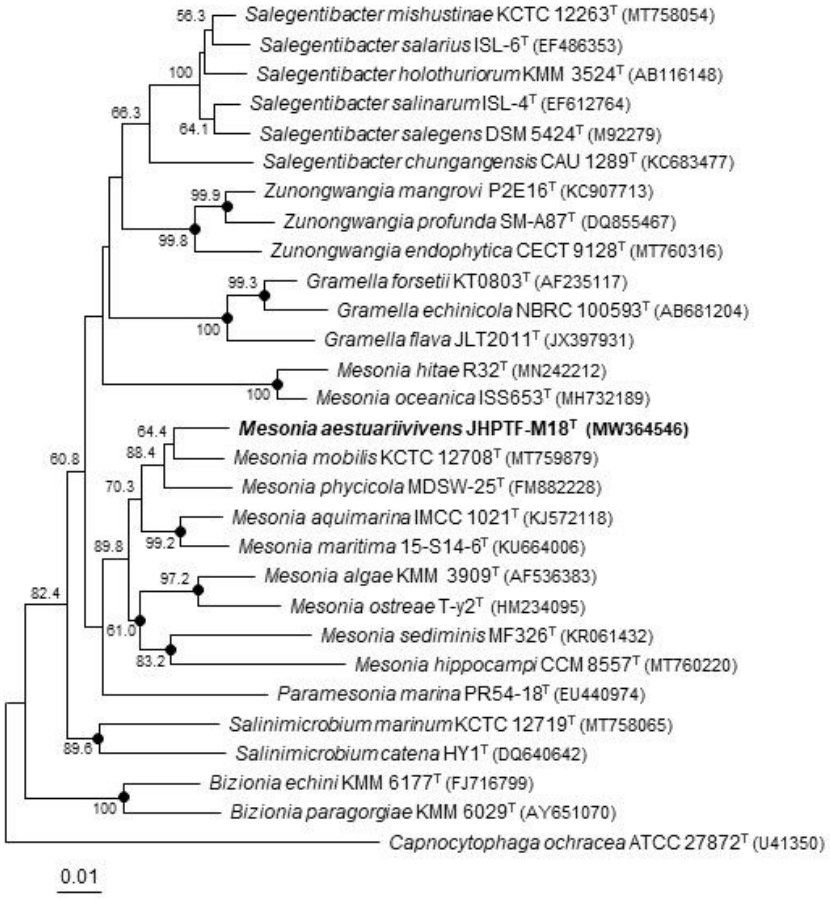

Fig. 1

Figure 1

Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Mesonia aestuariivivens* JHPTF-M18^T, the type strains of *Mesonia* species and representatives of some other related taxa. Only bootstrap values greater than 50% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-

likelihood and maximum-parsimony algorithms. *Capnocytophaga ochracea* ATCC 27872^T (GenBank accession number, U41350) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.pdf