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NOTE

Homoserinimonas aerilata gen. nov., sp. nov., a Novel Member of the Family *Microbacteriaceae* Isolated from an Air Sample in Korea§

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A bacterial strain isolated from an air sample, strain 5317J-19^T, was characterized. The isolate was an aerobic, motile, Gram-positive rod. The organism was able to grow between 4 and 35°C and between pH 6 and 9. The predominant fatty acids were anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The major respiratory menaquinones were MK-12 and MK-11, and the minor ones were MK13, MK-10, and MK-9. Genomic DNA G+C content was 66 mol%. The diagnostic diamino acid of the peptidoglycan is presumably D-Orn. The peptidoglycan is supposed to be B2β type. The 16S rRNA gene sequence analysis indicated that this isolate belongs to the family *Microbacteriaceae* and had the highest sequence similarities with *Salinibacterium xinjiangense* 0543^T (97.6%), *Salinibacterium amurskyense* KMM 3673^T (97.2%), and *Leifsonia bigeumensis* MSL-27^T (97.2%). Phylogenetic analysis and phenotypic characteristics support the proposal of a new genus and a novel species, with the name *Homoserinimonas aerilata* gen. nov., sp. nov. The type strain of *Homoserinimonas aerilata* is 5317J-19^T (=KACC 15522^T =NBRC 108729^T).

Keywords: *Homoserinimonas aerilata*, *Microbacteriaceae*, 16S rRNA gene sequence, DNA-DNA hybridization

The family *Microbacteriaceae* was first proposed by Park *et al.* (1993), and then, emended by Stackebrandt *et al.* (1997). This family consists of actinobacteria that are aerobic, non-motile, non-spore-forming, Gram-positive, and have G+C-rich genomic DNA. At present, 35 genera are validly published in the family *Microbacteriaceae*. Members of this family have been found in diverse environments, including plants, soil, dairy products, sewage, mushrooms, insects, and groundwater (Evtushenko *et al.*, 2000; Männistö *et al.*, 2000; Han *et al.*, 2003; Sheridan *et al.*, 2003; Tiago *et al.*, 2005, 2006; Yoon *et al.*, 2006; An *et al.*, 2008; Vaz-Moreira *et al.*, 2008; Baik *et al.*, 2010). Recently, *Schumannella*, *Glaciibacter*, *Chryseoglobus*, *Marisediminicola*, *Amnibacterium*, and *Herbiconiux*, which have been isolated from environments such as water, ice, phylosphere, antarctic sediment, and lichen, were validly listed as genera of the family *Microbacteriaceae* (An *et al.*, 2009; Katayama *et al.*, 2009; Baik *et al.*, 2010; Li *et al.*, 2010; Behrendt *et al.*, 2011; Kim and Lee, 2011). We isolated one novel actinobacteria strain from an air sample on Jeju Island, Republic of Korea.

Strain 5317J-19^T was isolated from an outdoor air sample in the Jeju region of Korea. The air sample was collected using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck, Germany), which contained Petri dishes with R2A agar (BBL, USA) amended with 200 μg cycloheximide/ml (Sigma, USA). After sampling, plates were incubated at 30°C for five days, and strain 5317J-19^T was recovered. The pure culture was maintained in 20% (v/v) glycerol suspensions at -70°C.

The cell morphology and motility of strain 5317J-19^T was examined by light microscopy (AXIO; Zeiss, Germany) and transmission electron microscopy (LEO model 912AB), with cells grown on R2A agar at 28°C for 2 days. For transmission electron microscopy, cells were negatively stained with 0.5% (w/v) uranyl acetate. Optimum growth conditions were determined by culturing at different temperatures, pH values, and NaCl concentrations for up to 14 days. Growth was monitored in R2A broth at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, and 45°C. The pH range (pH 5.0–10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth that was buffered with 0.2 M citrate/phosphate buffer or 0.05 M Tris/hydrochloride buffer (Breznak and Costilow, 1994). NaCl tolerance was tested by culturing on R2A broth containing NaCl at various concentrations (0, 1, 2, 3, 5, and 7% [w/v]). Gram staining was performed according to the method of Hucker (Smibert and Krieg, 1994). Catalase and oxidase activities were examined by bubble production in 3% (v/v) hydrogen peroxide solution and 1% (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux, France), respectively. Casein, starch and tyrosine degradations were examined on R2A plates containing milk powder (5%, w/v), starch (1%, w/v)

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Table 2. Comparison of chemotaxonomic characteristics among closely related taxa
Strain: 1, KACC 15522; 2, *Leifsonia bigeumensis* MSL-27^T; 3, *Leifsonia rubra* CMS 76r^T; 4, *Rhodoglobus vestalii* LV3^T; 5, *Salinibacterium amurskyense* KMM 3673^T; 6, *Salinibacterium xinjiangense* 0543^T. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; UGL, unknown glycolipid; +, present; -, absent; NA, no data available.

Characteristics	1	2	3	4	5	6
Major menaquinone(s)	12, 11	11	11	12,11	11, 10	10, 11
Major polar lipid(s)	DPG, PG, UGL	DPG, PG, UGL	DPG, PG, UGL	NA	PG, DPG	PG, DPG
Components of peptidoglycan[a]						
Alanine	2.9	NA	+	1.1	2.5	+
Glycine	1.5	NA	+	1.9	2.5	+
Homoserine	0.6	NA	-	-	-	-
Lysine	-	NA	-	-	0.6	+
Glutamic acid	1.0	NA	+	1.0	1.0	+
Ornithine	1.2	NA	-	-	2.5	+
2,4-Diaminobutyric acid	-	+	+	0.6	-	-

[a] Figure means molar ratio of components.

and tyrosine (0.1%, w/v), respectively. CM-cellulose and Tween 80 degradation was examined using R2A supplemented with 1% (w/v) of each substrate. DNase activity was determined with DNase test agar (Difco). Anaerobic growth was determined by incubation in the BBL GasPak Anaerobic System (Difco) for 14 days at 28°C on R2A agar. Enzyme activity, utilization of various carbon sources and acid production from substrates were tested in duplicate with commercial API ZYM, API 20NE, API ID 32GN, and API 50CH kits (bioMérieux) according to the manufacturer's protocols.

Isolation of chromosomal DNA, polymerase chain reaction (PCR) amplification, and direct sequencing of the purified product were carried out as described previously (Weon *et al.*, 2006). The resultant 16S rRNA sequence was aligned using the ARB software package (Ludwig *et al.*, 2004) and was added to the alignment of the SILVA SSURef 106 database (released April 2011; Pruesse *et al.*, 2007). The aligned nucleotide positions using 30 or 50% conservation filters and without filters were used for phylogenetic analysis with MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were inferred using neighbor joining with Kimura two-parameter model and maximum parsimony. To determine the sequence similarities among strains, the EzTaxon server (<http://www.eztaxon.org>; Chun *et al.*, 2007) was used.

Menaquinones and polar lipids were extracted and analyzed using cells grown on trypticase soy agar (TSA; Difco) at 28°C for 48 h by the method of Minnikin *et al.* (1984). For detection of polar lipids, molybdatophosphoric acid (for total lipids), phosphomolybdic acid (for phospholipids), ninhydrin (for aminolipids) and α-naphthol/sulfuric acid reagent (for glycolipids) were sprayed. The cells were grown on TSA at 28°C for 48 h, and the cellular fatty acids were extracted, methylated and separated by gas chromatography (model 6890; Hewlett Packard, USA) according to the protocol of the Sherlock Microbial Identification System (MIDI; Sasser, 1990). Fatty acid methyl esters were identified and quantified using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI). For peptidoglycan analysis, cells were grown in shake flasks containing liquid NBRC medium 802 [1.0% polypeptone (Wako, USA), 0.2% yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$; pH 7.0] on a rotary shaker at 28°C for 72 h. Cell-wall samples were prepared from approximately 1 g of wet cells by mechanical disruption with an ultrasonic oscillator and glass beads. The cell walls were separated from unbroken cells by differential centrifugation in distilled water, and further purified in boiling 4% sodium dodecyl sulfate (SDS) at 100°C for 40 min, followed by several washings with distilled water. Molar ratios of the amino acids in cell-wall hydrolysates (4 M HCl, 16 h) were determined using the method described by Hamada *et al.* (2009). Amino acid isomers in the cell-wall hydrolysates were examined using the method described by Nozawa *et al.* (2007) and a liquid chromatograph-mass spectrometer (LC- MS; model LCMS-2020; Shimadzu, Japan). Mycolic acids were extracted and analyzed as described by Minnikin *et al.* (1980). DNA-DNA hybridization was carried out in triplicate as described by Seldin and Dubnau (1985). Probe labeling was conducted using the nonradioactive DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Molecular Biochemicals, USA). The hybridized DNA was visualized using the DIG Luminescent Detection kit (Roche Molecular Biochemicals, Germany). DNA-DNA relatedness was quantified with a densitometer (Bio-Rad, USA). DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989).

Cells of strain 5317J-19^T were Gram-positive, aerobic, motile, and rod-shaped (Supplementary data Fig. S1). Colonies were light yellow with irregular margins after three days of growth at 28°C on R2A agar. Growth occurred on R2A, nutrient agar (NA; Difco), and TSA, but did not on MacConkey agar (Difco). The temperature and pH ranges for growth were 4–35°C (optimum, 30°C) and pH 6–9 (optimum, pH 7). The strain could grow in the presence of 0–5% (w/v) NaCl (optimum, 0–1%). Growth did not occur at ≥37°C or in the presence of ≥7% (w/v) NaCl. Other physiological and biochemical data are given in the genus and species descriptions, and Tables 1 and 2.

The 16S rRNA gene sequence of strain 5317J-19^T (1,469 bp) was most closely related to *Salinibacterium xinjiangense* 0543^T (97.6%), *Salinibacterium amurskyense* KMM 3673^T (97.2%), and *Leifsonia bigeumensis* MSL-27^T (97.2%). All the other species, except for the three mentioned above species, within

the family *Microbacteriaceae* had the sequence similarities below 97%. The neighbor-joining tree without filters showed that strain 5317J-19^T was clustered with members of the genera *Salinibacterium*, *Rhodoglobus*, and *Leifsonia*; however, the bootstrap values were not high enough to confirm grouping of strain 5317J-19^T with specific genera (Fig. 1). Other neighbor-joining and maximum parsimony trees also showed no reliable clustering of strain 5317J-19^T with a specific genus, although there was a slightly different topology among them (data not shown).

5317J-19^T contained several menaquinones, including MK-12 (50%) and MK-11 (31%) as major ones, and MK-13 (11%), MK-10 (7%), and MK-9 (1%) as minor ones. Diphosphatidylglycerol, phosphatidylglycerol and two unknown glycolipids were detected (Supplementary data Fig. S2). The fatty acid composition was anteiso-$C_{15:0}$ (74.4%), iso-$C_{16:0}$ (11.1%), iso-$C_{15:0}$ (4.9%), anteiso-$C_{17:0}$ (4.0%), iso-$C_{14:0}$ (3.9%), and $C_{16:0}$ (1.9%). Mycolic acids were absent. The peptidoglycan of strain 5317J-19^T contained alanine (Ala), glycine (Gly), homoserine (Hsr), threo-3-hydroxyglutamic acid (Hyg), and ornithine (Orn) in a molar ratio of 2.9 : 1.5 : 0.6 : 1.0 : 1.2. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Hyg, L-Hsr, D-Ala, L-Ala, and D-Orn. The diagnostic diamino acid of the peptidoglycan was presumably D-Orn. The peptidoglycan was supposed to be B2β (Schleifer and Kandler, 1972). Levels of DNA-DNA relatedness between strain 5317J-19^T and the three reference strains *Salinibacterium xinjiangense* KACC 15535^T, *Salinibacterium amurskyense* KACC 21020, and *Leifsonia bigeumensis* KACC 21122^T were 32±3 (reciprocal, 33±4), 30±3 and 22±5%, respectively. The DNA G+C content was 66 mol%.

5317J-19^T can be differentiated from closely related genera within the family *Microbacteriaceae* on the basis of its chemotaxonomic characteristics such as fatty acids, polar lipids, menaquinones, and peptidoglycan structure (Tables 1 and 2). The presence of L-Hsr in the peptidoglycan structure of strain 5317J-19^T can be clearly differentiated from all other closely related species within the family *Microbacteriaceae* (Table 2). In additon, strain 5317J-19^T did not contain 2,4-diaminobutyric acid which is a peptidoglycan component of *Leifsonia bigeumensis*, *Leifsonia rubra*, and *Rhodoglobus vestalii*. While *Salinibacterium amurskyense* and *Salinibacterium xinjiangense* contain lysine, strain 5317J-19^T did not. Menaquinones and polar lipids can also be distinguishing characteristics between strain 5317J-19^T and two *Leifsonia* species and the genera *Rhodoglobus* and *Salinibacterium* (Table 2).

The above phylogenetic and phenotypic characteristics indicated that strain 5317J-19^T represents a novel species of a new genus, for which the name *Homoserinimonas aerilata* gen. nov., sp. nov. is proposed.

Table 1. Differential characteristics of the closely related genera of the family *Microbacteriaceae*

Genera: 1, 5317J-19^T; 2, *Agreia* (Evtushenko *et al.*, 2001; Behrendt *et al.*, 2002, Schumann *et al.*, 2003); 3, *Agrococcus* (Goth *et al.*, 1996; Wieser *et al.*, 1999; Zlamala *et al.*, 2002; Mayilraj *et al.*, 2006; Bora *et al.*, 2007; Lee, 2008; Behrendt *et al.*, 2008; Zhang *et al.*, 2010; Dhanjal *et al.*, 2011); 4, *Chryseoglobus* (Baik *et al.*, 2010); 5, *Glacibacter* (Katayama *et al.*, 2009); 6, *Herbiconiux* (Qiu *et al.*, 2007; Behrendt *et al.*, 2011; Kim *et al.*, 2012); 7, *Leifsonia* (Leifson, 1962; Davis *et al.*, 1984; Suzuki *et al.*, 1984; Evtushenko *et al.*, 2000; Reddy *et al.*, 2003, 2008; An and Yokota, 2007; Qiu *et al.*, 2007; Dastager *et al.*, 2008, 2009; An *et al.*, 2009, 2010; Pindi *et al.*, 2009; Madhaiyan *et al.*, 2010; Ganzert *et al.*, 2011); 8, *Microcella* (Tiago *et al.*, 2005, 2006); 9, *Microterricola* (Matsumoto *et al.*, 2008); 10, *Phycicola* (Lee *et al.*, 2008); 11, *Rhodoglobus* (Reddy *et al.*, 2003; Sheridan *et al.*, 2003; An *et al.*, 2010); 12, *Salinibacterium* (Han *et al.*, 2003; Zhang *et al.*, 2008); 13, *Schumannella* (An *et al.*, 2008); 14, *Subtercola* (Männistö *et al.*, 2006; Behrendt *et al.*, 2002); 15, *Yonghaparkia* (Yoon *et al.*, 2006). +, Positive; -, negative; NA, not available; DAB, diaminobutyric acid; Lys, lysine; Orn, Ornithine; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; UGL, unknown glycolipid; UL, unknown lipid; UPA, phosphatidic acid; UPL, unknown phospholipid.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Motility	+	+	-	+	+	-	-/+	-	+	+	+	-	-	-	-
Diagnostic diamino acid	D-Orn	L-DAB, D-Orn	L-DAB	Lys	DAB	DL-DAB	DL-DAB	Lys, D-Orn	DAB	DAB	Orn, DL-DAB	Lys, Orn	DAB	DAB, D-Orn	DL-DAB
Peptidoglycan type	B2β	B	B, B2γ	B2β	B2γ	B2γ	B, B2γ	B, B2β	NA	B	B2α	B	NA	B2γ	B2γ
Major fatty acids (>10%)	ai-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{15:0}$, $C_{16:0}$, $C_{17:0}$	i-$C_{16:0}$, ai-$C_{15:0}$, i-$C_{14:0}$, $C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, ω-cyclohexylundecanoic acid, cyclohexyl-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
Major menaquinone(s)	12, 11	10, 11	11, 12, 10, 9	12, 13, 14	11, 12, 13	11	11, 12, 10, 9	13, 12, 14	12	11	11, 12	11, 10	11, 10	9, 10, 11	12, 11
Polar lipids	DPG, UGL, PG	PG, DPG	PG, DPG, UGL, UL, UP	DPG, PG	NA	DPG, PG, UPL, UGL	DPG, PG, UGL, PE, UL, UPA	NA	NA	DPG, PC, PG, PI	PG, DPG	PG, DPG	NA	PG, DPD, UPL, UGL	DPG, PG, UPL, UGL
DNA G + C content (mol%)	66	65-67	73-76	68	65	66-70	62-73	67-69	70	70	62-64	61-64	59	64-68	71-72

Description of *Homoserinimonas* gen. nov.

Homoserinimonas (Ho.mo.se.ri.ni.mo'nas. N.L. n. *homoserinum*, homoserine; L. fem. n. *monas*, a unit, *monad*; N.L. fem. n. *Homoserinimonas*, homoserine *monad*,

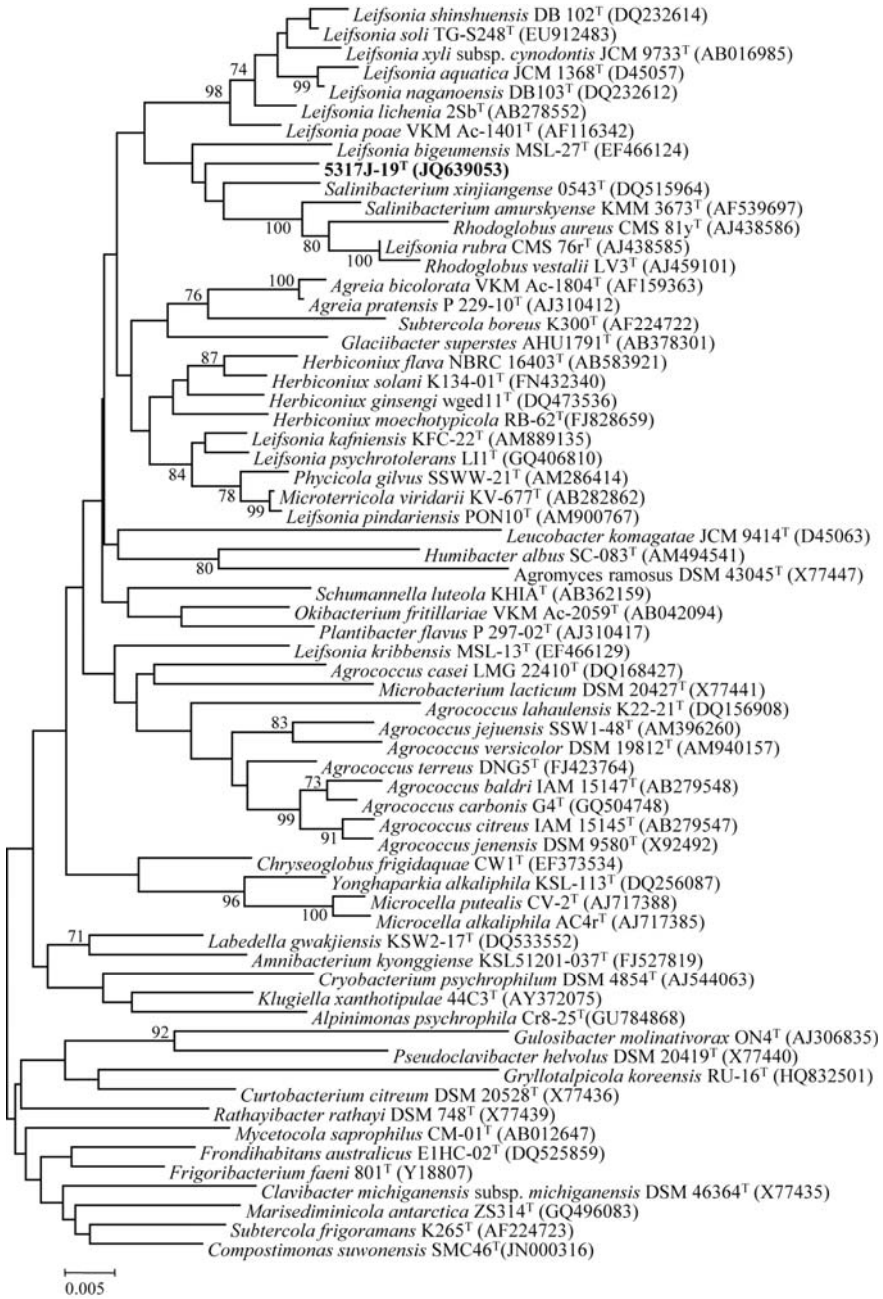

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences of strain 5317J-19^T (1469 nt) and others of the family *Microbacteriaceae*. The building was performed using the neighbor-joining method. Bootstrap percentages (based on 1,000 replications) >70% are shown at branching points. Bar, 0.005 accumulated changes per nucleotide.

referring to the presence of homoserine in the cell wall).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming, motile rods. The major menaquinones are MK-12 and MK-11, and the minor ones are MK-13, MK-10, and MK-9. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and two unknown glycolipids. The predominant cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{16:0}$. Mycolic acids are absent. The diagnostic diamino acid of the peptidoglycan is presumably D-Orn. The peptidoglycan is supposed to be B2β. The type species is *Homoserinimonas aerilata*.

Description of *Homoserinimonas aerilata* sp. nov.

Homoserinimonas aerilata (a.e.ri.la'ta. L. n. *aer*, air; L. part. adj. *latus* -a -um, carried; N.L. fem. part. adj. *aerilata*, airborne).

Cells are rod-shaped (0.4–0.6×1.0–1.5 μm). Colonies are smooth, circular, and colored light yellow after three days of growth at 28°C on R2A agar. Cells grow on R2A, NA, and TSA, but not on MacConkey agar. Growth occurs at 4–35°C (optimum, 30°C), pH 6–9 (optimum, pH 7) and in the presence of 0–5% (w/v) NaCl (optimum, 0–1%). Growth does not occur at ≥37°C in the presence of ≥7% (w/v) NaCl. The cells do not degrade casein, cellulose, chitin, DNA, hy-

poxanthine, starch, Tween 80, tyrosine, or xanthine. The following tests (API 20NE test strip) were negative: nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. Gelatin hydrolysis was weakly positive, and aesculin hydrolysis and β-galactosidase (PNG) were positive (API 20NE test strip). The cells assimilate D-glucose, D-maltose, D-mannitol, and D-saccharose, and they weakly assimilate L-arabinose and potassium gluconate. The cells do not assimilate *N*-acetylglucosamine, adipic acid, L-alanine, capric acid, L-fucose, glycogen, L-histidine, 3-hydroxybenzoic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, inositol, itaconic acid, lactic acid, malic acid, D-mannose, D-melibiose, potassium 2-ketogluconate, potassium 5-ketogluconate, propionic acid, L-rhamnose, phenylacetic acid, D-ribose, salicin, L-serine, sodium acetate, sodium malonate, D-sorbitol, suberic acid, trisodium citrate or valeric acid (API 20NE and API 32GN test strips). They are positive for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, and β-glucosidase and are negative for alkaline phosphatase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). The strain produces acids from L-arabinose, D-cellobiose, esculin ferric citrate, D-fructose, D-galactose, D-glucose, glycerol, D-maltose, D-mannitol, D-mannose, potassium gluconate, L-rhamnose, D-saccharose, D-turanose, and D-xylose. It also weakly produces acids from gentiobiose, potassium 5-ketogluconate, and D-trehalose, but does not from *N*-acetylglucosamine, D-adonitol, amidon, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, L-fucose, glycogen, inositol, inulin, D-lactose, D-lyxose, D-melezitose, D-melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, potassium 2-ketogluconate, D-raffinose, D-ribose, salicin, D-sorbitol, L-sorbose, D-tagatose, xylitol, or L-xylose (API 50CH). The DNA G+C content of the type strain is 66 mol%.

The type strain is 5317J-19^{T} (=KACC 15522^{T} =NBRC 108729^{T}) and was isolated from an air sample on Jeju Island, Republic of Korea.

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