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NOTE

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

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A novel isolate, designated 6408J-67^T, was isolated from an air sample collected from Jeju Island, Republic of Korea. Its phenotypic, genotypic, and chemotaxonomic properties were compared with those of members of the family *Microbacteriaceae*. The Gram-positive, aerobic, motile rod formed light yellow, smooth, circular and convex colonies. Optimal growth occurred at 30°C and pH 7.0. 16S rRNA gene sequence data showed that the isolate was a novel member of the family *Microbacteriaceae*, with the highest sequence similarity (97.4%) to *Labedella gwakjiensis* KSW2-17^T and less (<97%) sequence similarity with other taxa. The major cellular fatty acids (>10% of the total) were anteiso-$C_{15:0}$, iso-$C_{14:0}$, and iso-$C_{16:0}$. The strain also contained MK-13, MK-12, and MK-14 as the major menaquinones, as well as diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids. Its peptidoglycan structure was B1β with 2,4-diaminobutyric acid as a diamino acid. Mycolic acids were absent. The DNA G+C content was 68.3 mol%. Based on these phenotypic and genotypic findings, strain 6408J-67^T represents a novel species of a new genus within the family *Microbacteriaceae*, for which the name *Diaminobutyricimonas aerilata* gen. nov., sp. nov. is proposed. The type strain is 6408J-67^T (=KACC 15518^T =NBRC 108726^T).

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

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The family *Microbacteriaceae* was first proposed by Park *et al.* (1993), and then, emended by Stackebrandt *et al.* (1997). A total of 35 genera have been validly published. Members of the family *Microbacteriaceae* have been found in various environments such as soil, water, and air. Recently, one novel member, the genus *Compostimonas*, was isolated from an air (Kim *et al.*, 2012). Here, we report the identification of another air-borne bacterial strain as a new member of the family *Microbacteriaceae*.

Strain 6408J-67^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) containing Petri dishes with R2A agar (BD, USA) and 200 μg of cycloheximide per ml (Sigma, USA). The strain was isolated after growth on R2A medium (BD) at 28°C for 7 days. The strain was preserved in R2A broth (BD) with 20% (v/v) glycerol at -80°C or by lyophilization.

The 16S rRNA gene sequence of strain 6408J-67^T (1,464 bp) was determined as described previously (Weon *et al.*, 2006), and analyzed using mega version 4 (Tamura *et al.*, 2007) as well as arb (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 106 database (release April 2011; Pruesse *et al.*, 2007). The aligned nucleotide positions with 30 and 50% conservation filters and without filters were used for phylogenetic analyses. Phylogenetic trees were inferred using neighbor joining with the Kimura two-parameter model and maximum parsimony. The EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007) was used to determine the sequence similarities among the strains. A neighbor-joining tree (without filters) is shown in Fig. 1. Strain 6408J-67^T was clustered into one group with the genera or species *Labedella*, *Amnibacterium*, *Cryobacterium*, *Klugiella*, *Alpinimonas*, and *Agrococcus lahaulensis*. This large cluster was not shown as a coherent group due to low bootstrap values and disparity, as shown on the maximum parsimony tree (Fig. 1). Small group of strain 6408J-67^T with the genera *Labedella* and *Amnibacterium* was reliable since this cluster was also present in the maximum-parsimony tree, meaning that strain 6408J-67^T is closely related with these genera (Fig. 1). Other trees produced using filters also showed similar results (data not shown). Strain 6408J-67^T had the highest sequence similarity (97.4%) with *Labedella gwakjiensis* KSW2-17^T, but also showed high sequence similarities with *Herbiconiux ginsengi* wged11^T (96.8%), *Cryobacterium mesophilum* MSL-15^T (96.7%), *Cryobacterium psychrophilum* DSM 4854^T (96.6%), *Cryobacterium psychrotolerans*

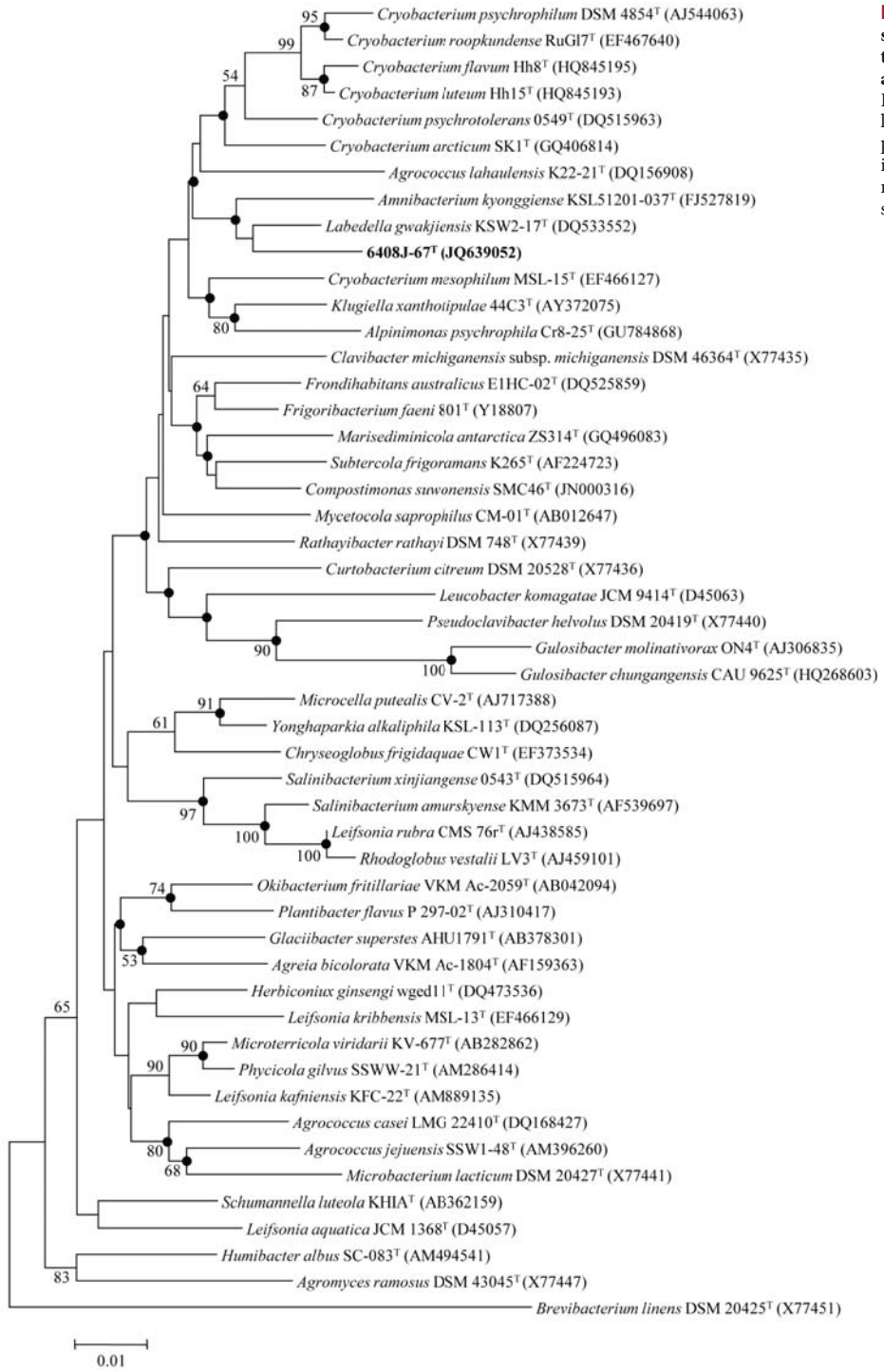

Fig. 1. Neighbor-joining phylogenetic tree showing the position of strain 6408J-67^T in the family *Microbacteriaceae*, based on an alignment of 16S rRNA gene sequences. Bootstrap percentages (based on 1000 replications) >50% are shown at the branch points. Dots indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.01 substitutions per nucleotide.

0549^T (96.6%), *Cryobacterium roopkundense* RuGl7^T (96.6%), and *Amnibacterium kyonggiense* KSL51201-037^T (96.2%). Excluding the above list, all other taxa within the family *Microbacteriaceae* showed ≤96.5% sequence similarity with strain 6408J-67^T.

DNA-DNA relatedness between strain 6408J-67^T and its closest phylogenetic neighbor, *Labedella gwakjiensis* KSW2-17^T, was determined according to the method of Seldin and Dubnau (1985) using three repeated experiments. The DNA-DNA relatedness value between strain 6408J-67^T and *Labedella gwakjiensis* KSW2-17^T was 23% (reciprocal, 27%), which was below the 70% cut-off recommended for the recognition of bacterial species (Wayne *et al.*, 1987).

The DNA G+C content of strain 6408J-67^T was 68.3 mol%

as determined by HPLC (Mesbah *et al.*, 1989).

To analyze whole-cell fatty acids, strain 6408J-67^T was grown for 48 h at 28°C in tryptic soy agar (TSA; BD). Cells were saponified, the fatty acids were methylated and extracted, and the fatty acid methyl esters were determined using the protocol of Sasser (1990). The fatty acid methyl esters were identified and quantified using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI). Menaquinones and polar lipids were extracted and analyzed by the method of Minnikin *et al.* (1984) using cells grown on TSA. For peptidoglycan analysis, cells were grown in shake flasks containing liquid NBRC medium 802 [1.0% polypeptone (Wako Pure Chemical Industries, Ltd., Japan), 0.2% yeast extract, and 0.1% $MgSO_4 \cdot H_2O$; 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% SDS (100°C, 40 min), followed by several washings with distilled water. The molar ratios of the amino acids in cell-all hydrolysates (4 M HCl, 16 h) were determined using the method of Hamada *et al.* (2010). Amino acid isomers in the cell-wall hydrolysates were examined by the method of Nozawa *et al.* (2007) using a liquid chromatograph-mass spectrometer (LC-MS; model LCMS-2020; Shimadzu Corp., Japan). Mycolic acids were extracted and analyzed as described by Minnikin *et al.* (1980). Strain 6408J-7^T contained the following menaquinones: MK-13 (38%), MK-12 (33%), MK-14 (21%), and MK-11 (7%). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol and two unknown glycolipids (Supplementary data Fig. S1). The peptidoglycan of strain 6408J-67^T contained alanine (Ala), glycine (Gly), homoserine (Hsr), threo-3-hydroxyglutamic acid (Hyg), and 2,4-diaminobutyric acid (DAB) in the molar ration of 1.4:1.9:0.6:1.0:1.1. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of Gly, D-Hyg, L-Hsr, D-Ala, and L-DAB. The peptidoglycan was determined to be B1β (Schleifer and Kandler, 1972). Mycolic acids were absent. The major cellular fatty acid of strain

Table 1. Differential characteristics of the investigated strains and closely related genera of the family Microbacteriaceae Genera: 1, strain 6408J-67^T; 2, *Amnibacterium* (Kim and Lee, 2011); 3, *Alpinimonas* (Schumann *et al.*, 2012.); 4, *Clavibacter* (Bendinger *et al.*, 1992; Collins and Bradbury, 1992; Groth *et al.*, 1996; Evtushenko *et al.*, 2000); 5, Compostimonas (Kim *et al.*, 2012); 6, *Cryobacterium* (Suzuki *et al.*, 1997; Zhang *et al.*, 2007a; Dastager *et al.*, 2008a; Reddy *et al.*, 2010); 7, *Frigoribacterium* (Kämpfer *et al.*, 2000; Dastager *et al.*, 2008b); 8, *Frondihabitans* (Zhang *et al.*, 2007b; Greene *et al.*, 2009; Lee, 2010); 9, *Klugiella* (Cook *et al.*, 2008); 10, *Labedella* (Lee, 2007); 11, *Marisediminicola* (Li *et al.*, 2010); 12, *Mycetocola* (Tsukamoto *et al.*, 2001; Bora *et al.*, 2008); 13, *Rathayibacter* (Zgurskaya *et al.*, 1993; Sasaki *et al.*, 1998; Dorofeeva *et al.*, 2002); 14, *Subtercola* (Männistö *et al.*, 2000). NA, Not available; C, cream; LY, light yellow; Y, yellow; O, orange; W, white; P, pink; DAB, 2,4-diaminobutyric acid; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; UGL, unknown glycolipid; PL, unknown phospholipid; UL, unknown lipid.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Source	Air	Water	Glacier cryoconite	Plant	Soil	Soil	Soil, air, dust, animal shed	Leaf litter, soil	Insect larvae	Seaweed	Sea sand	Cheese, mushroom	Plant	Groundwater
Colony color	LY	Y	Y	Y, W, O	W	P, Y	C, Y	Y, W	Y	Y	LY	LY	Y, O	Y
Motility	+	-	-	-	-	D	+,	-	-	-	+	-	-	-
Temperature for growth (optimum)	10–40 (30)	10–37 (30)	1–20 (1–15)	NA (20–29)	10–37 (28)	0–28 (9–28)	2–37 (4–28)	4–37 (20–30)	4–30 (28)	10–37	0–26 (18–23)	>4–33 (20–30)	>7-<37 (24–28)	-2 –28 (15–17)
Diamino acid	DAB	DAB	DAB	DAB	DAB	DAB	D-Lysine	Ornithine	Lysine	Ornithine	Ornithine	D-Lysine	DAB	DAB
Peptidoglycan type	B1β	NA	B	B2γ	B1	B2γ	B2β	B2β,	NA	NA	B2β	B2β	B2γ	B2γ
Major cellular fatty acids (>10 %)[a]	ai-$C_{15:0}$, i-$C_{14:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$[b], i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{15:1}$, i-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$, summed feature 7, ai-$C_{15:0}$ DMA, ai-$C_{17:0}$	summed feature 7, ai-$C_{15:0}$, $C_{14:0}$ 2OH, i-$C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$[§]	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
Menaquinones (MK)	13, 12, 14, 11	11, 12	11, 10	10, 9	11, 12	8, 9, 10, 11, 12	9	8, 7 , 9	12, 11, 10,13, 9	10, 11, 9, 7	10	10, 9, 11, 8	10, 8, 9, 11	9, 10, 12, 13, 11, 8
Polar lipids	DPG, PG, UGL	PG, PL	DPG, PG, GL	DPG, PG, UGL	DPG, UGL, PG	PG, DPG, UL, UGL	DPG, PG, UGL	DPG, PG, PL, UGL, UL	NA	PG, DPG	DPG, PG, UGL	DPG, PG, PL, UGL	PG, DPG, PL, UGL	NA
G+C content (mol%)	68.3	72.7	59	65–78	68.0	64.7–70	67.5–71.1	68.3–71.0	60.9	68.0	67.3	63.9–70	60.4–72	64.4–67.8

[a] Summed feature, a group of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 7 comprises $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$.
[b] Data from this study.

6408J-67^T was anteiso-$C_{15:0}$ (53.2%), and the strain also contained iso-$C_{14:0}$ (14.5%), iso-$C_{16:0}$ (14.6%), $C_{16:0}$ (8.5%), anteiso-$C_{17:0}$ (5.2%), $C_{14:0}$ (1.6%), iso-$C_{15:0}$ (1.5%), and anteiso-$C_{13:0}$ (1.0%).

Gram staining was performed according to the method of Hucker (Smibert and Krieg, 1994). The cell morphology and motility of strain 5317J-19^T were examined by light microscopy (AXIO; Zeiss, Germany) and transmission electron microscopy (LEO model 912AB, Germany) with cells grown on R2A agar at 28°C for 2 days. Catalase activity was assessed by bubble production in 3% (v/v) H_2O_2 and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization, enzyme activities and acid production from substrates were tested using API ZYM, API 20NE, API ID 32GN, and API 50CH kits (bioMérieux, France), according to the manufacturer's instructions. Growth at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, and 45°C was assessed after 14 days on R2A broth. Growth at pH 5.0–10.0 (at 1.0 unit intervals) was assessed after 14 days in R2A broth with the pH adjusted using citrate/phosphate buffer or Tris/HCl buffer (Breznak and Costilow, 1994). Growth in 1–10% (w/v) NaCl (at intervals of 1% NaCl) was assessed after 14 days in R2A broth by monitoring the OD_{600}. Anaerobic growth was checked using incubation in the BBL GasPak Anaerobic System (BD) for 14 days at 28°C on R2A agar. Casein, starch, and tyrosine degradation was examined on R2A plates containing milk powder (5%, w/v), starch (1%, w/v), and tyrosine (0.1%, w/v), respectively. The degradation of carboxymethyl cellulose (CM-cellulose) and Tween 80 was examined using R2A supplemented with 1% (w/v) substrate. DNase activity was determined using DNase test agar (BD). The strain was found to be a Gram-positive, aerobic, motile rod. The colonies formed were smooth, circular, convex and light yellow-colored. The strain grew on R2A, TSA, and nutrient agar (NA; BD); however, it did not grow on MacConkey agar (BD). Other physiological properties are shown in genus and species description, as well as in Table 1.

By comparing 6408J-67^T with the most closely related genus, *Labedella*, within the family *Microbacteriaceae*, strain 6408J-67^T was motile and contained type B1β peptidoglycan with L-DAB as the diamino acid; alanine, glycine, homoserine, threo-3-hydroxyglutamic acid, and 2,4-diaminobutyric acid in the molar ration of 1.4:1.9:0.6:1.0:1.1; anteiso-$C_{15:0}$, iso-$C_{14:0}$, and iso-$C_{16:0}$ as the major fatty acids; MK-13, MK-12, and MK-14 as the predominant menaquinones; and diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids as polar lipids. The genus *Labedella* was non-motile and had ornithine as its diamino acid; alanine, glycine, glutamic acid, and ornithine in the molar ratio of 0.9:1.1:1.0:1.1 (Lee, 2007); anteiso-$C_{15:0}$ and iso-$C_{16:0}$ as the major fatty acids (Supplementary data Table S1); MK-10 and MK-11 as the predominant menaquinones; and phosphatidylglycerol and diphosphatidylglycerol as polar lipids. Strain 6408J-67^T could be clearly differentiated from the genus *Amnibacterium* based on its motility, and the chemical compositions of its polar lipids, menaquinones, and fatty acids (Table 1 and Supplementary data Table S1). More comparisons between strain 6408J-67^T and other members of the family *Microbacteriaceae* are shown in Table 1.

Based on the genotypic and phenotypic evidences presented, strain 6408J-67^T represents a novel species of a new genus in the family *Microbacteriaceae*, for which the name *Diaminobutyricimonas aerilata* gen. nov., sp. nov. is proposed.

Description of *Diaminobutyricimonas* gen. nov.

Diaminobutyricimonas (Di.a.mi.no.bu.ty.ri.ci.mo'nas. N.L. n. *acidum diaminobutyricum*, diaminobutyric acid; L. fem. n. *monas*, a monad, unit; N.L. fem. n. *Diaminobutyricimonas*, a unit [bacterium] with 2,4-diaminobutyric acid in the peptidoglycan).

Aerobic, Gram-positive, catalase- and oxidase-negative, non-spore-forming, motile, rod-shaped cells. Branching or mycelium formation does not occur. The predominant menaquinones are MK-13, MK-12, and MK-14. The peptidoglycan in the cell wall is type B1β with DAB as the diamino acid. The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids. Mycolic acids are not present. The cellular fatty acid profile is dominated by anteiso-$C_{15:0}$, iso-$C_{14:0}$, and iso-$C_{16:0}$. Phylogenetically, the genus belongs to the family *Microbacteriaceae*, suborder *Micrococcineae*. The type species is *Diaminobutyricimonas aerilata*.

Description of *Diaminobutyricimonas aerilata* sp. nov.

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

Has the following characteristics in addition to those typical for the genus. The cells are rod shaped, 0.5–0.6 μm in width and 1.1–2.0 μm in length. The colonies are smooth, circular, convex, and light yellow-colored. Grows on R2A, TSA and NA; however, does not grow on MacConkey agar. Grows at 10–40°C, at pH 6–9 and in presence of 0–5% (w/v) NaCl. Optimal growth occurs at 30°C, pH 7.0, and in the presence of 0% NaCl. Nitrate is not reduced to nitrite. Indole production is not observed. Does not hydrolyze casein, chitin, CM-cellulose, DNA, hypoxanthine, starch Tween 80, tyrosine, and xanthine. Positive for aesculin hydrolysis and β-galactosidase (PNG), but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis (API 20NE). Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, D-ribose, D-saccharose, glycogen, and D-melibiose. Does not assimilate *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, or L-proline (API 20NE and API 32GN test strips). Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Negative for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosidase, and α-fuco-

sidase (API ZYM test strip). Produces acids from glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, methyl-α-D-mannopyranoside, esculin ferric citrate, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, amidon, glycogen, gentiobiose, and D-turanose. Dose not from erythritol, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, inulin, D-melezitose, D-raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, or potassium 5-ketogluconate (API 50CH test strip). The DNA G+C content is 68.3 mol%.

The type strain 6408J-67^T (=KACC 15518^T =NBRC 108726^T) was isolated from an air sample collected at Jeju Island, Republic of Korea.

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