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Chujaibacter soli gen. nov., sp. nov., isolated from soil[§]

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A Gram-staining-negative, aerobic, non-flagellated, rod-shaped bacterial strain, KIS55-21^T, was isolated from a soil sample from Chuja Island, Jeju Province, Republic of Korea. Strain KIS55-21^T grew optimally at pH 7.0, at 28–30°C and in the presence of 0% (w/v) NaCl. Neighbor-joining and maximum-likelihood trees based on the 16S rRNA gene sequences revealed that strain KIS55-21^T fell within the family *Xanthomonadaceae* and was closely related to *Metallibacterium scheffleri* DKE^T. Strain KIS55-21^T exhibited the highest 16S rRNA gene sequence similarity (92.6%) to that of *M. scheffleri* DKE^T, with similarities of less than 92.0% to those of the genera *Dokdonella*, *Rhodanobacter*, *Aquimonas*, and *Frateuria*. Strain KIS55-21^T contained ubiquinone-8 (Q-8) as the predominant ubiquinone, iso-$C_{17:0}$, summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl), anteiso-$C_{17:0}$ and $C_{16:0}$ as the major fatty acids, and phosphatidylethanolamine, aminophospholipid, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylmonomethylethanolamine as the main polar lipids. The DNA G+C content of strain KIS55-21^T was 65.9 mol%. Differential phenotypic and chemotaxonomic properties and phylogenetic data of strain KIS55-21^T demonstrated that this strain is distinguishable from closely related genera within the family *Xanthomonadaceae*. On the basis of the data presented, strain KIS55-21^T is considered to represent a novel genus and species, for which the name *Chujaibacter soli* gen. nov., sp. nov. is proposed. The type strain is KIS55-21^T (=KACC 16971^T =DSM 28578^T).

Keywords: *Chujaibacter*, novel species, polyphasic taxonomy

Introduction

The family *Xanthomonadaceae* is a member of the order *Xanthomonadales* within the class *Gammaproteobacteria*. At the time of writing, this family includes the following 23 genera: *Aquimonas*, *Arenimonas*, *Aspromonas*, *Dokdonella*, *Dyella*, *Frateuria*, *Fulvimonas*, *Ignatzschineria*, *Luteibacter*, *Luteimonas*, *Lysobacter*, *Metallibacterium*, *Panacagrimonas*, *Pseudofulvimonas*, *Pseudoxanthomonas*, *Rhodanobacter*, *Rudaea*, *Silanimonas*, *Stenotrophomonas*, *Thermomonas*, *Wohlfahrtiimonas*, *Xanthomonas*, and *Xylella* (http://www.bacterio.net/). Generally, members of the family *Xanthomonadaceae* are obligate aerobes, catalase-positive, unable to reduce nitrate (with exception of the *Stenotrophomonas*) and composed of complex fatty acids including branched chain and/or hydroxyl fatty acids, and ubiquinone-8 as the major respiratory quinone (Saddler and Bradbury, 2005).

In a survey of indigenous bacterial populations in Korea, one bacterial strain, isolated from a soil sample from Chuja Island, Jeju Province, Republic of Korea, was revealed to represent a novel genus of the family *Xanthomonadaceae* on the basis of phenotypic and phylogenetic inference.

Materials and Methods

Bacterial strain

Strain KIS55-21^T was isolated from a soil sample from Chuja Island, Jeju Province, Republic of Korea, as small brown-colored colonies after 8 days of incubation at 28°C on Reasoner's 2A (R2A) plates (Difco); the isolate was selected for polyphasic characterization. The novel strain was routinely grown on R2A agar at 28°C for 5 days and preserved at -70°C as a suspension in R2A broth supplemented with glycerol (20%, v/v).

Phylogenetic analysis

Genomic DNA of strain KIS55-21^T was extracted by the method of Ausubel *et al.* (1987), and the 16S rRNA gene was PCR-amplified using the universal primers 9F and 1512R (Weisburg *et al.*, 1991). 16S rRNA gene sequencing was conducted at Genotech using the sequencing primers 27F, 1492R (Weisburg *et al.*, 1991), 518R (5′-GTATTACCGCGGCTGCTGG-3′), and 785F (5′-GGATTAGATACCCTGGTA-3′) to generate a consensus sequence. The nearly complete 16S rRNA gene sequence (1,483 nt) for strain KIS55-21^T was compared against 16S rRNA gene sequences available from EzTaxon-e (Kim *et al.*, 2012) and the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence similarities were also calculated using the EzTaxon server. Alignments of the sequences were performed using the SILVA Incremental Aligner (Pruesse *et al.*, 2012), and the resulting multiple sequence alignment was corrected manually. Phylogenetic trees were constructed using MEGA version 5.0 (Tamura *et al.*, 2011) on the basis of the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969), and maximum-likelihood (Felsenstein, 1981) algorithms. In each

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case bootstrap values were calculated based on 1,000 replications.

Nucleotide accession number

The GenBank/European Molecular Biology Laboratory (EMBL)/DNA Data Bank of Japan (DDBJ) accession number for the 16S rRNA gene sequence of strain KIS55-21^T is KF934398.

Determination of DNA G+C content

The G+C content was determined by the fluorimetric method using SYBR Green 1 and a real-time PCR thermocycler (Bio-Rad) (Gonzalez and Saiz-Jimenez, 2002; Moreira *et al.*, 2011).

Morphological, physiological, and biochemical characterization

The morphology of the bacterial cells was observed by phase-contrast microscopy (AX10; Carl Zeiss), and transmission electron microscopy (TEM) (LEO 912AB; LEO Electron) using cells grown on R2A agar for 5 days at 28°C. Gram staining was determined using heat-fixed liquid cultures and the Difco Gram staining kit, according to the manufacturer's instructions. The pH range for growth was determined by culturing in R2A broth adjusted to pH 4.0–11.0 (at intervals of 1.0 pH unit) prior to sterilization using appropriate biological buffers (Breznak and Costilow, 1994): citrate/phosphate buffer, tris/hydrochloride buffer, HCl, or NaOH. The temperature range for growth was determined in R2A broth between 4 and 50°C (4°C, 10°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C, 37°C, 40°C, 45°C, and 50°C). To investigate the tolerance to NaCl, R2A broth was prepared by adjusting NaCl concentration to 0–6.0%, w/v (at intervals of 1.0%). Growth on nutrient agar (Difco), trypticase soy agar (Difco), and MacConkey agar (Difco) was checked. Growth under anaerobic conditions was determined after incubating strain KIS55-21^T on R2A agar in the BBL GasPak Anaerobic System (Difco). 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. Carboxymethyl cellulose (CM-cellulose), casein, chitin, hypoxanthine, Tween 80, tyrosine, starch, and xanthine degradations were examined on R2A plates containing CM-cellulose (1%, w/v), milk powder (5%, w/v), chitin (1%, w/v), hypoxanthine (0.5%, w/v), Tween 80 (1%, v/v), tyrosine (0.1%, w/v), starch (1%, w/v), and xanthine (0.5%, w/v), respectively. DNase hydrolysis was observed on DNase test agar (Difco). Susceptibility to antibiotics was determined using antibiotic discs containing the following (μg unless otherwise stated): amoxicillin (10), ampicillin (10), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), penicillin (10), polymyxin (300 IU), streptomycin (10) and vancomycin (30). Additional biochemical tests were performed using API 20NE, API ID 32GN, and API ZYM kits (bioMérieux) according to the manufacturer's recommendations.

Chemotaxonomy

For analysis of cellular fatty acid content, cells of strain KIS55-21^T were grown at 28°C on R2A for 5 days, at which time the strain was in the exponential phase. The cellular fatty acids were extracted, methylated and separated by gas chroma-

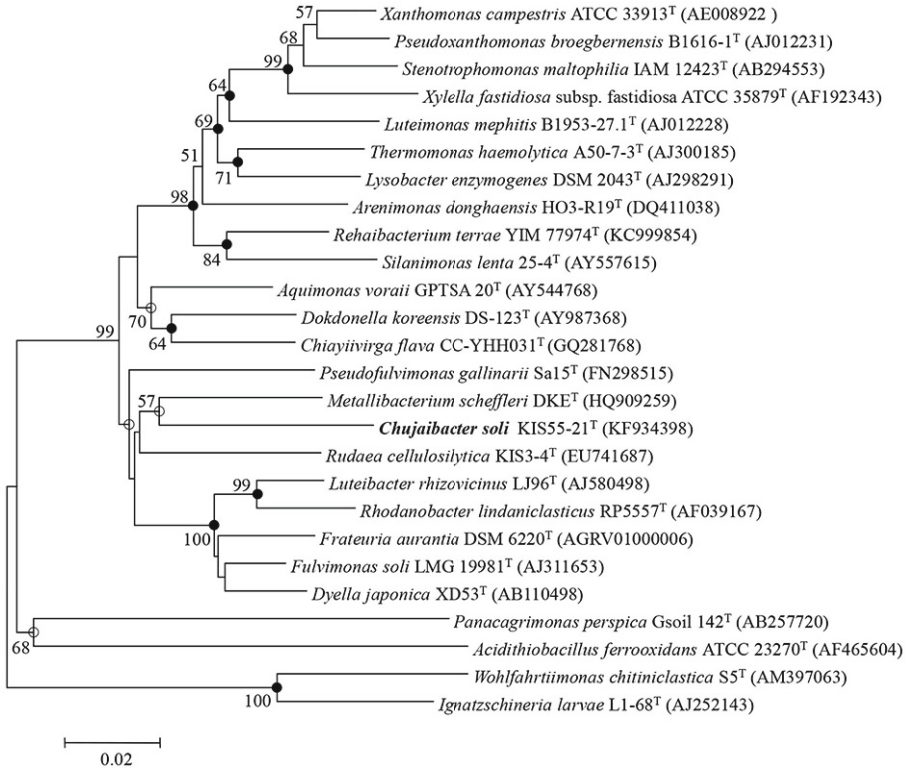

Fig. 1. Neighbor-joining tree, based on the 16S rRNA gene sequences, showing the phylogenetic relationships of strain KIS55-21^T and some other related taxa. The circles indicate nodes identical to those seen in the corresponding maximum-likelihood tree (open circles), or both of maximum-likelihood and maximum-parsimony trees (black circles). Percentage bootstrap values (1,000 replications) greater than 50% are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

tography (model 6890; Hewlett Packard) 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). Isoprenoid quinones and polar lipids were extracted from cells grown in R2A broth and analyzed using 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 reagents (for glycolipids) were applied.

Results and Discussion

Phylogenetic analysis

A nearly complete 16S rRNA gene sequence (1,483 bp) for strain KIS55-21^T was obtained. Phylogenetic analysis based on the 16S rRNA gene sequences using the neighbor-joining method revealed that strain KIS55-21^T was affiliated with the family *Xanthomonadaceae* and formed a cluster with *Metallibacterium scheffleri* DKE^T (Fig. 1). The clustering of strain KIS55-21^T and *M. scheffleri* DKE^T was also recovered in the maximum likelihood tree but not in the maximum parsimony tree, in which strain KIS55-21^T formed an independent clade within the family *Xanthomonadaceae*. Of the validly named species, strain KIS55-21^T was most closely related to *M. scheffleri* DKE^T with 92.6% 16S rRNA gene sequence similarity followed by *Dokdonella* sp. and *Rhodanobacter* sp. (<92.0%, respectively), *Aquimonas voraii* (91.8%), *Frateuria* sp. (<91.6%), and *Fulvimonas soli* (91.5%).

Morphological, physiological, and biochemical characteristics

The cells were aerobic, Gram-staining-negative, rod-shaped, and without flagella (Supplementary data Fig. S1). Strain KIS55-21^T grew optimally at pH 7.0, at 28–30°C and in the presence of 0% (w/v) NaCl. Detailed phenotypic characteristics of strain KIS55-21^T and a comparative analysis with its phylogenetically closest relatives are shown in Table 1 and in the genus and species descriptions.

Table 1. Differential characteristics of strain KIS55-21^T and other closely related species in the family *Xanthomonadaceae*

Strains: 1, KIS55-21^T; 2, *Aquimonas voraii* GPTSA 20^T (data from Saha *et al.*, 2005); 3, *Dokdonella koreensis* DS-123^T (Yoon *et al.*, 2006; Ten *et al.*, 2009); 4, *Frateuria aurantia* IFO 3245^T (Swings *et al.*, 1980; Anandham *et al.*, 2011; Zhang *et al.*, 2011); 5, *Fulvimonas soli* LMG 19981^T (Mergaert *et al.*, 2002; Anandham *et al.*, 2011); 6, *Metallibacterium scheffleri* DKE^T (Ziegler *et al.*, 2013); 7, *Rhodanobacter lindaniclasticus* RP5557^T (Nalin *et al.*, 1999; Woo *et al.*, 2012); 8, *Rudaea cellulosilytica* KIS3-4^T (Weon *et al.*, 2009). Summed feature 9 comprised iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl. +, Positive; w, weakly positive; -, negative; ND, no data available.

Characteristics	1	2	3	4	5	6	7	8
Colony color	Brown	Yellowish-brown	Yellow	Brown-yellow	Deep-yellow	Yellow	Yellow	Yellow
Motility	-	+	+	+	+	-	-	+
Gliding motility	+	ND	ND					
Catalase	-	+	+	+	+	w	+	+
Oxidase	+	+	+	-	+	w	+	+
Temperature range (°C)	20–35	25–42	10–39	10–45*	15–45*	ND	ND	5–35
pH range	6.0–9.0	6.0–11.0	5.0–9.0	4.0–9.0*	5.0–9.0*	2.0–6.5	ND	5.0–8.0
NaCl tolerance (%)	0	2	3	3*	3*	1	ND	1
Nitrate reduction	-	-	+	-	-	-	-	-
Hydrolysis								
Aesculin	+	-	-	-	+	ND	+	+
Casein	-	+	+	+*	+*	+	ND	-
Gelatin	-	+	+	-	-	ND	ND	+
Starch	-	+	-	+*	+	-	ND	-
Urea	-	-	-	+	-	ND	-	+
Assimilation of:								
D-Glucose	+	-	-	+	+	-	+	+
D-Mannose	-	-	-	-	-	-	-	+
N-Acetylglucosamine	+	ND	-	-	-	ND	-	+
D-Maltose	+	-	-	-	+	-	-	+
Capric acid	-	ND	-	-	-	ND	+	-
Malic acid	-	-	-	+*	-	ND	+	-
Trisodium citrate	-	-	-	-	-	-	+	-
Fatty acid(>10%)	i-$C_{17:0}$, summed feature 9, ai-$C_{17:0}$, $C_{16:0}$	i-$C_{15:0}$, i-$C_{17:1}$ ω9c, i-$C_{16:0}$	i-$C_{17:1}$ ω9c, i-$C_{17:0}$, i-$C_{15:0}$	i-$C_{15:0}$, i-$C_{17:0}$, i-$C_{17:1}$ ω9c	i-$C_{15:0}$, i-$C_{17:1}$ ω9c, i-$C_{17:0}$	i-$C_{17:0}$, i-$C_{17:1}$ ω9c	i-$C_{15:0}$, i-$C_{17:1}$ ω9c	i-$C_{17:1}$ ω9c, i-$C_{17:0}$, i-$C_{16:0}$
Major polar lipids	DPG, PE, PG, PME, APL	ND	DPG, PE, PG, AL	DPG, PE, PG, PME, AL	DPG, PE, PG, APL	PE, PG, PME, APL	ND	PE, APL, PL
G+C content (mol%)	65.9	75	71	63.5	71.7	66.6	63	64

*Data from this study.

Table 2. Cellular fatty acid content (%) of strain KIS55-21^T and other closely related species in the family *Xanthomonadaceae*

Strain: 1, KIS55-21^T; 2, *Aquimonas voraii* GPTSA 20^T (data from Saha *et al*., 2005); 3, *Dokdonella koreensis* DS-123^T (Yoon *et al*., 2006); 4, *Frateuria aurantia* DSM 6220^T (Zhang *et al*., 2011); 5, *Fulvimonas soli* LMG 19981^T (Mergaert *et al*., 2002); 6, *Metallibacterium scheffleri* DKE^T (Ziegler *et al*., 2013); 7, *Rhodanobacter lindaniclasticus* RP5557^T (Woo *et al*., 2012); 8, *Rudaea cellulosilytica* KIS3-4^T (Weon *et al*., 2009). Summed feature 3 comprised one or more of iso-$C_{15:0}$ 2-OH, $C_{16:1}$ *ω7c* and $C_{16:1}$ *ω6c*, and summed feature 9 comprised iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl. -, not detected or <0.5%.

Fatty acids	1	2	3	4	5	6	7	8
iso-$C_{11:0}$	3.2	7.4	4.7	-	4.0–5.6	3.2	3.7	1.7
iso-$C_{11:0}$ 3-OH	6.7	9.3	6.8	-	5.6–7.4	6.1	9.1	6.3
$C_{12:0}$ 2-OH	-	-	-	2.7	-	-	-	-
$C_{12:0}$ 3-OH	-	-	-	5.2	-	-	-	-
iso-$C_{12:0}$ 3-OH	-	-	-	1.0	0.6–1.4	-	2.4	-
iso-$C_{13:0}$ 3-OH	-	-	-	3.1	3.8–4.3	-	3.7	-
$C_{14:0}$	1.0	1.1	-	-	-	-	1.2	-
iso-$C_{14:0}$	-	1.4	-	-	-	-	1.8	-
anteiso-$C_{15:0}$	2.7	-	3.7	1.6	2.8–4.0	1.7	3.8	1.1
iso-$C_{15:0}$	6.3	25.0	10.1	36.9	25.8–31.2	9.1	21.3	9.5
iso-$C_{15:1}$ F	-	2.3	-	-	-	-	-	-
$C_{16:0}$	11.2	4.5	4.6	7.9	0.4–2.2	2.4	8.7	5.1
iso-$C_{16:0}$	9.1	17.7	6.2	-	3.7–13.2	2.2	4.8	12.7
$C_{17:0}$ cyclo	0.5	-	-	7.4	-	-	-	1.2
$C_{17:1}$ *ω6c*	-	-	-	-	0.3–1.5	-	3.4	-
anteiso-$C_{17:0}$	12.9	-	7.7	-	0.6–1.1	3.4	-	2.4
iso-$C_{17:0}$	24.4	2.8	19.8	11.9	10.9–15.0	33.8	5.3	18.0
iso-$C_{17:0}$ I	-	-	-	-	-	-	4.2	-
iso-$C_{18:0}$	1.1	-	-	-	-	-	-	-
Unknown 11.799	-	-	0.8	-	2.6–3.5	2.3	5.7	2.8
Summed feature 3	2.3	4.2	1.0	6.0	2.0–2.8	0.9	-	4.4
Summed feature 9	16.9	19.3	31.2	11.1	15.8–21.7	31.9	19.5	32.8

Chemotaxonomy

The major fatty acids of strain KIS55-21^T were iso-$C_{17:0}$, summed feature 9 (comprising iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl), anteiso-$C_{17:0}$ and $C_{16:0}$ (Table 2). In comparison, the fatty acid compositions of strain KIS55-21^T and closely related species were similar with respect to the fatty acid types, except for *Frateuria aurantia*, but exhibited differences in the relative quantities (Table 2). The predominant quinone was ubiquinone-8, which is consistent with members of the family *Xanthomonadaceae* (Saddler and Bradbury, 2005). Polar lipids of strain KIS55-21^T included phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unknown aminolipid (AL), three unknown aminophospholipids (APLs), and four unknown phospholipids (PLs) (Supplementary data Fig. S2). The major polar lipids of strain KIS55-21^T were DPG, PE, PG, PME, and APL, while those of the closely related species lacked one or more of the polar lipids that were present in strain KIS55-21^T, as shown in the closest relative, *M. scheffleri* DKE^T, which contained PE, PG, PME, and APL, but not DPG, as the major polar lipids. The DNA G+C content was 65.9 mol%.

Taxonomic conclusion

Phylogenetic analysis of the 16S rRNA gene sequences revealed that strain KIS55-21^T was affiliated with the family *Xanthomonadaceae* in the class *Gammaproteobacteria*. However, the isolate shared low 16S rRNA gene sequence similarities (<92.6%) with its closest related relatives. Meanwhile, strain KIS55-21^T could be differentiated from its closest relatives according to several features, including colony color, motility, growth responses to temperature, pH and NaCl, catalase and oxidase activities, nitrate reduction, assimilation ability for various substrates, cellular fatty acid composition, and polar lipid profiles. Based on phylogenetic and phenotypic characteristics, we propose that strain KIS55-21^T should be assigned to the family *Xanthomonadaceae* of the class *Gammaproteobacteria* as a new genus and species, for which we propose the name *Chujaibacter soli* gen. nov., sp. nov.

Description of *Chujaibacter* gen. nov.

Chujaibacter (Chu.ja.i.bac'ter. N.L. n. *Chuja*, Chuja, an island in Korea, where the organism was isolated; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Chujaibacter*, a rod named from Chuja).

Cells are aerobic, Gram-staining-negative, rod-shaped, and without flagella. Positive activity for oxidase and negative for catalase. NaCl is not required for growth. Ubiquinone-8 is the predominant quinone. The major cellular fatty acids are iso-$C_{17:0}$, summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl), anteiso-$C_{17:0}$, and $C_{16:0}$. Major polar lipids are phosphatidylethanolamine, aminophospholipid, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylmonomethylethanolamine. Phylogenetically, the genus belongs to the family *Xanthomonadaceae* of the order *Xanthomonadales* within the class *Gammaproteobacteria*. The type species is *Chujaibacter soli*.

Description of *Chujaibacter soli* sp. nov.

Chujaibacter soli (so'li. L. gen. n. *soli*, of soil).
The description is the same as that of the genus with the following additional characteristics. Cells are 0.6–0.7 μm in width and 3.0–9.0 μm in length. Colonies grown on R2A at 28°C for 5 days are brown-colored, small (<0.5 mm in diameter), and round. Shows growth on R2A, but does not grow on nutrient agar, trypticase soy agar, and MacConkey agar. Grows at 20–35°C (optimum, 28–30°C) and at pH 6.0–9.0 (optimum, pH 7.0). Grows in 0% (w/v) NaCl, but not 1% (w/v) NaCl. Does not hydrolyze casein, chitin, CM-cellulose, DNA, hypoxanthine, starch, Tween 80, tyrosine, and xanthine. In API 20NE tests, positive for aesculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis. Assimilates D-glucose, *N*-acetylglucosamine, D-maltose, D-ribose and valeric acid, weakly glycogen, and propionic acid, but does not assimilates L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, inositol, D-saccharose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, or L-proline (API 20NE and API ID 32GN test strips). Activities of esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase β-glucuronidase, and β-glucosidase are detected; activities of alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected (API ZYM test strip). Susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, neomycin, nalidixic acid, polymyxin, streptomycin, and vancomycin, but not to penicillin.
The type strain is KIS55-21^T (=KACC 16971^T =DSM 28578^T), which was isolated from a soil sample from Chuja Island, Jeju Province, Republic of Korea. The genomic DNA G+C content of the type strain is 65.9%.

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