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Acinetobacter kyonggiensis sp. nov., a *β*-Glucosidase-Producing Bacterium, Isolated from Sewage Treatment Plant[§]

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A Gram-negative, non-motile bacterium, designated KSL5401-037^T, was isolated from a sewage treatment plant in Gwangju in the Republic of Korea and was characterized using a polyphasic taxonomic approach. Comparative 16S rRNA gene sequence analysis showed that strain KSL5401-037^T belonged to the genus *Acinetobacter* in the family *Moraxellaceae* of the *Gammaproteobacteria* (Brisou and Prevot, 1954). According to a 16S rRNA gene sequence analysis, it was closely related to *Acinetobacter johnsonii* ATCC 17909^T (97.3%), *A. bouvetii* 4B02^T (97.2%), and *A. beijerinckii* 58a^T (96.8%). Chemotaxonomic data revealed that strain KSL5401-037^T possesses an ubiquinone system with Q-8 as the predominant compound and $C_{16:0}$ (19.2%), $C_{18:1}$ *ω9c* (19.5%), and summed feature 3 ($C_{16:1}$ *ω6c* / $C_{16:1}$ *ω7c*, 34.1%) as the predominant cellular fatty acids. The major polar lipids detected in strain KSL5401-037^T were diphosphatidylglycerol (DPG) and, phosphatidylethanolamine (PE), followed by phosphatidylglycerol (PG) and moderate amounts of phosphatidylcholine and phosphatidylserine. The G+C content of the genomic DNA was 41.2-42.1 mol%. Strain KSL5401-037^T exhibited relatively low levels of DNA-DNA relatedness with respect to *A. johnsonii* DSM 6963^T (17.7%) and *A. bouvetii* 4B02^T (9.3%). The DNA-DNA relatedness values, biochemical, and physiological characteristics of strain KSL5401-037^T strongly support its genotypic and phenotypic differentiation from other recognized type strains of the genus *Acinetobacter*. Based on these data, strain KSL5401-037^T (JCM 17071^T =KEMC 5401-037^T) should be classified in the genus *Acinetobacter* as a type strain of novel species, for which the name *Acinetobacter kyonggiensis* sp. nov. is proposed.

Keywords: *Acinetobacter kyonggiensis*, taxonomy

Acinetobacter is a Gram-negative, non-spore forming, rod-shaped bacteria belonging to the *Gammaproteobacteria*. At the time of this writing, the genus contains twenty-three validated, published species. Recently, two novel strains, *Acinetobacter berezinae* LMG 1003^T and *A. guillouiae* LMG 988^T, were reclassified from the genospecies (Nemec *et al*., 2009a, 2009b).

In the course of collecting microorganisms from a sewage treatment plant, a Gram-negative, non-motile strain, strain KSL5401-037^T, was isolated. Based on a 16S rRNA gene sequence analysis, strain KSL5401-037^T was considered to be an *Acinetobacter*-like strain. We subjected it to a polyphasic taxonomic investigation, and the results indicated that strain KSL5401-037^T is a novel species in the genus *Acinetobacter*.

Materials and Methods

Isolation of bacterial strain and culture conditions

Strain KSL5401-037^T was isolated from a sewage treatment plant in Gwangju in the Republic of Korea. One gram of the sewage was immersed in a 50 ml saline (0.85% NaCl) solution, vortexed, serially diluted, and 100 μl of the aliquot was inoculated on R2A agar that had been diluted 10 times (Difco). Single colonies from the plates were purified by transferring them to new plates and incubating them again under the same conditions. The isolates were routinely cultured on NB agar at 30°C and maintained in a glycerol suspension (20%, w/v) at -70°C. Type strains of *Acinetobacter* species that were used as reference strains for DNA-DNA hybridization and other experiments were obtained from the Korean Collection for Type Cultures (KCTC, South Korea) and the Culture Collection, University of Gőteborg (CCUG, Sweden).

Physiological and biochemical characteristics

Gram reactions were conducted according to the non-staining method, as described by Buck (1982). Cell morphology was examined with light microscopy (Olympus E600). Oxidase activity was evaluated via the oxidation of 1% *p*-aminodimethylaniline oxalate. Catalase activity was determined by testing for bubble production after the addition of 3% (v/v) hydrogen peroxide solution (Cappuccino and Sherman, 2002). Physiological characteristics were determined with API 20NE, API ID 32 GN, and API Zym galleries, according to manufacturer instructions (bioMérieux). Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, and 42°C) was assessed on Nutrient agar (NA, Difco) after 48 h incubation. Growth at various pH values (pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0) was assessed in Nutrient broth (NB, Difco) after 48 h incubation. Growth on NA, trypticase soy agar (TSA, Difco), R2A agar, and Luria-Bertani agar (LB, Difco) was also evaluated at 30°C.

Chemotaxonomic characteristics

Isoprenoid quinones were extracted with chloroform/methanol (2:1,

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v/v), purified via thin-layer chromatography (TLC), and subsequently analyzed with HPLC, as previously described (Collins and Jones, 1981; Shin *et al*., 1996). In order to analyze the methyl esters of cellular fatty acids, the strain was grown on TSA for 48 h at 30°C, and then two loopfuls of cell material were harvested. Fatty acid methyl esters were prepared, separated, and identified with the Sherlock Microbial Identification System (MIS, MIDI, Inc., USA) (Sasser, 1990). Polar lipids were extracted and examined by two-dimensional TLC with solvent 1 (chloroform: methanol: water, 65:25:4) and solvent 2 (chloroform: acetic acid: methanol: water, 80:15:12:4) (Minnikin *et al*., 1984). Phosphorus-containing lipids were detected with zinzadze reagent, free amino groups were detected with ninhydrin reagent, and total lipids were detected with molybdophospholic acid.

PCR amplification and 16S rRNA gene sequencing

The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set, fD1 and rP1 (Weisburg *et al*., 1991), and the purified PCR product was sequenced by Genotec (Daejeon, Korea) (Kim *et al*., 2005). The sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR Inc.). The 16S rRNA gene sequences of the related taxa were obtained from GenBank and edited using the program BioEdit (Hall, 1999). Multiple alignments were performed with the program CLUSTAL X (Thompson *et al*., 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the program MEGA 3 (Kumar *et al*., 2004). A bootstrap analysis with 1,000 replicates was also conducted to obtain confidence levels for the branches (Felsenstein, 1985).

Determination of DNA G+C content and DNA-DNA hybridization

For the determination of the DNA G+C content, genomic DNA was extracted using a commercial genomic DNA Extraction kit (Solgent, Korea) and was then enzymatically degraded into nucleosides. The nucleosides were analyzed using HPLC, as previously described (Tamaoka and Komagata, 1984; Mesbah *et al*., 1989). The DNA-DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. The hybridization was conducted for five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were used in the calculation of hybridization values. The DNA relatedness values quoted are expressed as the means of these three values.

Nucleotide sequence accession number

The nucleotide sequence determined in this study has been deposited with the NCBI GenBank under accession number FJ527818.

Results and Discussion

Physiological and biochemical characteristics

Strain KSL5401-037^T is Gram-negative, non-spore-forming bacteria whose cell morphology showed non-motile short rods 0.8-1.0 μm wide and 1.3-1.6 μm long. It grew well at 30°C. The phenotypic and chemotaxonomic characteristics that differentiate strain KSL5401-037^T from other *Acinetobacter* species are listed in Table 1.

Chemotaxonomic characteristics

The fatty acid profile of strain KSL5401-037^T is shown in Table 2 and compared with the profiles of other closely related *Acinetobacter* species. The seven species included contained $C_{16:0}$, $C_{18:1}\omega9c$, and summed feature 3 ($C_{16:1}\omega6c$ / $C_{16:1}\omega7c$) as their predominant fatty acids. Strain KSL5401-037^T contained a higher amount of summed feature 3 ($C_{16:1}\omega6c$ / $C_{16:1}\omega7c$). The result of a two-dimensional TLC analysis of polar lipids extracted from strain KSL5401-037^T is shown in Supplementary data Fig. 1 (available in IJSEM Online). The major polar lipids of strain KSL5401-370^T were diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE), followed by phosphatidylglycerol (PG) and moderate amounts of phosphatidylcholine (PC) and phosphatidylserine (PS). The polar lipids of strain KSL5401-370^T were detected with zinzadze reagent. PE and PS were detected with ninhydrin reagent. DPG, PG and PC were detected with molybdophospholic acid. Strain KSL5401-037^T contained ubiquinone Q-8 as its major respiratory quinine, which is a common feature in *Gammaproteobacteria*.

Phylogenetic analysis

We obtained a nearly complete 16S rRNA gene sequence of strain KSL5401-037^T (1,444 bp). We conducted a sequence similarity calculation using the EzTaxon server [http://www.eztaxon.org/; Chun *et al*. (2007)], which indicated that the closest relatives of strain KSL5401-037^T were *Acinetobacter johnsonii* ATCC 17909^T (97.3%) and *A. bouvetii* 4B02^T (97.2%).

Determination of DNA G+C content and DNA-DNA hybridization

The DNA G+C content of strain KSL5401-037^T was 41.2-42.1 mol%. To differentiate strain KSL5401-037^T from the closely related *Acinetobacter* species, DNA-DNA hybridizations were performed. Strain KSL5401-037^T exhibited relatively low levels of DNA-DNA relatedness with respect to *A. johnsonii* DSM 6963^T (17.3±1.1%) and *A. bouvetii* 4B02^T (7.1±2.6%), indicating that it is not related to them at the species level (Wayne *et al*., 1987).

Taxonomic conclusions

The results of the polyphasic analyses clearly showed that strain KSL5401-037^T represents a new species within the genus *Acinetobacter*, for which the name *Acinetobacter kyonggiensis* sp. nov. is proposed.

Description of Acinetobacter kyonggiensis sp. nov.

Acinetobacter kyonggiensis (kyong.gi.en'sis. N.L. neut. adj. kyonggiensis, of or belonging to Kyonggi University in the Republic of Korea).

Cells are Gram-negative, non-motile, and short rod-shaped with a width of 0.8-1.0 μm and a length of 1.3-1-6 μm. After 2 days on nutrient agar, colonies are 1-2 mm in diameter, smooth, pale-yellow in color, circular and convex in shape, oxidase-negative, and catalase-positive. Growth occurs at temperatures ranging from 15 to 30°C but not at 35°C, and the optimum growth temperature is 30°C on nutrient agar. The pH growth

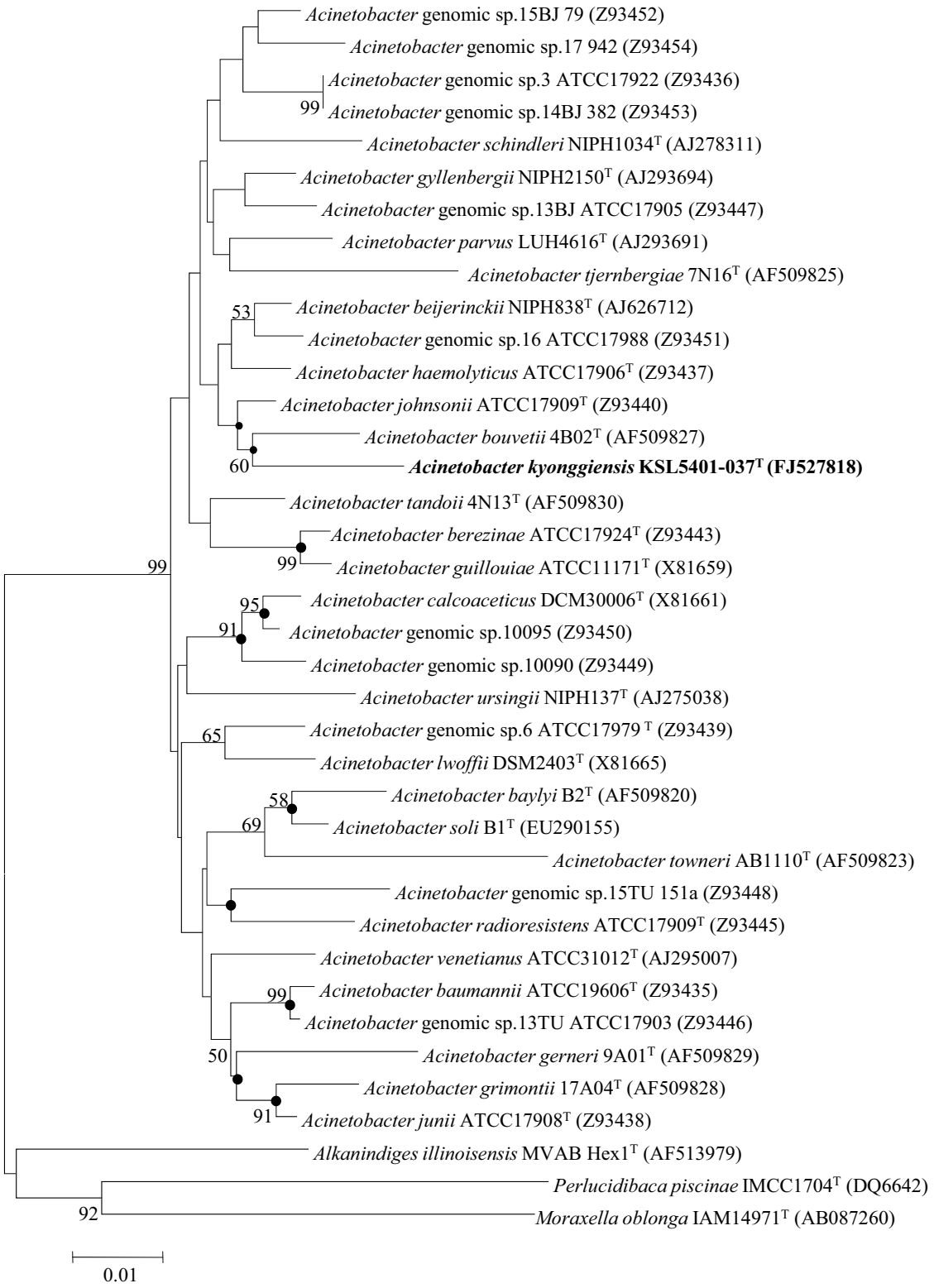

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain KSL5401-037^T and closely related species selected from the genus *Acinetobacter*. Bootstrap values >50% (percentage of 1,000 replications) are shown at branch points. The sequences used for the comparative study are included in parentheses. The neighbor-joining method was used, and filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony algorithms. The bar represents 0.01 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate *Acinetobacter kyonggiensis* sp. nov. from other *Acinetobacter* species
Strains: 1, *Acinetobacter kyonggiensis* KSL5401-037^T; 2, *A. beijerinckii* CCUG 51249^T; 3, *A. bouvetii* KCTC 12414^T; 4, *A. haemolyticus* KCTC 12404^T; 5, *A. johnsonii* KCTC 12405^T; 6, *A. junii* KCTC 12406^T; 7, *A. lwoffii* KCTC 12407^T; 8, *A. parvus* KCTC 12408^T.
Unless indicated, all data were obtained in the course of this study. All strains were yellow-pigmented, non-motile, gram-negative rods. All strains grew at 30°C, but not at 4°C. All strains showed positive activities for catalase and negative activities for oxidase.

Characteristic	1	2	3	4	5	6	7	8
Growth at 37°C	-	+	+	+	-	+	+	+
Enzyme activity								
Acid phosphatase	-	-	-	-	+	+	+	+
Alkaline phosphatase	+	-	-	-	+	+	+	-
Cystine arylamidase	+	-	-	+	+	+	+	-
Esterase (C4)	+	+	+	-	+	+	+	+
β-Glucosidase (Esculin hydrolysis)	+	-	-	-	+	+	-	-
Lipase (C14)	-	+	-	-	-	-	-	-
Naphtol-AS-BI-phosphohydrolase	+	-	+	+	+	+	+	-
Protease (gelatin hydrolysis)	-	-	-	+	-	-	-	-
Valine arylamidase	-	+	-	-	-	-	-	+
Assimilation								
4-Hydroxybenzoate	-	-	-	+	-	+	-	-
D,L-3-Hydroxybutyrate	w	+	w	-	+	-	+	-
Adipate	-	-	-	-	-	-	+	-
Caprate	-	+	+	+	+	+	+	+
Citrate	-	+	-	+	-	+	-	-
D,L-Lactate	+	-	+	-	+	-	+	-
Malate	+	+	+	+	+	-	+	-
Malonate	+	-	-	-	-	-	-	-
Phenyl acetate	-	-	-	-	-	-	+	-
Propionate	-	w	+	-	+	-	-	-
Suberate	-	-	-	-	-	-	+	-
n-Valerate	-	-	+	-	+	-	+	w
L-Fucose	-	-	+	-	-	-	-	-
L-Alanine	+	-	+	+	+	+	+	-
L-Histidine	-	+	+	w	-	w	-	-
L-Proline	w	+	+	+	+	+	w	-
L-Serine	-	-	-	+	-	+	-	-
Glycogen	+	-	-	-	+	-	+	-
G+C content	41.2-42.1	42.0	43.8	40.0-43.0[a]	44.0-45.0[b]	42.0[c]	46.0[d]	41.8[c]

Results are scored as: +, positive; -, negative; w, weak reactions.
[a, b, c, d] Data are taken from Bouvet and Grimont (1986).
[c] Data are taken from Nemec *et al.* (2003).

range is between 6.0 and 10.0, with optimum growth occurring at a pH of 7.0 in nutrient broth. Growth occurs on R2A agar, nutrient agar, TSA, and LB agar. Nitrate is not reduced in aerobic conditions. Cells show activity for alkaline phosphatase, crystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, and naphtol-AS-BI-phosphohydrolase. Cells show no activity for acid phospatase, N–acetyl-*β*-glucosaminidase, α-chymotrypsin, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase (PNPG), *α*-glucosidase (starch hydrolysis), gelatin hydrolysis, *β*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase, trypsin, and valine arylamidase. The following substrates may be utilized for growth: L-alanine, glycogen, 3-hydroxybutyric acid, lactic acid, malate, L-proline, sodium acetate, and sodium malonate. The following substrates cannot be utilized for growth: N-acetyl-glucosamine, adipate, L-arabinose, caprate, citrate, L-fucose, gluconate, D-glucose, 3-hydroxybenzoic acid, L-histidine, 4-hydroxybenzoic acid, inositol, itaconic acid, D-maltose, D-mannitol, mannose, D-melibiose, phenyl-acetate, potassium 5-ketogluconate, propionic acid, L-rhamnose, D-ribose, D-saccharose (sucrose), salicin, L-serine, D-sorbitol, suberic acid, and valeric acid. Cells do not produce acid from glucose. The major fatty acids of stain KSL5401-037^T are $C_{16:0}$ (19.2%), $C_{18:1}$ *ω9c* (19.5%), and summed feature 3 ($C_{16:1}$ *ω6c* / $C_{16:1}$ *ω7c*, 34.1%). The major

Table 2. Fatty acid composition of *Acinetobacter kyonggiensis* sp. nov. and other *Acinetobacter* species
Strains: 1, *Acinetobacter kyonggiensis* KSL5401-037^T; 2, *A. beijerinckii* CCUG 51249^T; 3, *A. bouvetii* KCTC 12414^T; 4, *A. calcoaceticus* KCTC 2357^T; 5, *A. gyllenbergii* CCUG 51248^T; 6, *A. haemolyticus* KCTC 12404^T; 7, *A. lwoffii* KCTC 12407^T; 8, *A. parvus* KCTC 12408^T; 9, *A. schindleri* KCTC 12409^T; 10, *A. ursingii* KCTC 12410^T; 11, *A. johnsonii* KCTC 12405^T.
All data were obtained in the course of this study. Only those fatty acids composing more than 1% are shown. *Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids											
10:0	4.5	1.0	1.7	ND	2.2	tr	tr	ND	1.0	ND	1.4
10:0 2OH	ND	1.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
12:0	**3.2**	**5.7**	**5.2**	**4.2**	**4.1**	**6.8**	**6.6**	**5.0**	**10.2**	**6.8**	**8.5**
12:0 2OH	ND	2.0	ND	1.9	5.8	4.3	ND	4.7	ND	1.7	1.4
12:0 3OH	**5.2**	**8.9**	**5.0**	**3.6**	**9.1**	**8.2**	**5.4**	**7.1**	**7.0**	**4.0**	**7.3**
14:0	1.5	tr	1.4	1.0	tr	tr	1.8	ND	1.5	tr	1.1
16:0	**19.2**	**14.0**	**26.9**	**15.4**	**16.1**	**17.5**	**17.4**	**19.3**	**17.8**	**19.9**	**19.6**
17:0	tr	3.5	tr	2.4	3.0	1.3	ND	ND	ND	ND	ND
18:0	3.6	1.4	1.3	1.5	tr	tr	1.3	ND	tr	tr	1.1
Unsaturated fatty acids											
16:0 N alcohol	3.6	ND	ND	tr	ND	ND	ND	ND	ND	ND	ND
16:1 *ω7c* alcohol	1.1	ND	ND	2.5	ND	ND	ND	ND	ND	ND	ND
17:1 *ω8c*	tr	2.6	tr	5.4	3.5	1.2	ND	1.9	tr	ND	ND
18:1 ω9c	**19.5**	**39.6**	**13.8**	**24.4**	**38.5**	**45.9**	**18.5**	**24.7**	**18.4**	**45.5**	**19.9**
18:3 ω6c (6, 9, 12)	1.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
summed feature 2 (16:1 iso I / 14:0 3OH)	ND	ND	ND	2.6	ND	12.4	1.0	ND	2.1	4.7	tr
summed feature 3 (16:1 ω7c/16:1 ω6c)	**34.1**	**17.6**	**40.5**	**27.8**	**14.7**	tr	**37.5**	**23.4**	**38.1**	**14.7**	**33.4**
summed feature 8 (18:1 ω7c)	1.9	tr	3.4	2.9	1.0	ND	3.1	ND	2.7	tr	5.7

Results are scored as: ND, not detected; tr, less than 1%

polar lipids of stain KSL5401-037^T are DPG and PE, follow by PG and moderate amounts of phosphatidylcholine and phosphatidylserine. The DNA G+C content of stain KSL5401-037^T is 41.2-42.1 mol%.

The type strain, KSL5401-037^T (JCM 17071^T =KEMC 5401-037^T) was isolated from a sewage treatment plant in Gwangju, Republic of Korea.

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References

Bouvet, P.J.M. and P.A.D. Grimont. 1986. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int. J. Syst. Bacteriol.* 36, 228-240.

Brisou, J. and A.R. Prevot. 1954. Genus II. *Acinetobacter*, pp. 425. *In* D.J. Brenner, N.R. Krieg, and J.T. Staley (eds.), Bergey's manual of systematic bacteriology, 2nd ed. The Springer Co., New York, N.Y., USA.

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* 44, 992-993.

Cappuccino, J.G. and N. Sherman. 2002. Microbiology: a Laboratory Manual, 6th ed. San Francisco: Benjamin Cummings.

Carr, E.L., P. Kämpfer, B.K.C. Patel, V. Gürtler, and R.J. Seviour. 2003. Seven novel species of *Acinetobacter* isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* 53, 953-963.

Chun, J., J.H. Lee, Y. Jung, M. Kim, S. Kim, B.K. Kim, and Y.W. Lim. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* 57, 2259-2261.

Collins, M.D. and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* 45, 316-354.

Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric DNA-DNA hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Evol. Microbiol.* 39, 224-229.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406-416.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment

editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser*. 41, 95-98.

Kim, M.K., W.T. Im, H. Ohta, M. Lee, and S.T. Lee. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J. Microbiol.* 43, 152-157.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Brief. Bioinform.* 5, 150-163.

Mesbah, M., U. Premachandran, and W.B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159-167.

Minnikin, D.E., A.G. O'Donnell, M. Goodfellow, G. Alderson, M. Athalye, A. Schaal, and J.H. Parlett. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* 2, 233-241.

Nemec, A., L. Dijkshoorn, I. Cleenwerck, T. De Baere, D. Janssens, T.J. Van Der Reijden, P. Ježek, and M. Vaneechoutte. 2003. *Acinetobacter parvus* sp. nov., a small-colony-forming species isolated from human clinical specimens. *Int. J. Syst. Evol. Microbiol.* 53, 1563-1567.

Nemec, A., M. Musílek, M. Maixnerová, T.D. Baere, J.K. Van Der Reijden, M. Vaneechoutte, and L. Dijkshoorn. 2009a. *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. *Int. J. Syst. Bacteriol.* 59, 118-124.

Nemec, A., M. Musílek, O. Šedo, T.D. Baere, M. Maixnerová, J.K. Van Der Reijden, Z. Zdráhal, M. Vaneechoutte, and L. Dijkshoorn. 2009b. *Acinetobacter berezinae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate, respectively, *Acinetobacter* genomic species 10 and *Acinetobacter* genomic species 11. *Int. J. Syst. Evol. Microbiol*. 60, 896-903.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Shin, Y.K., J.S. Lee, C.O. Chun, H.J. Kim, and Y.H. Park. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J. Microbiol. Biotechnol.* 6, 68-69.

Tamaoka, J. and K. Komagata. 1984. Determination of DNA base composition by reversed phase high-performance liquid chromategraphy. *FEMS Microbiol. Lett.* 25, 125-128.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.

Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, and *et al*. 1987. International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37, 463-464.

Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*. 173, 697-703.