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NOTE

Paralcaligenes ureilyticus gen. nov., sp. nov. Isolated from Soil of a Korean Ginseng Field

Soo-Jin Kim[1], Seung-Hee Yoo[1], Hang-Yeon Weon[1], Yi-Seul Kim[1], Rangasamy Anandham[2], Jang-Sun Suh[1], and Soon-Wo Kwon[1]*

[1]*Korean Agricultural Culture Collection (KACC), Agricultural Microbiology Team, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea*
[2]*Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai, India*

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A bacterial strain, designated GR24-5^T, was isolated from soil cultivated with Korean ginseng. Cells were Gram-negative, strictly aerobic, catalase- and oxidase-positive, non-spore-forming motile rods. Based on the 16S rRNA gene sequence, strain GR24-5^T could be assigned to the family *Alcaligenaceae*. Strain GR24-5^T showed the highest sequence similarities with *Parapusillimonas granuli* Ch07^T (97.1%), *Pusillimonas noertemannii* BN9^T (96.9%), *Pigmentiphaga kullae* DSM 13608^T (96.5%), and *Castellaniella defragrans* 54Pin^T (96.3%). Strain GR24-5^T demonstrated a low DNA-DNA relatedness (23%) with *P. granuli* Ch07^T. The major respiratory quinone is ubiquinone 8 (Q-8) and the major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 1 ($C_{14:0}$ 3-OH/iso-$C_{16:1}$ I/$C_{12:0}$ alde). Putrescine, spermidine, and 2-hydroxyputrescine are the major polyamines. The major polar lipids are phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown aminophospholipid. Polar lipid patterns of strain GR24-5^T were unique in having a large amount of phosphatidylmethylethanolamine. Based on phylogenetic analysis and physiological and biochemical characteristics, strain GR245^T represents a novel genus and species, for which the name *Paralcaligenes ureilyticus* gen. nov., sp. nov. is proposed. The type strain of *P. aralcaligenes ureilyticus* is GR24-5^T (=KACC 13888^T =DSM 24591^T).

Keywords: *Paralcaligenes ureilyticus*, 16S rRNA gene sequence, taxonomy

The family *Alcaligenaceae* was proposed for two genera, *Bordetella* and *Alcaligenes*, by De Ley *et al*. (1986). *Alcaligenaceae* comprises several genera, including the type genus *Alcaligenes* and the recently reported genera *Advenella* (Coenye *et al*., 2005), *Brackiella* (Willems *et al*., 2002), *Castellaniella* (Kämpfer *et al*., 2006), *Kerstersia* (Coenye *et al*., 2003), *Parapusillimonas* (Kim *et al*., 2010), *Parasutterella* (Nagai *et al*., 2009), *Pigmentiphaga* (Blümel *et al*., 2001), *Pusillimonas* (Stolz *et al*., 2005), *Tetrathiobacter* (Ghosh *et al*., 2005), and *Paenalcaligenes* (Kämpfer *et al*., 2010). Most members of the family *Alcaligenaceae* are characterized as Gram-negative, strictly aerobic or facultatively anaerobic rods or coccobacilli that have ubiquinone-8 (Q-8) as the predominant quinone. They have been isolated from various ecological niches, including human and veterinary clinical samples and environmental samples. Here, we performed a polyphasic taxonomic study to determine the taxonomic position of the new strain GR24-5^T that belongs to the family *Alcaligenaceae*.

During bacterial population analysis in Korean ginseng fields, we isolated the bacterial strain GR24-5^T. The dilution-plating method on R2A agar (Reasoner and Geldreich, 1985) was used and the plate was incubated at 28°C for 5 d. Gram staining, catalase activity, oxidase activity, and hydrolysis of casein, carboxymethylcellulose, DNA, and starch were tested using the methods described by Smibert and Krieg (1994). Temperature, salinity, and pH for growth were tested at 5, 10, 20, 25, 30, 33, 37, and 40°C in 0, 1, 2, 3, and 5% (w/v) NaCl and at pH 3, 4, 5, 6, 7, 8, 9, and 10. Bacterial cells

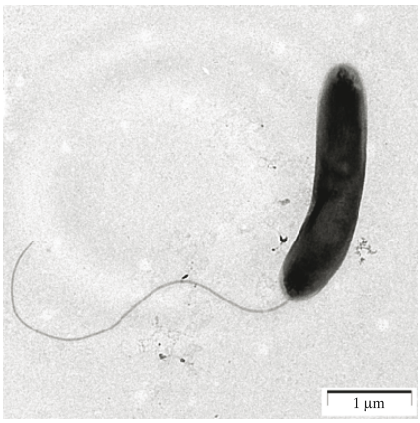

Fig. 1. Transmission electron micrograph of strain GR24-5^T showing polar flagellum.

* For correspondence. E-mail: swkwon@rda.go.kr; Tel: +82-31-299-1860; Fax: +82-31-299-1869

Table 1. Differential phenotypic characteristics of GR24-5^T and related genera
Strains: 1, GR24-5^T; 2, *Alcaligenes faecalis* subsp. *faecalis* LMG 1229^T; 3, *Castellaniella defragrans* DSM 12141^T; 4, *Parapusillimonas granuli* Ch07^T (Kim *et al*., 2010); 5, *Pigmentiphaga kullae* DSM 13608^T; 6, *Pusillimonas noertemannii* DSM 10065^T (Kim *et al*., 2010). All data are from this study unless indicated. All strains showed positive activities for urease and esterase (C4), as well as assimilated sodium acetate, propionic acid, valeric acid, and 3-hydroxybutyric acid. All strains showed negative activities for indole production, β-galactosidase, lipase (C14), cystine arylamidase α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, and did not assimilate L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, L-rhamnose, D-ribose, inositol, D-saccharose, potassium 5-ketogluconate, glycogen, salicin, D-melibiose, and L-fucose. +, Positive; (+), weak positive; -, negative.

Characteristics	1	2	3	4	5	6
Nitrate reduction	-	-	+	-	+	-
Glucose fermentation	-	-	-	+	-	-
Arginine dihydrolase	-	+	+	+	+	(+)
Gelatin hydrolysis	-	-	+	-	+	-
Assimilation of:						
D-Glucose	-	-	-	+	-	+
Potassium gluconate	-	-	+	+	-	-
Capric acid	-	+	+	-	-	-
Adipic acid	-	-	(+)	+	+	-
Malic acid	-	+	+	+	+	+
Trisodium citrate	-	+	+	+	+	+
Phenylacetic acid	-	+	+	+	-	-
Itaconic acid	+	-	-	(+)	+	+
Suberic acid	-	-	-	-	+	+
Sodium malonate	-	+	-	-	-	-
Lactic acid	+	+	+	+	-	+
L-Alanine	-	+	+	+	-	-
3-Hydroxybenzoic acid	-	-	+	+	+	+
L-Serine	-	-	+	+	-	+
D-Sorbitol	-	-	-	+	-	-
L-Histidine	-	+	-	(+)	-	(+)
Potassium 2-ketogluconate	+	-	+	+	-	-
4-Hydroxybenzoic acid	-	-	+	+	+	+
L-Proline	+	+	+	+	-	+
Enzymatic activities of :						
Alkaline phosphatase	+	+	-	-	+	-
Esterase lipase (C8)	-	+	+	+	+	-
Leucine arylamidase	+	+	+	+	+	-
Valine arylamidase	-	-	-	(+)	-	(+)
Trypsin	-	+	-	-	-	-
α-Chymotrypsin	-	-	-	-	+	-
Acid phosphatase	+	+	-	-	-	-
Naphthol-AS-BI-phosphohydrolase	+	+	+	-	+	(+)

were observed by phase-contrast microscopy (Axio; Zeiss, Germany) and transmission electron microscopy (TEM; model 912AB; LEO, Germany). For TEM observation, cells were grown for 2 d at 28°C on R2A agar (Difco, USA). Anaerobic growth was assessed on R2A agar incubated in a GasPak anaerobic system (BBL, USA). Other biochemical and physiological tests were performed using the commercial systems API 20NE, API ID 32GN, and API ZYM (bioMérieux, France) according to the manufacturer's instructions. The API ZYM test strip was read after 4 h of incubation at 37°C and the other API test strips were examined after 7 days at 28°C. Cells of strain GR24-5^T were rod-shaped and motile, having one polar flagellum (Fig. 1). Colonies of strain GR24-5^T were circular, convex, and milky white. Strain GR24-5^T grew on R2A and nutrient agar (NA; Difco). However, the strain rarely grew on tryptic soy agar (TSA; Difco) and did not grow on MacConkey agar (Difco). Phenotypic comparisons among strain GR24-5^T and type species of the closely related genera are shown in Table 1. Strain GR24-5^T differed from its closest relative, *P. granuli* Ch07^T, regarding glucose fermentation, arginine hydrolysis, substrate assimilation abilities, and enzymatic activities. In particular, strain GR24-5^T differed from related genera in terms of arginine dihydrolase and assimilation of malic acid and trisodium citrate.

The 16S rRNA gene of strain GR24-5^T was amplified using the polymerase chain reaction (PCR) with primers fD1 and rP2 (Weisburg *et al.*, 1991), and then the entire PCR fragment was directly sequenced (Hiraishi, 1992). After multiple alignments of data using CLUSTAL W (Thompson *et al.*, 1994), the MEGA software package (ver. 3.1; Kumar *et al.*, 2004)

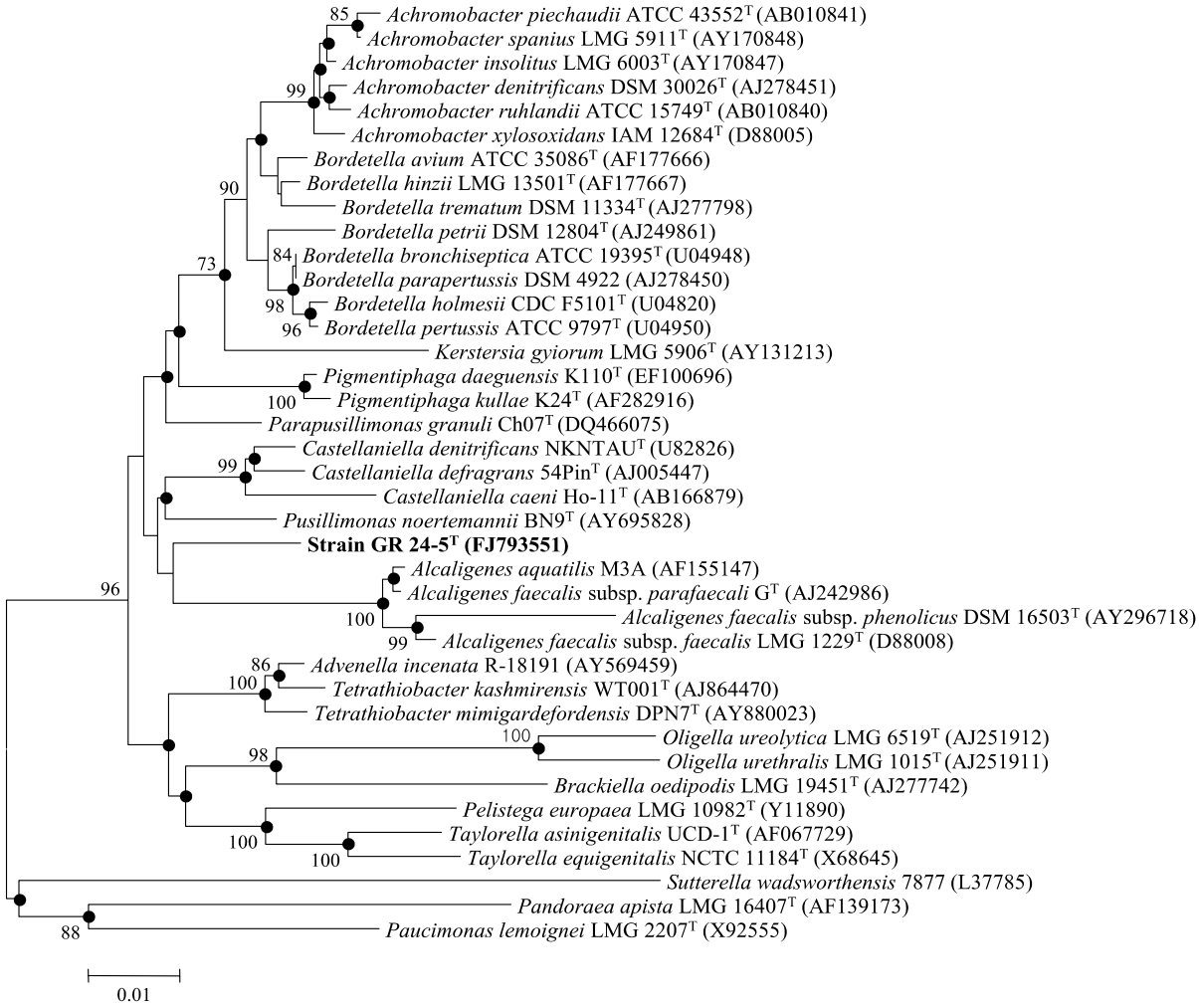

Fig. 2. Neighbor-joining tree based on partial 16S rRNA gene sequences showing the phylogenetic position of strain GR24-5^T. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap values (expressed as percentages of 1,000 replications) >70% are indicated at the nodes. Bar, 0.01 changes per nucleotide position.

was used for all analyses. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values, based on 1,000 replications. The 16S rRNA gene sequences of strain GR24-5^T determined in the present study comprised 1441 nucleotides. The highest sequence similarities were shown to be 97.1% with *P. granuli* Ch07^T, 96.9% with *P. noertemannii* BN9^T, 96.5% with *P. kullae* DSM 13608^T, and 96.3% with *C. defragrans* 54Pin^T. Strain GR24-5^T showed <96.2% sequence similarity to all other closely related species, including *Alcaligenes faecalis* LMG 1229^T (95.6%) and *A. aquatilis* M3A (95.1%). According to the neighbor-joining tree (Fig. 2), strain GR24-5^T was shown to be a member of the family *Alcaligenaceae* and clustered with the genus *Alcaligenes* at bootstrap values below 70%. Compared with the neighbor-joining tree, the maximum-parsimony tree revealed different phylogenetic topologies where strain GR24-5^T formed an independent lineage within the family *Alcaligenaceae*.

The following chemotaxonomic characteristics were analyzed: fatty acids (according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System; Sasser, 1990), phospholipids (Minnikin *et al.*, 1984), polyamines (Busse and Auling, 1988; Busse *et al.*, 1997), and G+C content of the DNA (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were prepared for bacteria grown on R2A medium for 2 d at 28°C. DNA-DNA hybridization was performed using the filter hybridization method described by Seldin and Dubnau (1985). Probe labeling was conducted using the non-radioactive DIG High Prime system and hybridized DNA was visualized using the DIG luminescent detection kit (Roche, Switzerland). DNA-DNA relatedness was quantified using a densitometer (Bio-Rad, USA). The fatty acid composition of strain GR24-5^T included $C_{16:0}$ (35.1%), $C_{17:0}$ cyclo (23.4%), summed feature 1 ($C_{14:0}$ 3-OH/iso-$C_{16:1}$ I/$C_{12:0}$ alde; 11.6%), summed feature 3 ($C_{16:1}$ ω7*c* and/or iso-$C_{15:0}$ 2-OH; 8.1%), $C_{12:0}$ (7.4%), $C_{18:1}$ *ω7c* (7.3%), and several trace elements (Table 2). The predominant isoprenoid quinone was ubiquinone 8 (Q-8). Polar lipids detected were phosphatidylmethylethanol-

Table 2. Fatty acid composition of strain GR24-5^T and closely related taxa
1, GR24-5^T; 2, *Alcaligenes faecalis* subsp. *faecalis* LMG 1229^T (data from this study); 3, *Castellaniella defragrans* DSM 12141^T (Liu *et al*., 2008); 4, *Parapusillimonas granuli* Ch07^T (Kim *et al*., 2010); 5, *Pigmentiphaga kullae* DSM 13608^T (data from this study); 6, *Pusillimonas noertemannii* BN9^T (Stolz *et al*., 2005). All strains were cultivated on R2A medium at 28°C for 2 days before harvesting cell mass. -, Not detected; tr, <1% of the total fatty acids.

Fatty acids	1	2	3	4	5	6
$C_{10:0}$	-	1.9	-	-	-	tr
$C_{10:0}$ 3-OH	-	-	-	-	2.6	-
$C_{12:0}$	7.4	1.4	5.7	-	-	4.4
$C_{12:0}$ aldehyde	-	-	tr	-	-	-
$C_{12:0}$ 2-OH	-	2.9	-	-	-	3.3
$C_{12:0}$ 3-OH	-	tr	-	-	-	-
$C_{13:0}$ at 12-13	-	-	-	-	-	tr
$C_{14:0}$	tr	1.0	tr	-	tr	-
$C_{14:0}$ 2-OH	-	-	-	-	3.4	-
$C_{15:0}$	-	-	tr	-	-	-
$C_{16:0}$	35.1	32.7	27.9	33.2	34.8	18.5
$C_{16:0}$ 2-OH	-	-	-	-	3.3	tr
$C_{16:0}$ 3-OH	-	tr	-	-	tr	tr
$C_{16:1}$ 2-OH	tr	-	-	-	tr	-
$C_{16:1}$ *ω5c*	-	-	-	-	-	-
$C_{17:0}$	-	tr	tr	-	-	-
$C_{17:0}$ cyclo	23.4	27.3	2.3	18.2	25.5	30.9
$C_{17:1}$ *ω8c*	-	-	tr	-	-	-
$C_{18:0}$	tr	1.6	-	tr	1.1	2.5
$C_{18:1}$ 2-OH	-	-	-	-	1.8	-
$C_{18:1}$ *ω7c*	7.3	8.4	18.6[a]	11.3[a]	3.0	tr
$C_{18:1}$ *ω7c* 11 methyl	-	-	-	-	-	tr
$C_{19:0}$ cyclo *ω8c*	4.2	2.8	-	tr	16.5	26.8
iso-$C_{19:0}$	tr	tr	-	-	tr	tr
$C_{20:2}$ *ω*6,9c	-	-	-	-	-	tr
Unknown 13.957	-	tr	-	-	-	-
Summed feature 1[b]	11.6	12.8	6.6	7.4	5.0	9.2
Summed feature 3[b]	8.1	5.3	36.0	28.5	1.0	-

[a] Summed feature 5 comprises $C_{18:1}$ *ω7c*, *ω9t*, and/or *ω12t*.
[b] Summed feature 1 comprises $C_{14:0}$ 3-OH, iso-$C_{16:1}$ I, and/or $C_{12:0}$ alde. Summed feature 3 comprises $C_{16:1}$ *ω7c*, and/or iso-$C_{15:0}$ 2-OH.

amine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown aminophospholipid (Fig. 3). The polyamine pattern exhibited characteristics of members of the Betaproteobacteria [μmol/g dry weight; 2-hydroxyputrescine, 13.3; putrescine, 39.1; cadaverine, 3.9; spermidine, 0.6]. The DNA G+C content was 55.1 mol%. Strain GR24-5^T showed a DNA-DNA relatedness of 23% (25% in a reciprocal experiment) with *P. granuli* Ch07^T.

Differentiation of the genera within the family *Alcaligenaceae* depends primarily on phylogenetic data, based on 16S rRNA genes, fatty acids, and polar lipid patterns (Blümel *et al*., 2001; Stolz *et al*., 2005; Kämpfer *et al*., 2006). Strain GR24-5^T shared the properties of isoprenoid quinone (Q-8), polyamines (putrescine, 2-hydroxyputrescine, and cadaverine as major components), and fatty acids ($C_{16:0}$, $C_{17:0}$ cyclo, summed feature 1, and summed feature 3 as the major components) with closely related genera within the family *Alcaligenaceae* (Table 2). Polar lipid patterns of strain GR24-5^T were unique in having a large amount of phosphatidylmethylethanolamine (Fig. 3; Blümel *et al*., 2001; Stolz *et al*., 2005; Kämpfer *et al*., 2006; Kim *et al*., 2009). Strain GR24-5^T could also be differentiated by several phenotypic characteristics from the type species of the related genera; in particular, it differed from its closest relative, *P. granuli* Ch07^T, in terms of glucose fermentation, arginine hydrolysis, substrate assimilation abilities, and enzymatic activities (Table 1). Based on these results, we consider that strain GR24-5^T is representative of a new taxon for which the name *Paralcaligenes ureilyticus* gen. nov., sp. nov. is proposed.

Description of *Paralcaligenes* gen. nov.

Paralcaligenes (Par.al.ca.li'ge.nes. Gr. Prep. *para* beside; M.L. masc. n. *Alcaligenes* a bacterial genus name; M.L. masc. n. *Paralcaligenes* beside *Alcaligenes*).

Cells are Gram-negative and rod-shaped. They are motile, having one polar flagellum. Growth is strictly aerobic. Oxidase- and catalase-positive. Major cellular fatty acids (>10%) are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 1 ($C_{14:0}$ 3-OH/iso-$C_{16:1}$ I/$C_{12:0}$ alde). The isoprenoid quinone is Q-8. The polar lipids detected are phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown aminophospholipid. Putrescine, 2-hydroxy-

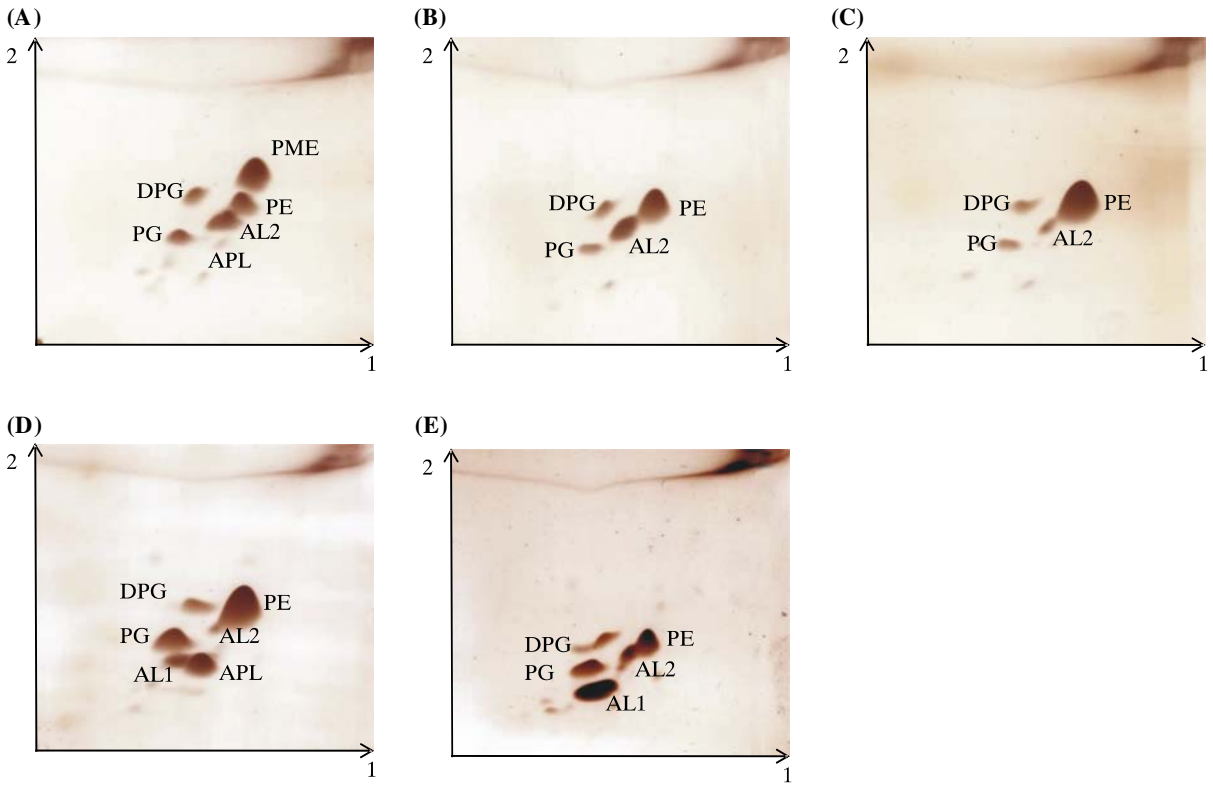

Fig. 3. Polar lipid profiles of strain GR24-5^T (A), *Alcaligenes faecalis* DSM 30030^T (B), *Castellaniella defragrans* DSM 12141^T (C), *Pigmentiphaga kullae* DSM 13608^T (D), and *Pusillimonas noertemannii* BN9^T (E) after separation by two-dimensional thin-layer chromatography (TLC). Chloroform/methanol/water (65:25:4) was used in the first dimension, and chloroform/methanol/acetic acid/water (80:12:15:4) was used in the second dimension. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PME, phosphatidylmethylethanolamine; APL, unknown aminophospholipid; AL1-2, unknown aminolipid.

putrescine, and cadaverine are the major polyamines. Phylogenetically, the genus is a member of the family *Alcaligenaceae*. The type species is *Paralcaligenes ureilyticus*.

Description of *Paralcaligenes ureilyticus* sp. nov.

Paralcaligenes ureilyticus [u.re.i.ly'ti.cus. N.L. n. *urea -ae* urea; -i- connecting vowel, N.L. adj. *lyticus -a -um* (from Gr. adj. *lytikos*) dissolving; N.L. masc. adj. *ureilyticus* urea-dissolving].

After 2 d on R2A agar plates at 28°C, colonies are circular, convex, and milky white. Cells are rod-shaped, approximately 0.4-0.5 μm wide and 1.2-1.8 μm long. Catalase is positive and oxidase is negative. Grows at 10-30°C (optimum, 28°C) and pH 4.0-8.0 (optimum, pH 6.0-7.0). Growth occurs at NaCl concentrations of 0-2% (w/v) (optimum, 0-1%). Casein, carboxymethylcellulose, DNA, tyrosine, and starch are not hydrolyzed. According to the API 20NE, positive for urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis, and β-galactosidase. According to the API 20NE and API ID 32GN test strips, assimilates itaconic acid, sodium acetate, lactic acid, propionic acid, valeric acid, potassium 2-ketogluconate, 3-hydroxybutyric acid, and L-proline, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, D-saccharose, suberic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, L-histidine, and 4-hydroxybenzoic acid. According to the API ZYM test strips, positive activities for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

Type strain is GR24-5^T (=KACC 13888^T =DSM 24591^T) isolated from soil in Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GR24-5^T is FJ793551.

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References

Blümel, S., B. Mark, H.J. Busse, P. Kämpfer, and A. Stolz. 2001. *Pigmentiphaga kullae* gen. nov., sp. nov., a novel member of the

family *Alcaligenaceae* with the ability to decolorize azo dyes aerobically. *Int. J. Syst. Evol. Microbiol*. 51, 1867-1871.

Busse, H.J. and G. Auling. 1988. Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst. Appl. Microbiol*. 11, 1-8.

Busse, H.J., S. Bunka, A. Hensel, and W. Lubitz. 1997. Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int. J. Syst. Bacteriol*. 47, 698-708.

Coenye, T., M. Vancanneyt, M.C. Cnockaert, E. Falsen, J. Swings, and P. Vandamme. 2003. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Rüger and Tan 1983 as *Achromobacter denitrificans* comb. nov. *Int. J. Syst. Evol. Microbiol*. 53, 1825-1831.

Coenye, T., E. Vanlaere, E. Samyn, E. Falsen, P. Larsson, and P. Vandamme. 2005. *Advenella incenata* gen. nov., sp. nov., a novel member of the *Alcaligenaceae*, isolated from various clinical samples. *Int. J. Syst. Evol. Microbiol*. 55, 251-256.

De Ley, J., P. Segers, K. Kersters, W. Mannheim, and A. Lievens. 1986. Intra- and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: Proposal for a new Family, *Alcaligenaceae*. *Int. J. Syst. Bacteriol*. 36, 405-414.

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

Ghosh, W., A. Bagchi, S. Manda, B. Dam, and P. Roy. 2005. *Tetrathiobacter kashmirensis* gen. nov., sp. nov., a novel mesophilic, neutrophilic, tetrathionate-oxidizing, facultatively chemolithotrophic betaproteobacterium isolated from soil from a temperate orchard in Jammu and Kashmir, India. *Int. J. Syst. Evol. Microbiol*. 55, 1779-1787.

Hiraishi, A. 1992. Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett. Appl. Microbiol*. 15, 210-213.

Kämpfer, P., K. Denger, A.M. Cook, S.T. Lee, U. Jäckel, E.B.M. Denner, and H.J. Busse. 2006. *Castellaniella* gen. nov., to accommodate the phylogenetic lineage of *Alcaligenes defragrans*, and proposal of *Castellaniella defragrans* gen. nov., comb. nov. and *Castellaniella denitrificans* sp. nov. *Int. J. Syst. Evol. Microbiol*. 56, 815-819.

Kämpfer, P., E. Falsen, S. Langer, N. Lodders, and H.J. Busse. 2010. *Paenalcaligenes hominis* gen. nov., sp. nov., a new member of the family *Alcaligenaceae*. *Int. J. Syst. Evol. Microbiol*. 60, 1537-1542.

Kim, Y.J., M.K. Kim, W.T. Im, S. Srinivasan, and D.C. Yang. 2010. *Parapusillimonas granuli* gen. nov., sp. nov., isolated from the granule in a wastewater-treatment bioreactor. *Int. J. Syst. Evol. Microbiol*. 60, 1401-1406.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*. 5, 150-163.

Liu, Q.M., L.N. Ten, W.T. Im, and S.T. Lee. 2008. *Castellaniella caeni* sp. nov., a denitrifying bacterium isolated from sludge of a leachate treatment plant. *Int. J. Syst. Evol. Microbiol*. 58, 2141-2146.

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 isoprenoid quinones and polar lipids. *J. Microbiol. Methods* 2, 233-241.

Nagai, F., M. Morotomi, H. Sakon, and R. Tanaka. 2009. *Parasutterella excrementihominis* gen. nov., sp. nov., a novel member of the family *Alcaligenaceae*, isolated from human faeces. *Int. J. Syst. Evol. Microbiol*. 59, 1793-1797.

Reasoner, D.J. and E.E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol*. 49, 1-7.

Saitou, N. and M. Nei. 1987. The neighbor-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.

Seldin, L. and D. Dubnau. 1985. Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int. J. Syst. Bacteriol*. 35, 151-154.

Smibert, R.M. and N.R. Krieg. 1994. Phenotypic characterization, p. 607-654. *In* P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, DC, USA.

Stolz, A., S. Bürger, A. Kuhm, P. Kämpfer, and H.J. Busse. 2005. *Pusillimonas noertemannii* gen. nov., sp. nov., a new member of the family *Alcaligenaceae* that degrades substituted salicylates. *Int. J. Syst. Evol. Microbiol*. 55, 1077-1081.

Thompson, J.D., D.F. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. 22, 4673-4680.

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.

Willems, A., H. Gilhaus, W. Beer, H. Mietke, H.R. Gelderblom, B. Burghardt, W. Voigt, and R. Reissbrodt. 2002. *Brackiella oedipodis* gen. nov., sp. nov., Gram-negative, oxidase-positive rods that cause endocarditis of cotton-topped tamarin (*Saguinus oedipus*). *Int. J. Syst. Evol. Microbiol*. 52, 179-186.