RESEARCH LETTER

'*Lysobacter enzymogenes* ssp. *cookii*' Christensen 1978 should be recognized as an independent species, *Lysobacter cookii* sp. nov.

Yoshiaki Kawamura[1], Junko Tomida[1], Yuji Morita[1], Takashi Naka[2,3], Seiko Mizuno[2,4] & Nagatoshi Fujiwara[2]

[1]Department of Microbiology, School of Pharmacy, Aichi Gakuin University, Aichi, Japan; [2]Department of Bacteriology, Osaka City University Graduate School of Medicine, Osaka, Japan; [3]MBR Co. Ltd, Osaka, Japan; and [4]Department of Development Nourishment, Faculty of Human Development, Soai University, Osaka, Japan

Correspondence: Yoshiaki Kawamura, 1-100 Kusumoto-cho, Nagoya, Aichi 4648650, Japan. Tel.: +81 52 757 6780; fax: +81 52 757 6799; e-mail: kawamura@dpc.agu.ac.jp

Received 9 June 2009; accepted 18 June 2009. Final version published online 16 July 2009.

DOI:10.1111/j.1574-6968.2009.01703.x

Editor: Aharon Oren

Keywords
gram-negative bacteria; gliding bacteria; *Lysobacter cookii*; '*Lysobacter enzymogenes* ssp. *cookii*'.

Abstract

'*Lysobacter enzymogenes* ssp. *cookii*' was proposed by Christensen and Cook in 1978; however, this subspecies name has not been cited in the Approved Lists of Bacterial Names and therefore the nomenclature has not been validated. In our genetic approach to clarify the relationships of the designated type strain of '*L. enzymogenes* ssp. *cookii*' PAGU 1119 (GenBank accession number ATCC29488) within the genus *Lysobacter* revealed that the strain was closely related to *Lysobacter capsici* YC5194^T (99.4%) rather than *L. enzymogenes* DSM2043^T (97.2%). The value for whole genome DNA–DNA relatedness between strain PAGU 1119 and *L. enzymogenes* DSM 2043^T or *L. capsici* YC5194^T was 20.7–26.1% or 60.9–62.0%, respectively. Although PAGU 1119 and *L. capsici* YC5194^T showed relatively high DNA relationships, the fatty acid profiles and some phenotypic characteristics were different, and we concluded that PAGU 1119 should be placed in a new species. We therefore propose a new species with the name *Lysobacter cookii* sp. nov. The type strain is PAGU 1119^T (ATCC29488).

Introduction

The genus *Lysobacter* was established by Christensen & Cook (1978) for gliding bacteria with high G+C contents that do not produce fruiting bodies, with *Lysobacter enzymogenes* as the type species. These authors mainly used phenotypic characteristics to establish this genus; the taxonomic position and phylogenetic features of the organisms were confirmed by Bae *et al.* (2005). Since then, 11 species have been proposed as a new species within *Lysobacter* (Bae *et al.*, 2005; Lee *et al.*, 2006, Weon *et al.*, 2006, 2007; Yassin *et al.*, 2007; Park *et al.*, 2008; Romanenko *et al.*, 2008; Aslam *et al.*, 2009; Wang *et al.*, 2009). To date, 15 species have been recognized as members of the genus *Lysobacter*.

In 1978, Christensen & Cook also proposed two subspecies: '*Lysobacter enzymogenes* ssp. *enzymogenes*' and '*Lysobacter enzymogenes* ssp. *cookii*.' These subspecies were not cited in the Skerman *et al.* (1980 and 1989), even though they were listed in the Index of Bacterial and Yeast Nomenclatural Changes (Moore & Moore, 1989), and the nomenclature has therefore not been validated. In 2006, Tindall & Euzéby requested that the Judicial Commission rule that these names be treated as having been included on the approved lists, on the amended edition of the lists. Up to now, no opinion related to this issue has been announced, and the nomenclatural status for these subspecies is still not fixed.

During our investigation of the taxonomic relationships of *L. enzymogenes* strains, we found that '*L. enzymogenes* ssp. *cookii*' were actually not closely related to *L. enzymogenes* and should be recognized as an independent species within the genus *Lysobacter*, namely *Lysobacter cookii* sp. nov.

Materials and methods

Strains used in this study

We used the following type strains: *L. enzymogenes* (PAGU 1067^T = DSM2043^T), *Lysobacter antibioticus* (PAGU 1068^T = DSM2044^T), *Lysobacter capsici* (PAGU 1064^T = YC5194^T), *Lysobacter gummosus* (PAGU 1069^T = DSM6980^T), *Lysobacter koreensis* (PAGU 1128^T = NBRC101156^T), *Lysobacter*

FEMS MICROBIOLOGY LETTERS

niastensis (PAGU 1071^T = DSM18481^T), and *Lysobacter yangpyeongensis* (PAGU 1070^T = DSM17635^T). All strains were purchased directly from each culture collection, except *L. capsici* YC5194^T, which was kindly provided by Dr J.H. Park (Park *et al.*, 2008). We also used the strain PAGU 1119 (ATCC29488), purchased direct from American Type Culture Collection (ATCC), which was designated as the type strain of '*Lysobacter enzymogenes* ssp. *cookii*.' All strains were grown on R2A agar (Wako Pure Chemical Ltd, Osaka, Japan) plates or 2% trypticase soy agar at 30 °C under aerobic conditions.

Genotypic characterization

First, we determined the 16S rRNA gene sequences of strain PAGU 1119, and investigated the genetic position of the strain within the genus *Lysobacter*. The PCR primers used for amplification of 16S rRNA gene were as described previously (Kawamura *et al.*, 1999, 2003). After confirming single amplification products on 1% agarose gels, sequences were determined with an automatic sequencer (Model 3130, Applied Biosystems) using a dye-terminator reaction kit (Applied Biosystems). The CLUSTAL-X software originally described by Thompson *et al.* (1997) was used to align sequences, and phylogenetic distances were calculated by the neighbor-joining method. Phylogenetic trees were drawn using TREEVIEW software (Page, 1996).

To clarify the exact genomic relationships of PAGU 1119 strain, we decided to measure the whole genomic DNA reassociation rate. DNA from each strain was prepared by the standard procedure of Marmur (1961). We also used the silica–guanidinium thiocyanate DNA purification method described previously (Boom *et al.*, 1990). Quantitative microplate DNA–DNA hybridization was carried out as described previously (Ezaki *et al.*, 1989). Hybridization experiments were carried out at 42 °C (optimal conditions) and 52 °C (stringent conditions) using 2 × SSC and 50% formamide. The optimal temperature was 55 °C below the thermal denaturation temperature, because formamide lowered the hybridization temperature (Meinkoth & Wahl, 1984).

Chemotaxonomic analyses

Cellular lipids and fatty acids were analyzed as described previously (Naka *et al.*, 2000; Li *et al.*, 2003). Briefly, bacterial cells grown on R2A medium were harvested, and cellular lipids were extracted twice with chloroform : methanol (2 : 1, v/v). The cellular lipids were analyzed using two-dimensional TLC. For the fatty acids, the harvested cells were hydrolyzed with 3.75 M NaOH in methanol : water (1 : 1, v/v) at 100 °C for 30 min. After neutralization with 6 N HCl, the fatty acids were extracted twice with *n*-hexane. Methyl ester derivatives of fatty acids were performed by the treatment of 10% trimethylsilyldiazomethane in *n*-hexane (Nacalai Tesque Inc., Kyoto, Japan), and analyzed by GC/MS.

Biochemical characteristics

Biochemical traits were determined with API20NE, API ZYM (bioMérieux) and Nonfergram (Wako Pure Chemical

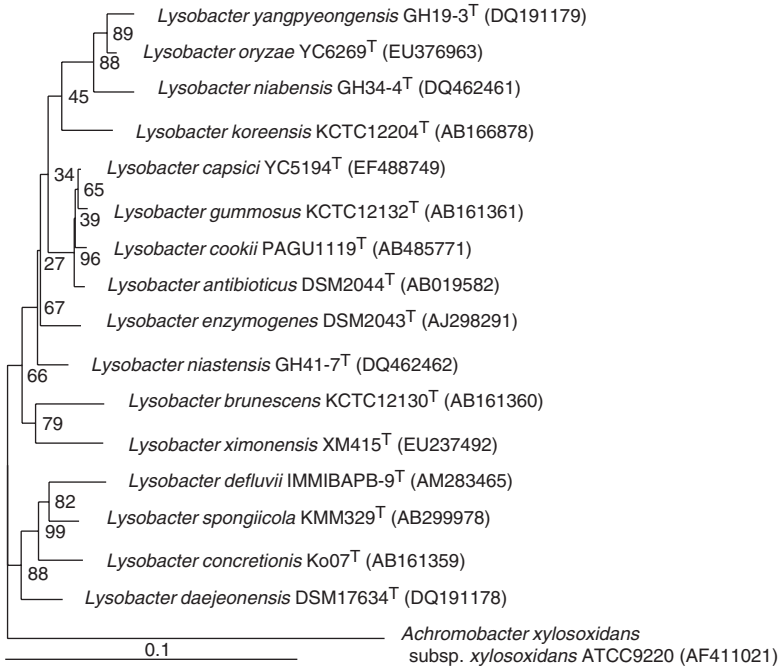

Fig. 1. Phylogenetic relationships among all members of the genus *Lysobacter*. Distances were calculated by the neighbor-joining method. The numbers at the branching points are bootstrap values. *Achromobacter xylosoxidans* ssp. *xylosoxidans* was used as the outgroup.

Table 1. DNA–DNA hybridization similarity values

	DNA-hybridization (%) with biotin-labeled DNA from					
	L. cookii PAGU 1119^T		*L. enzymogenes* PAGU 1067^T		*L. capsici* PAGU 1064^T	
Strains	Optimal	Stringent	Optimal	Stringent	Optimal	Stringent
L. cookii PAGU 1119^T	100.0	100.0	26.1 ± 2.6	26.6 ± 0.9	62.0 ± 0.1	53.3 ± 2.2
L. enzymogenes PAGU 1067^T	20.7 ± 0.3	20.8 ± 1.0	100.0	100.0	36.0 ± 2.3	18.9 ± 1.4
L. capsici PAGU 1064^T	60.9 ± 2.2	60.6 ± 2.9	28.7 ± 0.9	28.8 ± 1.4	100.0	100.0
L. antibioticus PAGU 1068^T	24.9 ± 0.3	23.9 ± 0.4	23.6 ± 2.6	25.2 ± 1.0	31.2 ± 2.0	19.7 ± 0.7
L. gummosus PAGU 1069^T	35.9 ± 7.4	35.6 ± 9.0	33.1 ± 3.1	32.8 ± 4.1	45.3 ± 6.5	31.0 ± 6.9
L. yangpyeongensis PAGU 1070^T	11.6 ± 0.4	11.3 ± 0.7	14.0 ± 1.2	14.3 ± 2.0	18.8 ± 1.3	9.2 ± 0.8
L. niastensis PAGU 1071^T	13.5 ± 2.0	13.4 ± 2.5	15.4 ± 0.9	15.4 ± 1.8	20.1 ± 1.5	10.2 ± 1.1
L. koreensis PAGU 1128^T	14.9 ± 0.1	15.2 ± 1.1	18.7 ± 2.4	18.0 ± 2.6	26.2 ± 0.9	12.3 ± 1.4
Salmon DNA	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Cellular fatty acid compositions (%) of strain PAGU 1119^T and type strains of the genus *Lysobacter*

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
11:0 iso	0.5	–	0.7	3.1	3.8	6.4	4.1	4.3	5.3	5.9	1.8	5.7	3.7	9.5	3.9	3.7
11:0 iso 3-OH	2.1	4.4	3.0	8.0	9.7	9.3	8.0	5.5	9.0	7.2	7.2	6.9	6.0	15.5	3.2	5.2
14:0 iso	0.6	0.1	1.5	1.3	–	8.7	4.2	4.5	4.0	3.7	Tr	2.3	11.2	3.3	Tr	6.1
15:1 iso	1.8	0.7	0.1	Tr	1.7	3.4	1.6	3.1	4.4	1.7	Tr	3.2	3.2	–	3.9	1.8
15:0 iso	18.4	12.3	20.8	24.9	25.2	12.7	21.9	14.5	12.5	19.6	40.9	33.6	13.1	23.0	12.5	22.6
15:0 anteiso	4.0	3.3	4.6	3.8	5.5	5.9	3.8	5.1	–	2.6	–	1.2	3.2	–	2.2	6.3
16:0 iso	5.5	6.3	10.6	10.3	5.7	23.7	23.3	27.5	26.3	23.5	19.2	20.4	33.7	32.5	8.5	24.0
16:0	11.4	10.2	9.1	8.0	6.0	1.1	–	3.1	–	1.5	2.9	1.5	1.4	–	2.7	7.0
16:1	6.7	5.0	–	5.7	7.2	8.8	4.5	11.0	10.8	–	Tr	–	–	–	–	–
17:1 iso	10.5	8.5	7.4	6.4	12.2	10.0	10.9	6.7	16.7	15.5	5.8	15.1	6.7	13.2	21.5	6.7
17:0 iso	6.6	7.3	6.7	3.4	7.8	1.6	1.7	1.9	1.8	2.3	11.1	4.1	–	2.8	12.3	1.5
17:0 cyclo	8.5	15.1	9.9	7.2	Tr	–	–	–	–	–	3.2	1.9	–	–	–	–
18:1	5.9	5.3	6.5	1.7	2.5	–	–	–	–	–	Tr	–	–	–	–	–
18:1 branched	0.6	6.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–
16:1ω7c/15:0 iso 2-OH	10.8	3.0	13.9	8.3	6.4	2.0	6.5	3.3	1.4	9.5	–	–	6.1	–	1.1	2.9
19:0 cyclo	0.8	4.6	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–
Unknown (ECL 11.799)	–	–	–	2.0	1.8	–	1.4	–	–	–	–	–	–	–	–	–

Strains: 1, PAGU1119^T; 2, *Lysobacter capsici* YC5194^T; 3, *Lysobacter enzymogenes* DSM2043^T (data from the present study); 4, *Lysobacter antibioticus* DSM2044^T; 5, *Lysobacter gummosus* DSM6980^T; 6, *Lysobacter niabensis* DSM18244^T; 7, *Lysobacter niastensis* DSM18481^T; 8, *Lysobacter yangpyeongensis* GH19-3^T; 9, *Lysobacter koreensis* KCTC12204^T; 10, *Lysobacter brunescens* DSM6979^T; 11, *Lysobacter defluvii* DSM18482^T; 12, *Lysobacter concretionis* KCTC12205^T; 13, *Lysobacter daejeonensis* GH1-9^T; 14, *Lysobacter spongicola* KMM329^T [data in columns 4–14 from Romanenko *et al*. (2008)]; 15, *Lysobacter oryzae* YC6269^T (data from Aslam *et al*., 2009); 16, *Lysobacter xymonensis* XM415^T (data from Wang *et al*., 2009).
–, not detected; Tr, trace amount (≤ 1%); ECL, equivalent chain length.

Ltd) according to the manufacturers' recommendations. All phenotypic characterization experiments were performed in duplicate.

Results and discussion

Phylogenetic analysis

On the phylogenetic tree based on 16S rRNA gene sequences, three species groups were formed: *L. capsici*, *L. gummosus* and *L. antibioticus* formed one cluster; *L. yangpyeongensis*, *Lysobacter niabensis*, *L. koreensis* and *Lysobacter oryzae* formed another cluster; *Lysobacter defluvii*, *Lysobacter spongiicola*, *Lysobacter concretionis* and *Lysobacter daejeonensis* also formed a different cluster. PAGU 1119 strain formed a cluster with *L. capsici*, *L. antibioticus* and *L. gummosus* with high similarity values (99.4%, 99.2%, and 99.1%, respectively) but the strain was somewhat remote from *L. enzymogenes* (97.2%) and other members of the genus *Lysobacter* (Fig. 1).

Table 3. Differential phenotypic characteristics of strain PAGU 1119^T and species of the genus *Lysobacter*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cell size (μm)	0.3–0.5 × 4–50	0.3–0.5 × 2.0–20	0.4 × 2.0	0.4 × 6.5	0.5 × 38.0	0.5–0.6 × 2.0–4.0	0.5 × 2.0–5.0	0.4–0.6 × 3.0–4.0	0.3–0.5 × 1.8–2.0	0.5–0.8 × 1.5–2.0	0.5 × 1–3	0.3 × 11.0	1–2 (length)	0.7 × 1.0–13.5	0.4–0.6 × 3.0–4.0	0.5–0.6 × 1.3–1.5
Colony color	C	Y–C	PY	C	DY–C	C	Y	Y–G	PY	C	Y	PY	Y	Y	DY	C-PY
DNA G+C content (mol%)	66.2	65.4	65.7	69.2	69.0	66.6	62.5	67.3	67.4	68.9	63.5	67.7	67.1	63.8	61.7	69.0
Gliding motility	+	+	+	+	+	+	−	−	+	ND	+	+	+	+	−	−
Catalase	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+
Oxidase	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+
Nitrate reduction	−	−	−	+	−	+	−	−	−	−	−	−	−	−	+	−
Hydrolysis of																
Aesculin	+	+	+	+	+	+	−	−	−	−	+	+	−	−	+	−
Starch	−	−	−	−	−	+	+	+	−	−	+	−	−	−	−	−
API 20NE tests for assimilation of																
D-Glucose	+	+	+	+	+	+w	−	−	−	−	+	−	−	−	+	−
L-Arabinose	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
D-Mannose	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−
D-Mannitol	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Maltose	+	+	+	+	+	+	−	−	−	−	+	−	−	−	+	−
Malic acid	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Enzyme activities (API ZYM)																
Trypsin	+	+	−	+	+	ND	ND	+	+	−	−	ND	ND	ND	+	−
α-Chymotrypsin	+w	−	−	−	+	ND	ND	−	+	−	+w	ND	ND	ND	−	+
α-Galactosidase	+	+w	+	−	−	ND	ND	−	−	−	−	−	−	−	−	−
β-Galactosidase	+	−	+	+	+	ND	ND	−	−	−	+w	−	−	−	−	−
α-Glucosidase	+	+	−	−	+	ND	ND	+	+	−	−	+	−	−	+	−
N-Acetyl-β-glucosaminidase	+	−	+	+	−	ND	ND	+	+	−	+	−	−	−	−	−

Strains: 1, PAGU 1119^T (data from the present study); 2, *Lysobacter capsici* YC5194^T (data from the present study); 3, *Lysobacter gummosus* DSM6980^T (data from the present study); 4, *Lysobacter antibioticus* DSM2044^T (data from the present study); 5, *Lysobacter enzymogenes* DSM2043^T (data from the present study); 6, *Lysobacter niastensis* GH41-7^T; 7, *Lysobacter niabensis* GH34-4^T (Weon *et al*., 2007); 8, *Lysobacter yangpyeongensis* DSM17635^T (Weon *et al*., 2006); 9, *Lysobacter oryzae* YC6269^T (Aslam *et al*., 2009); 10, *Lysobacter koreensis* KCTC12204^T (Lee *et al*., 2006); 11, *Lysobacter ximonensis* XM415^T (Wang *et al*., 2009); 12, *Lysobacter brunescens* ATCC29482^T (Christensen & Cook, 1978; Bae *et al*., 2005); 13, *Lysobacter defluvii* IMMIB APB-9^T (Yassin *et al*., 2007); 14, *Lysobacter concretionis* Ko07^T (Bae *et al*., 2005); 15, *Lysobacter daejeonensis* DSM17634^T (Weon *et al*., 2006); 16, *Lysobacter spongiicola* KMM329^T (Romanenko *et al*., 2008).
+, positive; −, negative; +w, weak; ND, not determined; C, cream colored; DY, deep yellow; G, green; PY, pale yellow; Y, yellow.

DNA relatedness

The DNA–DNA hybridization values are shown in Table 1. Surprisingly, $< 27\%$ DNA relatedness was shown between strain PAGU 1119 and *L. enzymogenes* PAGU 1067^T, whereas $> 50\%$ reassociation values were observed with *L. capsici* PAGU 1064^T (62.0% and 53.3% under the optimal and stringent conditions, respectively). From these data, we further confirmed that PAGU 1119 ('*L. enzymogenes* ssp. *cookii*') was genetically close to *L. capsici* but not to *L. enzymogenes*.

Chemotaxonomic characteristics

The cellular fatty acid profiles of PAGU 1119 and related species are shown in Table 2. The major cellular fatty acids in PAGU 1119 were 15:0 iso (18.4%), 16:0 (11.4%), 17:1 iso (10.5%), 16:1ω7c/15:0 iso 2-OH (10.8%), 17:0 cyclo (8.5%), 16:1 (6.7%), 17:0 iso (6.6%), 18:1 (5.9%), 16:0 iso (5.5%), 15:0 anteiso (4.0%), 11:0 iso 3-OH (2.1%), and 15:1 iso (1.8%). No significant distinctive features were found in the fatty acid profiles of strain PAGU 1119 compared with the profiles of *Lysobacter* species. The presence of 16:1 could distinguish PAGU 1119 (6.7%) from *L. enzymogenes* (undetected). The presence of somewhat large amounts of 18:1 branched and 19:0 cyclo could also distinguish PAGU 1119 (0.6% and 0.8%, respectively) from *L. capsici* (6.3% and 4.6%, respectively).

The polar lipids of PAGU 1119 strain and the two closely related type strains, *L. enzymogenes* (PAGU 1067^T) and *L. capsici* (PAGU 1064^T) were determined. The major polar lipids, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, and diphosphatidylglycerol, were same in these three strains (data not shown).

Phenotypic characteristics

The summary of some biochemical and phenotypic characteristics are shown in Table 3. Colonies grown on an R2A agar plate after 2 days at 30 °C are creamy white to light brown. The DNA G+C content is 66.2 ± 0.4 mol% as determined by HPLC methods (Kawamura *et al.*, 1998). PAGU 1119 strain could be differentiated from other members of the genus *Lysobacter* by many biochemical traits, for example nitrate reduction, aesculin hydrolysis, assimilation of D-glucose, D-mannose, malic acid, and others. Some enzyme activities such as α-chemotrypsin, α-galactosidase, β-galactosidase and *N*-acetyl-β-glucosaminidase were useful as characteristics differentiating PAGU 1119 from genetically closely related species (*L. enzymogenes*, *L. capsici*, and *L. antibioticus*).

Although PAGU 1119 and *L. capsici* YC5194^T showed relatively high DNA relationships, the fatty acid profiles and some phenotypic characteristics were different. We therefore conclude that PAGU 1119 should be placed in a new species rather than a subspecies of *L. capsici*. We propose the name *Lysobacter cookii* sp. nov. for this new species.

Description of *Lysobacter cookii* sp. nov.

Lysobacter cookii (coo'ki.i. N.L. gen. n. *cookii* of Cook; named from F.D. Cook, the microbiologist who first isolated lysobacters).

Cells are aerobic, gram-negative, rod or filamentous shaped, of various sizes ($0.3–0.5 \times 4–50\,\mu m$), non-spore-forming, and nonmotile, but having gliding activity. Colonies grown on an R2A agar plate after 2 days at 30 °C are creamy white to light brown. No growth on MacConkey agar. The DNA G+C content is 66.2 ± 0.4 mol% as determined by HPLC. The major cellular fatty acids data are shown in Table 2. Catalase and oxidase positive. Does not reduce nitrate. Can hydrolyze aesculin but not arginine and starch. Liquidize gelatin. Does not produce indole. Cannot produce acid from xylitol, lactose or mannitol. Negative for lysine and ornithine decarboxylase. Positive for alkaline phosphatase, C4 and C8 esterase, and naphthol hydrase, but negative for urease, cystine aryl amidase, β-glucuronidase, α-mannosidase, and α-fucosidase. Other phenotypic characteristics are described in Table 1.

The type strain is PAGU 1119 (ATCC29488), isolated from soil in Ottawa, Canada.

Statement

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of the strains PAGU 1119^T is AB485771^T.

References

Aslam Z, Yasir M, Jeon CO & Chung YR (2009) *Lysobacter oryzae* sp. nov. isolated from the rhizosphere of rice (*Oryza sativa* L.). *Int J Syst Evol Micr* **59**: 675–680.

Bae HS, Im WT & Lee ST (2005) *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Micr* **55**: 1155–1161.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM & van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* **28**: 495–503.

Christensen P & Cook FD (1978) *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* **28**: 367–393.

Ezaki T, Hashimoto Y & Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid 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 Bacteriol* **39**: 224–229.

Kawamura Y, Hou XG, Todome Y, Sultana F, Hirose K, Shu S, Ezaki T & Ohkuni H (1998) *Streptococcus peroris* sp. nov. and *Streptococcus infantis* sp. nov., new members of the *Streptococcus mitis* group, isolated from human clinical specimens. *Int J Syst Bacteriol* **48**: 921–927.

Kawamura Y, Whiley RA, Shu S, Ezaki T & Hardie M (1999) Genetic approaches to the identification of the mitis group within the genus *Streptococcus*. *Microbiology* **145**: 2605–2613.

Kawamura Y, Fujiwara H, Mishima N, Tanaka Y, Tanimoto A, Ikawa S, Itoh Y & Ezaki T (2003) First *Streptococcus agalactiae* isolates highly resistant to Quinolones, with point mutations in *gyr*A and *par*C. *Antimicrob Agents Chemother* **47**: 3605–3609.

Lee JW, Im WT, Kim MK & Yang DC (2006) *Lysobacter koreensis* sp. nov., isolated from a ginseng field. *Int J Syst Evol Micr* **56**: 231–235.

Li Y, Kawamura Y, Fujiwara N, Naka T, Liu H, Huang X, Kobayashi K & Ezaki T (2003) *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. *Syst Appl Microbiol* **26**: 523–528.

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Mol Biol* **3**: 208–218.

Meinkoth J & Wahl G (1984) Hybridization of nucleic acids immobilized on solid supports. *Anal Chem* **138**: 267–284.

Moore WEC & Moore LVH (1989) *Index of the Bacterial and Yeast Nomenclatural Changes*. American Society for Microbiology, Washington, DC.

Naka T, Fujiwara N, Yabuuchi E, Doe M, Kobayashi K, Kato Y & Yano I (2000) A novel sphingoglycolipid containing galacturonic acid and 2-hydroxy fatty acid in cellular lipids of *Sphingomonas yanoikuyae*. *J Bacteriol* **182**: 2660–2663.

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Comp Appl Biosci* **12**: 357–358.

Park JH, Kim R, Aslam Z, Jeon CO & Chung YR (2008) *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Micr* **58**: 387–392.

Romanenko LA, Uchino M, Tanaka N, Frolova GM & Mikhailov VV (2008) *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Micr* **58**: 370–374.

Skerman VBD, McGowan V & Sneath PHA (1980) Approved lists of bacterial names. *Int J Syst Bacteriol* **30**: 225–420.

Skerman VBD, McGowan V & Sneath PHA (1989) *Approved Lists of Bacterial Names (Amended Edition)*. American Society for Microbiology, Washington, DC.

Tindall BJ & Euzéby JP (2006) *Lysobacter enzymogenes* subsp. *enzymogenes* Christensen and Cook 1978, *L. enzymogenes* subsp. *cookii* Christensen 1978 and *Streptococcus casseliflavus* (Mundt and Graham 1968) Vaughan *et al.* 1978 should have been cited in the Approved Lists of Bacterial Names. Request for an Opinion. *Int J Syst Evol Micr* **56**: 2707–2709.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Res* **25**: 4876–4882.

Wang Y, Dai J, Zhang L, Luo X, Li Y, Chen G, Tang Y, Meng Y & Fang C (2009) *Lysobacter ximonensis* sp. nov., isolated from soil. *Int J Syst Evol Micr* **59**: 786–789.

Weon HY, Kim BY, Baek YK, Yoo SH, Kwon SW, Stackebrandt E & Go SJ (2006) Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int J Syst Evol Micr* **56**: 947–951.

Weon HY, Kim BY, Kim MK, Yoo SH, Kwon SW, Go SJ & Stackebrandt E (2007) *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Micr* **57**: 548–551.

Yassin AF, Chen WM, Hupfer H, Siering C, Kroppenstedt RM, Arun AB, Lai WA, Shen FT, Rekha PD & Young CC (2007) *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Micr* **57**: 1131–1136.