Paenibacillus alkaliterrae sp. nov., isolated from an alkaline soil in Korea

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A Gram-positive, rod-shaped, motile and endospore-forming bacterial strain, KSL-134^T, was isolated from an alkaline soil in Korea, and its taxonomic position was investigated by a polyphasic study. Strain KSL-134^T grew optimally at pH 7.5 and 30 °C. Its cell wall peptidoglycan contained *meso*-diaminopimelic acid. Strain KSL-134^T was characterized as having MK-7 as the predominant menaquinone and anteiso-C_{15:0} as the major fatty acid. The DNA G+C content was 49.4 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain KSL-134^T formed a distinct lineage within the evolutionary radiation encompassed by the genus *Paenibacillus*. Similarity levels between the 16S rRNA gene sequence of strain KSL-134^T and those of the type strains of recognized *Paenibacillus* species ranged from 90.4 to 96.5 %. DNA–DNA relatedness levels and some differential phenotypic properties were enough to distinguish strain KSL-134^T from several phylogenetically related *Paenibacillus* species. On the basis of phenotypic and phylogenetic data, strain KSL-134^T (= KCTC 3956^T = DSM 17040^T) was classified in the genus *Paenibacillus* as a member of a novel species, for which the name *Paenibacillus alkaliterrae* sp. nov. is proposed.

The genus *Paenibacillus* was proposed by reclassification of 11 *Bacillus* species by Ash *et al.* (1993). Since the description of the genus, continuous reclassifications of some other *Bacillus* species and descriptions of new species have increased considerably the number of species belonging to the genus *Paenibacillus* (for example Heyndrickx *et al.*, 1996; Shida *et al.*, 1997a, b; Pettersson *et al.*, 1999; Roux & Raoult, 2004). At the time of writing, the genus *Paenibacillus* comprises at least 60 species with validly published names, including the recently described species *Paenibacillus xylanilyticus* (Rivas *et al.*, 2005a), *Paenibacillus phyllosphaerae* (Rivas *et al.*, 2005b), *Paenibacillus hodogayensis* (Takeda *et al.*, 2005), *Paenibacillus barcinonensis* (Sánchez *et al.*, 2005) and *Paenibacillus rhizosphaerae* (Rivas *et al.*, 2005c). In this study, we report on the taxonomic characterization of a *Paenibacillus*-like bacterial strain, KSL-134^T, which was isolated from an alkaline soil in Korea.

Strain KSL-134^T was isolated by the standard dilution plating technique at 30 °C on 10-fold diluted nutrient agar (NA; Difco) with pH adjusted to 9.0. *Paenibacillus glycanilyticus* KCTC 3808^T, *Paenibacillus agarexedens* KCTC 3848^T and *Paenibacillus agaridevorans* KCTC 3849^T, which were used as reference strains for DNA–DNA hybridization and some physiological characterization, were obtained from the Korean Collection for Type Cultures, Taejeon, Korea. To investigate its morphological and physiological characteristics, strain KSL-134^T was routinely cultivated at 30 °C on twofold diluted NA with pH adjusted to 7.5. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Presence of flagella was examined by TEM using cells from exponentially growing cultures. Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth at various temperatures (4–45 °C) was measured on twofold diluted NA (pH 7.5). Growth at 5 % (w/v) NaCl concentration was investigated in twofold diluted NA (pH 7.5). The pH range for growth was determined in twofold diluted nutrient broth (NB; Difco) supplemented with 1 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) that was adjusted to various pH values (initial pH 4.5–11.5 at intervals of 0.5 pH units). The pH of twofold diluted NB was adjusted prior to sterilization to various levels by the addition of HCl and Na₂CO₃ (below pH 10.5) or KOH (above pH 10.5). Growth under

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A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of *Paenibacillus alkaliterrae* KSL-134^T, *Paenibacillus* species and representatives of some other related taxa is available as supplementary data in IJSEM Online.

anaerobic conditions was determined after incubation in an anaerobic chamber on twofold diluted NA (pH 7.5) and on twofold diluted NA (pH 7.5) supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lanyi, 1987). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968). Sensitivity to antibiotics was tested using antibiotic discs containing the following concentrations: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; erythromycin, 15 µg; tetracycline, 30 µg. Enzyme activity was determined by using the API ZYM system (bioMérieux) with a modification that 0.1 M phosphate buffer (pH 7.5) was used to prepare the cell suspension of strain KSL-134^T. Other physiological and biochemical tests were performed with the API 20E system (bioMérieux).

Cell biomass for isoprenoid quinone analysis and for DNA extraction was obtained by cultivation at 30 °C in twofold diluted NB (pH 7.5) supplemented with 1% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957). Menaquinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that ribonuclease T1 was treated in combination with ribonuclease A to minimize contamination with RNA. For fatty acid methyl ester analysis, cell mass of strain KSL-134^T was harvested from agar plates after incubation for 5 days on twofold diluted NA (pH 7.5) at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard

protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain KSL-134^T are given in the species description (see later) or are shown in Table 1. The 16S rRNA gene sequence of strain KSL-134^T determined in this study comprised 1509 nucleotides, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analyses showed that strain KSL-134^T falls within the radiation of the cluster comprising *Paenibacillus* species (Fig. 1). Strain KSL-134^T exhibited 16S rRNA gene sequence similarity levels of 90.4% (*Paenibacillus nematophilus*) to 96.5% (*P. agarexedens*) with respect to the type strains of *Paenibacillus* species with validly published names.

The results obtained from chemotaxonomic analyses were in agreement with the results of 16S rRNA gene sequence analysis and phylogenetic inference. Strain KSL-134^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone found in strain KSL-134^T was unsaturated menaquinone with seven isoprene units (MK-7). Strain KSL-134^T had a cellular fatty acid profile that contained large amounts of branched and straight-chain fatty

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data showing the phylogenetic positions of strain KSL-134^T and representatives of the genus *Paenibacillus*. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50% or greater. Scale bar, 0.01 substitutions per nucleotide position. The tree from which Fig. 1 was taken is available as supplementary data in IJSEM Online.

Table 1. Differential phenotypic characteristics of *Paenibacillus alkaliterrae* sp. nov. and phylogenetically related *Paenibacillus* species

Species: 1, *Paenibacillus alkaliterrae* sp. nov.; 2, *Paenibacillus glycanilyticus*, data from Dasman *et al.* (2002) and this study for type strain; 3, *Paenibacillus agarexedens*, Uetanabaro *et al.* (2003) and this study for type strain; 4, *Paenibacillus agaridevorans*, Uetanabaro *et al.* (2003) and this study; 5, *Paenibacillus granivorans*, data from van der Maarel *et al.* (2000); 6, *Paenibacillus curdlanolyticus*; 7, *Paenibacillus kobensis*, data from Kanzawa *et al.* (1995), Shida *et al.* (1997a) and Rivas *et al.* (2005b); 8, *Paenibacillus phyllosphaerae*, data from Rivas *et al.* (2005b); 9, *Paenibacillus lentimorbus*; 10, *Paenibacillus popilliae*, data from Claus & Berkeley (1986) and Pettersson *et al.* (1999); 11, *Paenibacillus thiaminolyticus*, data from Nakamura (1990) and Shida *et al.* (1997a); 12, *Paenibacillus alvei*, data from Claus & Berkeley (1986) and Shida *et al.* (1997a); 13, *Paenibacillus apiarius*, data from Nakamura (1996) and Shida *et al.* (1997a). +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined; v, variable reaction. Data in parentheses are for the type strain. All species are positive for motility (or not determined) and formation of swollen sporangia, and negative for H₂S production (or not determined).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Gram stain	+	+	v or –	v or –	+	+	+	v	v or –	v or –	+	ND	v
Flagellation*	P	Pr	ND	ND	Pr	Pr	Pr	Pr	ND	ND	ND	ND	ND
Colony colour†	Ivory	Pinkish yellow	Whitish	Whitish	ND	ND	ND	Whitish cream	ND	ND	Non-pigmented	ND	Non-pigmented
Anaerobic growth	–	–	–	–	–	–	–	+	+	+	+	+	+
Oxidase	+	–	+	+	–	–	–	+	(–)	(–)	+	ND	–
Catalase	+	+	+	+	+	+	+	+	–	–	+	+	+
Urease	–	–	–	–	ND	+	+	–	–	ND	–	–	+
Anaerobic growth	–	–	–	–	–	–	–	+	+	+	+	+	+
Nitrate reduction	–	–	–	–	+	+	+	+	–	–	+	–	+
Growth at pH 5.7	–	+	–	–	–	ND	ND	ND	–	–	–	–	+
Growth at 5% (w/v) NaCl	–	–	–	–	–	–	–	–	–	–	v	v	+
Growth at 40 °C	–	+	v (–)	–	+	ND	ND	–	–	–	+	ND	+
Production of indole	–	–	–	–	–	–	–	–	–	–	+	+	–
V-P reaction	–	–	–	–	–	–	–	–	–	–	–	+	–
Hydrolysis of:													
Agar	–	ND	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
Casein	–	–	–	–	–	–	–	–	–	–	+	+	+
Gelatin	–	–	–	–	–	ND	ND	ND	–	–	ND	+	ND
Starch	+	+	+	+/–‡	+	+	+	+	–	–	+	+	+
Tween 80	+	–	–	–	ND	ND	ND	ND	ND	ND	–	ND	–
Tyrosine	–	–	v (+)	–	–	ND	ND	ND	–	–	+	v	+
Utilization of:													
D-Fructose	+	+	(–)	(–)	ND	+	–	+	ND	ND	ND	ND	ND
D-Mannose	+	+	(–)	(–)	ND	–	–	w	ND	ND	ND	ND	ND
D-Xylose	+	+	(+)	(–)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetate	–	–	(–)	(–)	–	–	–	ND	ND	ND	+	ND	ND
Citrate	–	–	(–)	(–)	–	–	–	–	–	–	+	–	+
L-Malate	–	–	(–)	(–)	ND	–	–	–	ND	ND	+	ND	ND
Succinate	+	–	(–)	(–)	ND	–	–	ND	ND	ND	+	ND	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Enzyme activity													
Esterase (C4)	—	+	(+)	(+)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esterase lipase (C8)	—	W	(+)	(W)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acid phosphatase	—	W	(—)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
α -Galactosidase	+	+	(—)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
α -Glucosidase	+	+	(—)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
β -Glucosidase	+	+	(—)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sensitivity to antibiotics													
Streptomycin	+	+	(+)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cephalothin	—	+	(+)	(+)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lincomycin	+	+	(+)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oleandomycin	+	W	(—)	(—)	ND	ND	ND	ND	ND	ND	ND	ND	ND
DNA G + C mol%	49.4	50.5	47–49 (47)	50–52 (51)	47.8	50–52	50–52	50.7	37.7§	(41.3)	52–54	45–47	52–54

*P, single polar flagellum; Pr, peritrichous flagella.

†Differences might be caused by different cultivation conditions.

‡Positive in ethanol precipitation method but negative in flooding with Lugol's iodine solution.

§Data for a non type strain.

acids; the components (>1%) were anteiso-C_{15:0} (46.3%), C_{16:0} (17.8%), iso-C_{16:0} (10.7%), C_{18:0} (5.2%), C_{15:0} (3.8%), anteiso-C_{17:0} (3.7%), C_{16:0} 2-OH (3.4%), iso-C_{14:0} (3.3%), iso-C_{15:0} (2.3%), C_{14:0} (2.2%) and C_{16:1}ω11c (1.5%). This fatty acid profile was similar to those of *Paenibacillus* species (Shida *et al.*, 1997a; Rivas *et al.*, 2005a, b, c; Takeda *et al.*, 2005; Sánchez *et al.*, 2005). The DNA G + C content of strain KSL-134^T was 49.4 mol%.

The phylogenetic distinctiveness was sufficient to categorize strain KSL-134^T as a member of a species that is distinct from the previously recognized *Paenibacillus* species (Stackebrandt & Goebel, 1994). There were differences between strain KSL-134^T and several phylogenetically related *Paenibacillus* species in phenotypic characteristics (Table 1). Mean levels of DNA–DNA relatedness were low (9–17%) enough to genetically distinguish strain KSL-134^T from the type strains of three phylogenetically and physiologically related *Paenibacillus* species, *P. glycanilyticus*, *P. agarexedens* and *P. agaridevorans* (Wayne *et al.*, 1987). Therefore, on the basis of the data presented, strain KSL-134^T should be classified in the genus *Paenibacillus* as a member of a novel species, for which the name *Paenibacillus alkaliterrae* sp. nov. is proposed.

Description of *Paenibacillus alkaliterrae* sp. nov.

Paenibacillus alkaliterrae (al.ka.li.ter'rae. N.L. n. *alkali* alkali; L. gen. n. *terrae* of the soil or earth; N.L. gen. n. *alkaliterrae* of high-pH soil).

Cells are aerobic rods, 0.4–0.5 × 1.5–3.0 µm. Gram-positive. Motile by means of a single polar flagellum. Central or subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies on twofold diluted NA (pH 7.5) are circular to slightly irregular, smooth, sticky, glistening, raised, ivory-coloured and 2.0–4.0 mm in diameter after 5 days incubation at 30 °C. Optimal temperature for growth is 30 °C; growth occurs at 10 and 37 °C, but not at 4 and 38 °C. Optimal pH for growth is 7.5; growth occurs at pH 7.0 and 9.5, but not at pH 6.5 and 10.0. Anaerobic growth does not occur on twofold diluted NA (pH 7.5) and on twofold diluted NA (pH 7.5) supplemented with nitrate. Aesculin is hydrolysed, but Tweens 20, 40 and 60, hypoxanthine and xanthine are not. D-Glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xylose, L-arabinose, sucrose, maltose and salicin are utilized, but benzoate, pyruvate, formate and L-glutamate are not. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with API ZYM, alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. Sensitive to polymyxin B, penicillin G, chloramphenicol, gentamicin, novobiocin, tetracycline and kanamycin, but not to ampicillin. The cell-wall peptidoglycan

contains meso-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acid is anteiso-C_{15:0}. The DNA G+C content is 49.4 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, KSL-134^T (= KCTC 3956^T = DSM 17040^T), was isolated from an alkaline soil in Kwangchun, Korea.

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