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-134T, was isolated from an alkaline soil in Korea, and its taxonomic position was investigated by a polyphasic study. Strain KSL-134T grew optimally at pH 7.5 and 30 °C. Its cell wall peptidoglycan contained meso-diaminopimelic acid. Strain KSL-134T was characterized as having MK-7 as the predominant menaquinone and anteiso-C15 : 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-134T formed a distinct lineage within the evolutionary radiation encompassed by the genus Paenibacillus. Similarity levels between the 16S rRNA gene sequence of strain KSL-134T 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-134T from several phylogenetically related Paenibacillus species. On the basis of phenotypic and phylogenetic data, strain KSL-134T (=KCTC 3956T = DSM 17040T) was classified in the genus Paenibacillus as a member of a novel species, for which the name Paenibacillus alkaliterrae sp. nov. is proposed.
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-134T. 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-134T 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-134T are given in the species description (see later) or are shown in Table 1. The 16S rRNA gene sequence of strain KSL-134T 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-134T falls within the radiation of the cluster comprising Paenibacillus species (Fig. 1). Strain KSL-134T 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-134T contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone found in strain KSL-134T was unsaturated menaquinone with seven isoprene units (MK-7). Strain KSL-134T had a cellular fatty acid profile that contained large amounts of branched and straight-chain fatty acids.
Table 1. Differential phenotypic characteristics of *Paenibacillus alkaliterrae* sp. nov. and phylogenetically related *Paenibacillus* species

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</table>

| (3–4) | (3–4) | (2–3) | (2–2) | C16:0:10(11c (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-134T was 49.4 mol%.

The phylogenetic distinctiveness was sufficient to categorize strain KSL-134T as a member of a species that is distinct from the previously recognized Paenibacillus species (Stackebrandt & Goebel, 1994). There were differences between strain KSL-134T 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-134T from the type strains of three phylogenetically and physiologically related Paenibacillus species, P. glycinaityticus, P. agarexedens and P. agaridevorans (Wayne et al., 1987). Therefore, on the basis of the data presented, strain KSL-134T 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, glistening, raised, ivory-coloured and 2–4–0.4–0.0 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 Tween 20, 40 and 60, hypoxanthine and xanthine are not. D-Glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xylene, L-arabinose, sucrose, maltose and salicin are utilized, but benzoate, ppyruvate, 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 β-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<sub>15:0</sub>. 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<sup>T</sup> (=KCTC 3956<sup>T</sup> = DSM 17040<sup>T</sup>), was isolated from an alkaline soil in Kwangchun, Korea.

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References


