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.

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 hodagayensis (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-134ᵀ, which was isolated from an alkaline soil in Korea.

Strain KSL-134ᵀ 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 glycanolyticus KCTC 3808ᵀ, Paenibacillus agararexedens KCTC 3848ᵀ and Paenibacillus agaridevorans KCTC 3849ᵀ, which were used as reference strains for DNA–DNA hybridization and some physiological characterization, were obtained from the Korean Collection for Type Cultures, Taejon, Korea. To investigate its morphological and physiological characteristics, strain KSL-134ᵀ 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). The pH range for growth was determined in twofold diluted nutrient broth (NB; Difco) supplemented with 1% (v/v) Htuner’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 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
an aerobic 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 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) 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).

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 contained 16S rRNA gene sequence similarity levels of 90-4% (Paenibacillus nematophilus) to 96-5% (P. agarexdenis) 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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Species: 1, *Paenibacillus alkaliterrae* sp. nov.; 2, *Paenibacillus glycanilyticus*, data from Dasman et al. (2002) and this study for type strain; 3, *Paenibacillus agaridevorans*, 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 kohensis*, 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).
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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. glycanilyticus, P. agardelevorans and P. agaridelevorans (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 swelled sporangia. Colonies on twofold diluted NA (pH 7.5) are circular to slightly irregular, smooth, 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, butTweenes 20, 40 and 60, hypoxanthine and xanthine are not. D-Glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xyllose, 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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References


