**Lysinibacillus xylanilyticus** sp. nov., a xylan-degrading bacterium isolated from forest humus

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A novel xylan-degrading bacterium, designated XDB9\(^{\text{T}}\), was isolated from forest humus collected from Gyeryong Mountain in Korea. Cells were Gram-positive, aerobic, motile and endospore-forming rods. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain XDB9\(^{\text{T}}\) was most closely related to members of the genus *Lysinibacillus*. 16S rRNA gene sequence similarities between strain XDB9\(^{\text{T}}\) and the type strains of species of the genus *Lysinibacillus* ranged from 98.0 to 98.5\%. The cell-wall peptidoglycan type of strain XDB9\(^{\text{T}}\) was A4\(_{\text{b}}\), which is based on L-Lys–D-Asp. Strain XDB9\(^{\text{T}}\) contained iso-C\(_{15:0}\) and C\(_{16:1}\)\(\gamma\)7c alcohol as the major fatty acids and MK-7 as the predominant menaquinone. The major polar lipids were diphasphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content was 37.2 mol\%. The DNA–DNA hybridization results and differential phenotypic properties showed that strain XDB9\(^{\text{T}}\) could be distinguished from recognized species of the genus *Lysinibacillus*. It was concluded that strain XDB9\(^{\text{T}}\) represents a new taxon for which the name *Lysinibacillus xylanilyticus* sp. nov. is proposed. The type strain is XDB9\(^{\text{T}}\) (=KCTC 13423\(^{\text{T}}\)=CCUG 57438\(^{\text{T}}\)).

The genus *Lysinibacillus* was proposed by Ahmed et al. (2007) by the description of one novel species and the reclassification of two *Bacillus* species on the basis of a polyphasic taxonomic study, and especially with regard to characteristics such as cell-wall peptidoglycan structure. At the time of writing, the genus *Lysinibacillus* was composed of four species with validly published names: *Lysinibacillus boronitolerans*, *Lysinibacillus fusiformis* and *Lysinibacillus sphaericus* (Ahmed et al., 2007) and *Lysinibacillus parviborononicapiens* (Miwa et al., 2009).

During the course of a study on the diversity of xylan-degrading bacteria from natural environments, a novel bacterial strain, XDB9\(^{\text{T}}\), was isolated from forest humus. Xylan, a polymer of β-1,4-D-xylopyranosyl units, is one of the most ubiquitous polysaccharides in nature and a main constituent of hemicellulose, which is found in the cell walls of plants (Timmell, 1967). Xylan-degrading bacteria have been isolated in order to screen for xylanases, such as β-1,4-D-xylan xylanohydrolase and β-1,4-D-xylan xylanohydrolase, which are useful in the food and pulp industries (Coughlan & Hazlewood, 1993).

For the screening of xylan-degrading bacteria, forest humus was collected from Gyeryong Mountain, Taejon, Korea. The samples were serially diluted in 0.85 % (w/v) saline solution and then aliquots of each serial dilution were spread onto solid medium containing 0.5 % (w/v) birch wood xylan (Sigma) and 1.5 % (w/v) Noble agar (Difco). The medium was incubated for 7 days at 25 °C under aerobic conditions. One of the isolates, XDB9\(^{\text{T}}\), was selected and subcultivated on trypticase soy agar (TSA; Difco) at 30 °C for further study. *L. boronitolerans* JCM 21713\(^{\text{T}}\), *L. fusiformis* KACC 10903\(^{\text{T}}\) and *L. sphaericus* KCTC 3346\(^{\text{T}}\) were used as reference strains for phenotypic characterization, cellular fatty acid analysis and DNA–DNA hybridization. *L. parviborononicapiens* KCTC 13154\(^{\text{T}}\) was also used as a reference strain for phenotypic characterization and DNA–DNA hybridization.

To investigate the xylan-degrading activity of strain XDB9\(^{\text{T}}\), Congo red (Wood et al., 1988) and dinitrosalicylic acid (DNS) methods (Miller, 1959) were used. For the DNS test, strain XDB9\(^{\text{T}}\) was cultured and suspended in 100 ml of 50 mM potassium phosphate buffer (pH 6.5) and the inoculum density was adjusted to a McFarland standard of 0.5. Birch wood xylan (1 g) was added and the mixture was incubated for 6 h at 30 °C. A 100 μl aliquot of the mixture was then transferred into an Eppendorf tube, 1 ml DNS reagent was added and the tube was heated in a boiling water bath for 10 min. Finally, the absorbance was measured at 575 nm with a spectrophotometer (DU800; Beckman Coulter).
Cell morphology and motility were examined by light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). The Gram reaction was performed using the bioMérieux Gram-stain kit according to the manufacturer’s instructions. Growth at various temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) was measured on TSA. The pH range for growth was determined in trypticase soy broth (TSB; Difco) adjusted to various pH values (pH 4.0–10.0, in increments of 0.5 pH units) by the addition of HCl or Na2CO3. Growth at various NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 %, w/v) was investigated using TSB prepared according to the formula of the Difco medium except that NaCl was excluded. Oxidase and catalase activities and hydrolysis of casein, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine and xanthine were tested on appropriate media and substrates as described by Cowan & Steel (1965). Hydrolysis of aesculin and reduction of nitrate were examined as described by Lányi (1987).

Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 50CH and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. The cells were suspended in AUX medium to inoculate the API 50CH system. Resistance to antibiotics was assessed by spreading bacterial suspension on TSA and applying paper discs impregnated with the following (µg per disc unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbencillin (100). Boron tolerance was tested as described by Ahmed et al. (2007) at concentrations of 0, 50, 100 and 150 mM.

For cellular fatty acid analysis, cell biomass was harvested from TSA plates after cultivation for 24 h at 28 °C. Cell biomass of strain XDB9 was extracted and purified as described by Schleifer & Kandler (1972). The peptidoglycan amino acids were determined using an automated amino acid analyser (L-8500A; Hitachi). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed using reversed-phase HPLC equipped with a YMC ODS-A (250 × 4.6 mm) column. Polar lipids were extracted and identified by two-dimensional TLC followed by spraying with appropriate detection reagents as described by Minnikin et al. (1984). Extraction of chromosomal DNA and PCR-mediated amplification and sequencing of the 16S rRNA gene sequence were performed as described previously (Yoon et al., 1996, 1998, 2003). The 16S rRNA gene sequence was aligned with sequences retrieved from EMBL by using CLUSTAL_X (Thompson et al., 1997) and edited by using BioEdit (Hall, 1999).

Phylogenetic trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms with MEGA version 3.1 (Kumar et al., 2004). The stability of relationships in the neighbour-joining tree was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications. The DNA G + C content was determined as described by Tamaoka & Komagata (1984) with the modification that, after hydrolysis of the DNA, the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and 96-well plates (Nunc). Hybridization was performed with five replications for each sample. The highest and lowest values obtained for 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 XDB9 are given in the species description and in Table 1. The 16S rRNA gene sequence of strain XDB9 determined in this study consisted of 1349 nucleotides and showed the closest affiliations to those of members of the genus Lysinibacillus (Fig. 1). In the phylogenetic trees based on 16S rRNA gene sequences, strain XDB9 formed an evolutionary lineage within the cluster comprising species of the genus Lysinibacillus, clustering most closely with L. boronitolerans 10aT with a bootstrap resampling value of 88 % (Fig. 1). The relationship between strain XDB9 and L. boronitolerans 10aT was also found in the trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). 16S rRNA gene sequence similarity values between strain XDB9 and the type strains of species of the genus Lysinibacillus ranged from 98.0 % (L. sphaericus NRRL B-23268T) to 98.5 % (L. fusiformis NRRL NRS-350T and L. boronitolerans 10aT).

Quantitative analysis of the cell-wall peptidoglycan showed that strain XDB9 and L. boronitolerans JCM 21713 had similar molar ratios of alanine, aspartic acid, glutamic acid and lysine (approximately 1.43 : 0.43 : 1.0 : 0.33 and 1.45 : 0.65 : 1.0 : 0.45, respectively). The smaller amounts of lysine and aspartic acid were due to the presence of the stable peptide L-Lys–D-Asp. From these data, it was concluded that strain XDB9 had the peptidoglycan type A4z, which is based on L-Lys–D-Asp, as described by Schleifer & Kandler (1972). This supported the result of the phylogenetic analysis, which showed an affiliation of strain XDB9 to the genus Lysinibacillus, because the A4z peptidoglycan type is a key marker used to discriminate the genus Lysinibacillus, as well as the genus Kurthia, from other members of Bacillus group 2 (Shaw & Keddie, 1983; Stackebrandt et al., 1987; Claus & Fritze, 1989; Ash et al., 1991; Rheims et al., 1999; Nakamura et al., 2002). The predominant isoprenoid quinone detected in strain XDB9 was menaquinone-7 (MK-7), which is the same as that
found in members of the genus *Lysinibacillus* (Ahmed et al., 2007; Miwa et al., 2009). The cellular fatty acid profile of strain XDB9\(^T\) is shown in Table 2 together with those of three *Lysinibacillus* species, also analysed in this study, and *L. parviboronicapiens* BAM-582\(^T\), analysed by Miwa et al. (2009). The major fatty acids (>10% of total fatty acids)
were iso-C<sub>15</sub>:0 (50.7 %) and C<sub>16</sub>:1<sub>v</sub>7c alcohol (10.4 %).

This fatty acid profile was similar to those of the type strains of species of the genus *Lysinibacillus*, although there were differences in the proportions of some fatty acids (Table 2). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine.

The DNA G+C content of strain XDB9<sup>T</sup> was 37.2 mol%. Mean DNA–DNA relatedness values between strain XDB9<sup>T</sup> and the type strains of four *Lysinibacillus* species were in the range of 11–27 %: *L. parviboronicapiens* KCTC 13154<sup>T</sup> (11 %), *L. sphaericus* KCTC 3346<sup>T</sup> (16 %), *L. fusiformis* KACC 10903<sup>T</sup> (21 %) and *L. boronitolerans* JCM 21713<sup>T</sup> (27 %). These values indicate that strain XDB9<sup>T</sup> can be assigned to a distinct genomic species (Wayne et al., 1987).

Strain XDB9<sup>T</sup> is distinguishable from other species of the genus *Lysinibacillus* on the basis of several phenotypic differences, including boron tolerance and hydrolysis and utilization of several substrates (Table 1). Strain XDB9<sup>T</sup> is similar to other species of the genus *Lysinibacillus* in that the predominant fatty acid is iso-C<sub>15</sub>:0, but it is distinguishable from them in that a significant amount of iso-C<sub>11</sub>:0 is also present (Table 2). Therefore, on the basis of differential phenotypic properties and phylogenetic and genetic distinctiveness, strain XDB9<sup>T</sup> is sufficiently different from other *Lysinibacillus* species to be classified as a member of a novel taxon, for which the name *Lysinibacillus xylanilyticus* sp. nov. is proposed.

**Description of *Lysinibacillus xylanilyticus* sp. nov.**

lutikos) able to loosen, able to dissolve; N.L. masc. adj. *xylanilyticus* xylan-dissolving).

Cells are Gram-positive, motile and endospore-forming rods (0.8–1.0 × 3.0–5.0 μm). Colonies on TSA are dark yellow, opaque and circular with entire margins. Growth occurs at 10 and 40 °C (optimum 30 °C), but not at 4 or 45 °C, at pH 5.0 and 9.0, but not at pH 4.5 or pH 9.5 and with 0–5 % (w/v) NaCl. Casein is hydrolysed, but starch and Tween 20, 40, 60 and 80 are not. Susceptible to boron and does not grow with 50, 100 or 150 mM boron. In assays with the API ZYM system, alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, α- and β-glucosidases, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. The predominant menaquinone is MK-7. The major fatty acids (>10 % of total fatty acids) are iso-C₁₅:₀ and C₁₆:₀7c alcohol. The cell-wall peptidoglycan type is A4α. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidyethanolamine. Other phenotypic properties are shown in Table 1.

The type strain, XDB9^T^ (=KCTC 13423^T^=CCUG 57438^T^), was isolated from forest humus of Gyeryong Mountain, Taejon, Korea. The DNA G+C content of the type strain is 37.2 mol%.

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References


