**Paenibacillus daejeonensis** sp. nov., a novel alkaliphilic bacterium from soil

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The taxonomy of soil isolates AP-20⁷ and AP-37 was examined. These alkaliphilic organisms grew over a wide pH range (pH 7.0–13.0). The growth rate was higher at pH 8.0–12.0 than at pH 7.0. The G+C composition of these strains averaged 53 mol%. These strains contained MK-7 as the main respiratory quinone and meso-diaminopimelic acid in the cell-wall peptidoglycan. The major cellular fatty acid of the isolates was 12-methyltetradecanoic acid. Levels of 16S rDNA similarity between strain AP-20⁷, AP-37 and other *Paenibacillus* species were 94–90.2%. Phylogenetic analysis based on 16S rDNA sequence revealed that strains AP-20⁷ and AP-37 formed an evolutionary lineage distinct from other *Paenibacillus* species. Based on the evaluation of morphological, physiological and chemotaxonomic characteristics, and on 16S rDNA sequence comparison, the new species *Paenibacillus daejeonensis* sp. nov. is proposed; the type strain is AP-20⁷ (= KCTC 3745T = JCM 11236T).

Keywords: *Paenibacillus daejeonensis*, alkaliphilic, taxonomy

Aerobic, rod-shaped, endospore-forming bacteria have generally been classified in the genus *Bacillus*. However, this genus was shown to be a systematically diverse taxon, because of the lack of distinct differentiating criteria (Claus & Berkeley, 1986). 16S rDNA sequence analyses suggested that the genus *Bacillus* consisted of at least 10 phylogenetic groups (Ash et al., 1991, 1993; Farrow et al., 1992; Nielsen et al., 1994; Rainey et al., 1994; Shida et al., 1996; Spring et al., 1996; Suzuki & Yamasato, 1994; Wisotzkey et al., 1992). Ash et al. (1993) proposed the reclassification of ‘group 3’ as the genus *Paenibacillus*. The genus *Paenibacillus*, first proposed by Ash et al. (1993) and emended by Shida et al. (1997), consists of 25 species and one subspecies at the time of writing.

Members produce ellipsoidal spores in swollen sporangia, are facultatively anaerobic or strictly aerobic rod-shaped, and have G+C contents ranging from 45 to 54 mol% (Ash et al., 1993). Some of these organisms excrete diverse assortments of polysaccharide-hydrolysing enzymes (Kanzawa et al., 1995; Nakamura, 1987; Priest et al., 1988) and produce antibacterial compounds such as polymyxin, octopytin and baciphelacin (Slepecky & Hemphill, 1991) and an antifungal compound (Chung et al., 2000).

In this study, we describe the morphological, biochemical and phylogenetic characteristics of an alkaliphilic bacterium isolated from soil. Although isolates produce protease activity, this is not described here. We propose that the isolates be assigned to the new species *Paenibacillus daejeonensis* sp. nov.

Strains AP-20⁷ and AP-37 were isolated from soil in Daejeon, Korea. They were cultured on trypticase soy agar plates adjusted to pH 10.0 with 1% Na₂CO₃ and incubated for 48 h at 30 °C.

Cell morphology was examined by light microscopy. Motility was determined with an optical microscope using the hanging drop technique (Skerman, 1967). Flagellation type was examined by transmission electron microscopy using cells from the exponential phase of growth. The cells were negatively stained with 1% (w/v) phosphotungstic acid, and after air-drying the grids were examined with a model CM-20 transmission electron microscope. Spores fixed with glutaraldehyde/osmium were sectioned, stained and observed with a transmission electron microscope (model CM-20; Philips). API (bioMérieux) 20NE, API 20E and API

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The GenBank accession numbers for the 16S rDNA sequences of AP-20⁷ and AP-37 are AF290916 and AY032949, respectively.
50CHB were used for physiological and biochemical characterization. All API tests were performed in accordance with the manufacturer’s directions. Catalase activity was determined by bubble production from 3% (v/v) H_{2}O_{2} and oxidase activity was tested using 1% (w/v) tetramethyl-p-phenylenediamine. The GP Biolog microplate containing 95 different carbon compounds was used to test for oxidation of substrates.

Cells were Gram-variable, rod-shaped and motile with peritrichous flagella (Fig. 1a). They produced ellipsoidal spores in swollen sporangia (Fig. 1b, c). Strains AP-20\textsuperscript{T} and AP-37 formed circular, flat, smooth, opaque and white colonies. The isolates tested positive for aesculin hydrolysis, \( \beta \)-galactosidase, oxidase, catalase and acid production from some carbohydrates. In Biolog microplates, the organisms oxidized \( \alpha \)-cyclohexadexrin, \( \beta \)-cyclodextrin, dextrin, glycogen, amygdalin, gentiobiose, \( \beta \)-d-glucose, lactulose, maltotriose, d-mannitol, d-melibiose, methyl \( \beta \)-d-galactosidase, d-raffinose, d-ribose, stachyose and glycerol. They did not oxidize Tween 40, \( N \)-acetyl-\( D \)-glucosamine, \( L \)-arabinose, \( D \)-fructose, \( \alpha \)-d-glucose, \( D \)-sorbitol, \( \alpha \)-ketoglutaric acid, \( L \)-lactic acid, \( D \)-malic acid, \( L \)-malic acid, propionic acid, pyruvic acid, succinic acid or \( L \)-glutamic acid. Weak reactions were observed with cellubiose, d-galactose, methyl \( \alpha \)-d-galactoside, d-xylene and acetic acid.

The growth experiment was performed using screw-cap tubes containing 10 ml trypticase soy broth adjusted to pH values ranging from 6.0 to 13.0. Growth was estimated by monitoring the OD
\_650. Trypticase soy broth containing 100 mM Na\textsubscript{2}HPO\textsubscript{4}/NaH\textsubscript{2}PO\textsubscript{4} buffer at pH 6.0–8.0, 100 mM NaHCO\textsubscript{3}/Na\textsubscript{2}CO\textsubscript{3} buffer at pH 9.0–10.0, 50 mM Na\textsubscript{2}HPO\textsubscript{4}/100 mM NaOH buffer at pH 11.0 and 100 mM KCl/NaOH buffer.

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**Table 1. Cellular fatty acid profiles (%) of strains AP-20\textsuperscript{T} and AP-37**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>AP-20\textsuperscript{T}</th>
<th>AP-37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_13:0</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C_14:0</td>
<td>2.8</td>
<td>3.9</td>
</tr>
<tr>
<td>C_15:0</td>
<td>15.9</td>
<td>13.7</td>
</tr>
<tr>
<td>C_16:0</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>C_17:0</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>C_18:0</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>Unsaturated fatty acids:*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_16:1 ( \omega )7c alcohol</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C_16:1 ( \omega )11c</td>
<td>2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Branched fatty acids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_13:0 anteiso</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C_14:0 iso</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>C_15:0 iso</td>
<td>7.2</td>
<td>7.6</td>
</tr>
<tr>
<td>C_15:0 anteiso</td>
<td>36.4</td>
<td>37.9</td>
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<td>9.7</td>
</tr>
<tr>
<td>C_17:0 iso</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>C_17:0 anteiso</td>
<td>2.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

TR, trace (< 0.5%).

*The position of the double bond can be located by counting from the methyl (\( \beta \)) end of the carbon chain. A cis isomer is indicated by the suffix c.

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**Fig. 1.** Electron micrographs of strain AP-20\textsuperscript{T}. (a) Transmission electron micrograph showing peritrichous flagella. (b) Transmission electron micrograph of an ultrathin section showing an endospore in a swollen sporangium. (c) Scanning electron micrograph showing vegetative cells, swollen cells and spores. Bar, 1 \( \mu m \).
buffer at pH 12.0–13.0 was used. This experiment was done on the basis of the methods of Yumoto et al. (1998). Strains AP-20 and AP-37 did not grow at pH 6–0, but grew at pH 7–13. Growth was more rapid at pH 8–12 than at neutral pH. Although generally good at pH 8–12, the growth rate at pH 8 was a little higher than at pH 9–12. Yoon et al. (1998) reported the alkaliphilic property of Paenibacillus campinasensis. Likewise, strains AP-20 and AP-37 grew over a wide range of alkaline pH. This was an important character for distinguishing strains AP-20 and AP-37 from most of the other Paenibacillus species and indicated that these isolates were alkaliphilic organisms (Yumoto et al., 1998).

For total cellular fatty acid analysis, cells were cultured on trypticase soy agar for 48 h at 30 °C. Fatty acids were extracted by following the Microbial Identification System instructions as previously described (Lee et al., 1996; Yang et al., 1993). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), and purified by TLC on Merck Kieselgel 60 F254 plates (20 x 20 cm, 0.5 mm thickness) using petroleum ether:diethyl ether (9:1, v/v) as the solvent. The identity of the quinone was determined by HPLC analysis as described by Shin et al. (1996).

The whole-cell fatty acid profiles in the strains are shown in Table 1. The major fatty acid of the isolates was 12-methyltetradecanoic acid. MK-7 was the major menaquinone, and meso-diaminopimelic acid was present in the cell-wall peptidoglycan.

DNA was extracted and purified by a modification of the method of Marmur (1961). The DNA G+C content of the strains, determined by the reversed-phase HPLC method described by Tamaoka & Komagata (1984), was 53 mol%.

The nearly complete 16S rDNA sequence of AP-20 and the partial 16S rDNA sequence of AP-37 were determined by the method of Chun & Goodfellow (1995). The 16S rDNA sequences of these strains were aligned with the 16S rRNA and rDNA sequences of representatives of the genus Paenibacillus and related taxa by using clustal w software (Thompson et al., 1994). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) on the basis of distance matrix data. Evolutionary distances were calculated by using the Jukes & Cantor (1969) model. The phylogenetic package (Felsenstein, 1993) was used for all analyses. The topology of the phylogenetic tree was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 replications.

The primary structures of the 16S rDNA sequence of these strains were compared with those of closely related reference strains. A phylogenetic tree based on K\textsubscript{muc} value (Fig. 2) indicates that these strains belongs to the genus Paenibacillus. The GenBank accession numbers for the 16S rDNA sequences used for the phylogenetic analysis are shown in Fig. 2.

The partial 16S rDNA sequence of strain AP-37 was almost identical to that of strain AP-20 (99.9%). They also had identical morphological, physiological and chemotaxonomical characteristics. Therefore these isolates were considered to be the same species. The GenBank accession number for strain AP-37 is
AY032949. Isolates AP-20T and AP-37 were phylogenetically closely related to Paenibacillus curdulanolyticus IFO 15724T and Paenibacillus kobensis IFO 15729T. The levels of sequence similarity between these strains and other Paenibacillus species ranged from 94.2 to 90.2%. The most closely related species to AP-20T and AP-37 was P. illinoisensis NRRL NRS-1356 (94.2%), but they formed a distinct cluster (Fig. 2). Discrepancies between similarity values and phylogenetic relationships commonly occur when evolution rates are different between lineages. Species with high similarity values with strain AP-20T have short branch lengths in the phylogenetic tree, indicating that they have low evolution rates, and P. curdulanolyticus IFO 15724T and P. kobensis IFO 15729T have long branch lengths, indicating that they have high evolution rates.

Results of a similarity search with Paenibacillus alvei IFO 3343T, Paenibacillus apiarius NRRL NRS-1438T, Paenibacillus thiaminolyticus JCM 8360T, Paenibacillus popilliae ATCC 14706T and Paenibacillus lentimorbus ATCC 14707T as query sequences also supported the arguments by showing that similarity values with P. illinoisensis NRRL NRS-1356T were higher than with P. curdulanolyticus IFO 15724T and P. kobensis IFO 15729T.

The phylogenetic definition of a species generally includes `strains with approximately 70% or greater DNA–DNA relatedness' (Wayne et al., 1987). According to the available compilation of data, organisms that have less than 97% sequence similarity will not reassort to more than 60%, irrespective of the hybridization method applied (Stackebrandt & Goebel, 1994). This phylogenetic result demonstrated that the isolates were not related to any of the previously described Paenibacillus taxa at the species level.

On the basis of morphological, physiological and chemotaxonomic characteristics and 16S rDNA sequence comparison data, we propose a new species of the genus Paenibacillus, Paenibacillus daejeonensis.

Description of Paenibacillus daejeonensis sp. nov.

Paenibacillus daejeonensis (dae.jeon.en'sis. N.L. masc. adj. daejeonensis referring to Daejeon, Korea, the geographical origin of the new species).

Gram-variable, rod-shaped organism. Cells are motile with peritrichous flagella. Ellipsoidal spores formed in swollen sporangia. Colonies are circular, flat, smooth, opaque and white on trypticase soy agar. Aesculin hydrolysis, β-glucosidase, oxidase and catalase are positive. Gelatin is not liquefied, and glucose is not fermented. Reduction of nitrate, urease and H2S production are negative. Acid is produced from mannitol, amygdalin, cellobiose, lactose, melibiose, d-raffinose, starch and glycogen. The organism oxidizes α-cyclohexcin, β-cyclohexdin, dextrin, glycogen, amygdalin, gentiobiose, α-d-lactose, lactulose, maltotriose, d-mannitol, d-melibiose, methyl β-d-galactoside, d-raffinose, d-ribose, stachyose and glycerol. Alkaliphilic. Cells do not grow at pH 6.0, but grow at pH 7.0–13.0; optimum pH is 8.0. The G+C content of the DNA is 53 mol%. The major isoprenoid quinone is menaquione MK-7. The major cellular fatty acid is 12-methyltetradecanoic acid. Cell-wall peptidoglycan contains meso-diaminopimelic acid. Source: isolated from soil from Daejeon City, Korea. The type strain is AP-20T (= KCTC 3745T = JCM 11236), and the reference strain is AP-37 (= KCTC 3750 = JCM 11237).

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References


