Paenibacillus pectinilyticus sp. nov., isolated from the gut of Diestrammena apicalis

Doo-Sang Park, Won-Jin Jeong, Kang Hyun Lee, Hyun-Woo Oh, Byung-Chun Kim, Kyung Sook Bae and Ho-Yong Park

The genus *Paenibacillus*, proposed in 1993 by Ash et al. (1993) to accommodate the rRNA group 3 bacilli, comprises many recently described species that were isolated from a wide variety of sources, including antarctic sediments (Montes et al., 2004), warm springs (Saha et al., 2005), rice fields (Sánchez et al., 2005), garden peas (Šmerda et al., 2005), air (Rivas et al., 2005a), plant rhizospheres (Rivas et al., 2005b) and alkaline soils (Yoon et al., 2005). Some strains of this genus have a distinct ability to hydrolyse hydrocarbons or complex carbohydrates, including alginate (Nakamura, 1987), cellulose (Rivas et al., 2006), chondroitin (Nakamura, 1987), curdlan (Kanzawa et al., 1995), extracellular polysaccharides (Takeda et al., 2005), naphthalene (Daane et al., 2002) and xylan (Lee et al., 2000; Velázquez et al., 2004; Rivas et al., 2005a).

Insects and their gut bacteria often have a symbiotic relationship (Breznak, 1982; Chen & Purcell, 1997), and high bacterial diversity was observed when the bacterial communities of the gut compartments of the gypsy moth and European cockchafer were evaluated (Broderick et al., 2004; Egert et al., 2005). It is believed that the digestion of wood constituents such as cellulose, xylan, lignin and pectin is catalysed by digestive enzymes produced by symbiotic micro-organisms harbouring in the guts of insects (Brune & Friedrich, 2000; Suh et al., 2003), and a large number of exo-enzyme-producing bacteria were reported recently in an analysis of bacteria isolated from the guts of beetle species (Park et al., 2007).

In a study of bacteria capable of producing enzymes that degrade polymer, a number of novel bacterial strains were isolated from the gut of an insect, *Diestrammena apicalis*, collected near the Daejeon district, Republic of Korea. One isolate, designated strain RCB-08\(^\mathrm{T}\), hydrolysed pectin and was considered to be *Paenibacillus*-like based on its 16S rRNA gene sequence. Therefore, this study was conducted to elucidate the taxonomic position of strain RCB-08\(^\mathrm{T}\) by means of phenotypic and genetic analyses.

Strain RCB-08\(^\mathrm{T}\) was selected by plating serial dilutions onto a pectinase activity test medium, R2A (Difco) agar that contained 0.3 % citric pectin. The samples were incubated at 30 °C for 3 days. The isolate was then cultivated aerobically in tryptic soy broth (TSB) or on TSB agar (Difco) for 2 days at 30 °C and the physiological characteristics were determined. Gram staining was performed using a Difco Gram stain set. Spore formation and shape were determined by using microscopic observation after staining with malachite green (Sigma).

During a search for exo-enzyme-producing bacteria in the gut of an insect, *Diestrammena apicalis*, a novel bacterium capable of degrading pectin was isolated. The isolate, designated strain RCB-08\(^\mathrm{T}\), comprised Gram-positive, endospore-forming, motile rods capable of growth at 15–30 °C and pH 6.0–8.7. The DNA G+C content of the isolate was 51.5 mol% and the predominant cellular fatty acid was anteiso-C\(_{15:0}\) (74.1 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain RCB-08\(^\mathrm{T}\) was affiliated with a cluster within the *Paenibacillaceae*, and was related most closely to *Paenibacillus chondroitinus* NBRC 15376\(^\mathrm{T}\), with a sequence similarity of 96.7 %. The DNA–DNA relatedness value for strain RCB-08\(^\mathrm{T}\) with *P. chondroitinus* NBRC 15376\(^\mathrm{T}\) was 15.0 %. Strain RCB-08\(^\mathrm{T}\) hydrolysed pectin, but not cellulose, casein, starch or xylan. Strain RCB-08\(^\mathrm{T}\) could be clearly distinguished from other *Paenibacillus* species on the basis of characteristics observed using a polyphasic approach. Therefore strain RCB-08\(^\mathrm{T}\) is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus pectinilyticus* sp. nov. is proposed. The type strain is RCB-08\(^\mathrm{T}\) (=KCTC 13222\(^\mathrm{T}\)=CECT 7358\(^\mathrm{T}\)).
Anaerobic growth was tested by culturing the isolate on a TSB plate supplemented with nitrate in a sealed container that contained a BBL GasPak Pouch (Becton Dickinson). Xylanase activity was determined by using Congo red staining by using the following procedure (Skipper et al., 1985). Briefly, cells were grown on an M9 minimal agar plate (Difco) that contained 0.5 % yeast extract and 0.5 % birchwood xylan (Sigma), and then washed with water. Next, the plate was stained with Congo red (2 mg ml⁻¹) for a few minutes, and then rinsed with 1 M NaCl. The unstained areas on the agar plate were assumed to indicate the hydrolysis of xylan. API 20NE test strips (bioMérieux) and an API 50 CH kit (bioMérieux) were used to analyse the biochemical and physiological traits, as well as the sugar fermentation patterns of the bacterial strain over a period of 48 h.

The morphology and size of cells was examined using phase-contrast microscopy with a Nikon Optiphot-2 light microscope at a magnification of 1500. In addition, cells that were negatively stained with 1 % (w/v) uranyl acetate were used for electron microscopy analysis, with thin sections of cells being prepared as described by Paster & Canale-Parola (1982) and then examined using a model H-7600 transmission electron microscope (Hitachi).

The presence of oxidase activity was determined using an Oxy-swab (bioMérieux), and catalase activity was detected by placing drops of 3 % (v/v) H₂O₂ on cultures growing on Oxy-swab (bioMérieux), and catalase activity was detected. The presence of oxidase activity was determined using an API 20NE test strips (bioMérieux) and an API 50 CH kit (bioMérieux) were used to analyse the biochemical and physiological traits, as well as the sugar fermentation patterns of the bacterial strain over a period of 48 h. Cells of strain RCB-08T that had been cultured on TSB agar for 2 days were used to analyse the cellular fatty acid composition. Saponification, methylation and extraction were performed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The fatty acids were then analysed using a gas chromatograph (model 6890N and autosampler 7683; Agilent) and identified using the Microbial Identification Sherlock software package.

The DNA G+C content was determined using the method described by Tamaoka & Komagata (1984). Briefly, chromosomal DNA was extracted and purified according to the method described by Sambrook & Russell (2001), and then treated with nuclease P1 and alkaline phosphatase. The resultant nucleotides were then analysed by HPLC using a reversed-phase column (Supelcosil LC-18 S; Supelco).

The 16S rRNA gene of the isolate was amplified by PCR using the universal primers 27F and 1492R, as described by Yoon et al. (1998). The 16S rRNA gene sequences of the isolates were compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/) and the Ribosomal Database Project (Maidak et al., 2001). The 16S rRNA gene sequence of strain RCB-08T was aligned with the 16S rRNA gene sequences of representatives of the genus Paenibacillus and related taxa, using CLUSTAL_X software (Thompson et al., 1997). Phylogenetic trees were constructed with MEGA (Molecular Evolutionary Genetics Analysis; Kumar et al., 2004) using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods based on distance-matrix data. The topology of the neighbour-joining phylogenetic tree was evaluated with bootstrap analysis (Felsenstein, 1985), based on 1000 replications.

DNA–DNA hybridization was performed based on the method described by Kusuda et al. (1991) and Willcox (1996). DNA was transferred to a nylon membrane (Hybond-N+; Amersham). The membrane was incubated for 1 h at 45 °C for pre-hybridization and then for 12 h at 45 °C for hybridization. A DIG High Prime DNA Labelling and Detection starter kit II (Roche Molecular Biochemicals) was used for the detection of DNA. After washing, the membrane was exposed to autoradiography film (Hyperfilm-ECL; Amersham) for 10 min and signal intensities were determined using the TINA 2.0 program (Lee et al., 2003). The signal produced by self-hybridization was taken as 100 %, and relative intensities of genomic DNAs of other strains were determined to be the DNA–DNA percentage relatedness.

Single cells of strain RCB-08T were observed to be rods that measured 2.0–3.0 μm in length and 0.5–0.7 μm in diameter (Supplementary Fig. S1, available in IJSEM Online). The strain was capable of growth on TSB medium containing 0–0.5 % (w/v) NaCl; however, the strain did not grow on the same medium that contained ≥1 % (w/v) NaCl.
NaCl. Physiological properties of strain RCB-08<sup>T</sup> and some other related type strains are shown in Table 1.

Strain RCB-08<sup>T</sup> did not contain diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone was unsaturated menaquinone with seven isoprene units (MK-7). The polar lipids of strain RCB-08<sup>T</sup> were diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and an unidentified polar lipid. The polar lipid pattern of strain RCB-08<sup>T</sup> was similar to that of *Paenibacillus polymyxa* DSM 36<sup>T</sup>, reported by Kämpfer et al. (2006); however, phosphatidylmonomethylethanolamine was present in strain RCB-08<sup>T</sup> as a major compound. The polar lipid content of strain RCB-08<sup>T</sup> after separation by two-dimensional TLC is shown in Fig. 1. The principal cellular fatty acids of cells of strain RCB-08<sup>T</sup> grown on TSB agar were anteiso-C<sub>15 : 0</sub> (74.1 %) followed by iso C<sub>16 : 0</sub> (9.1 %). Similar to other *Paenibacillus* species, the predominant cellular fatty acid of the isolate was anteiso-C<sub>15 : 0</sub>. The cellular fatty acid profile of strain RCB-08<sup>T</sup> is shown in Table 2, together with those of *P. chondroitinus* NBRC 15376<sup>T</sup>, *Paenibacillus alginolyticus* CIP 103122<sup>T</sup> and *P. larvae*.

A complete sequence (1402 bp) of the 16S rRNA gene of strain RCB-08<sup>T</sup> was determined. Analysis of the sequence showed that the highest similarity was with that of *P. chondroitinus* NBRC 15376<sup>T</sup> (96.7 %), with the next highest similarities being with *P. alginolyticus* CIP 103122<sup>T</sup> (95.8 %) and *Paenibacillus elgii* SD17<sup>T</sup> (93.5 %). The relationship between strain RCB-08<sup>T</sup> and representatives of the genus *Paenibacillus* is shown in a phylogenetic tree constructed with the neighbour-joining method (Fig. 2). A phylogenetic tree constructed using the maximum-likelihood method also gave a similar topology (Supplementary Fig. S2, in IJSEM Online). Strain RCB-08<sup>T</sup> was clearly discriminated from the type species of the genus *Paenibacillus*. The DNA–DNA hybridization value between strain RCB-08<sup>T</sup> and the reference strain *P. chondroitinus* NBRC 15376<sup>T</sup> was 15.0 %. Organisms that have less than 97.0 % 16S rRNA gene sequence similarity

### Table 1. Physiological properties of strain RCB-08<sup>T</sup> and some related *Paenibacillus* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Taxa 1</th>
<th>Taxa 2</th>
<th>Taxa 3</th>
<th>Taxa 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth at pH 5.7</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Agar</td>
<td>−</td>
<td>−</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tween 80</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>51.5</td>
<td>47–49</td>
<td>47–48</td>
<td>42.3</td>
</tr>
</tbody>
</table>

### Table 2. Whole-cell fatty acid compositions of strain RCB-08<sup>T</sup> and some related *Paenibacillus* species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14 : 0&lt;/sub&gt;</td>
<td>1.2</td>
<td>0.9</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>0.5</td>
<td>2.0</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>2.4</td>
<td>6.4</td>
<td>2.6</td>
<td>9.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.9</td>
</tr>
<tr>
<td>Branched fatty acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;13 : 0&lt;/sub&gt;</td>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;14 : 0&lt;/sub&gt;</td>
<td>4.1</td>
<td>1.7</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>4.0</td>
<td>2.4</td>
<td>4.7</td>
<td>12.2</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>74.1</td>
<td>69.7</td>
<td>69.6</td>
<td>32.6</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>9.1</td>
<td>10.4</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>0.5</td>
<td>0.7</td>
<td>1.7</td>
<td>9.6</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>2.6</td>
<td>3.2</td>
<td>6.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Summed feature 4†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Mean values of three *P. larvae* strains.
†Summed feature 4 contains one or more of the following fatty acids: iso-C<sub>15 : 0</sub> 2-OH and/or C<sub>16 : 0</sub> 2.07.
will not reassociate to more than 60% DNA–DNA relatedness, according to the available compiled data (Stackebrandt & Goebel, 1994). Strains with approximately 70% or greater DNA–DNA relatedness were considered as representing the same species (Wayne et al., 1987). The results of the phylogenetic and DNA–DNA hybridization analyses demonstrated that strain RCB-08\(^T\) was not related to any of the recognized species of the genus *Paenibacillus*.

On the basis of the data presented above, we propose a novel species of the genus *Paenibacillus*, *Paenibacillus pectinilyticus* sp. nov., to accommodate strain RCB-08\(^T\).

**Description of *Paenibacillus pectinilyticus* sp. nov.**

*Paenibacillus pectinilyticus* [pec.ti’ni.ly.ti’cus. N.L. n. pecti-num (from French n. pectine), pectin; N.L. adj. lyticus (from Gr. adj. lutikos), dissolving; N.L. masc. adj. pectinilyticus pectin-dissolving bacteria].

Cells are Gram-positive, catalase-positive, oxidase-positive, motile rods and have a terminal ellipsoidal spore in a swollen sporangium. Colonies on TSB agar are round, smooth, cream-white and approximately 1–2 mm in diameter. Single cells are slightly curved rods, measuring 2.0–3.0 × 0.5–0.7 μm. Growth occurs at temperatures between 15 and 30 °C in TSB medium, with optimum growth at 30 °C. Grows in the presence of 0–0.5% NaCl and at pH 6.0–8.7. Good growth is observed on TSB agar when incubated under aerobic or anaerobic conditions. Gelatin, pectin and Tween 80 are hydrolysed, but casein, cellulose, starch or xylan are not. Positive reactions are obtained for acetoin production, citrate utilization, β-galactosidase, lysine decarboxylase, ornithine decarboxylase and nitrate reduction. Negative reactions are obtained for arginine dihydrolase, H\(_2\)S production, indole production, phenylalanine deaminase, tryptophan deaminase and urease. Acid is produced from L-arabinose, arbutin, fructose, ascesulin, galactose, glycerol, glucose, inositol, maltose, melibiose, melezitose, mannose, mannotol, raffinose, ribose, salicin, sorbitol, sucrose, trehalose and turanose, but not from adonitol, D-arabinose, D-arabitol, D-arabitol, D-cellobiose, Dulcitol, erythritol, D-fucose, D-fucose, glycogen, inulin, lactose, D-lyxose, methyl α-D-mannose, methyl β-D-glucose, rhamnose, sorbose, methyl β-D-xylose, D-tagatose, xylitol or L-xylose. MK-7 is the predominant isoprenoid quinone. Does not contain diaminopimelic acid in the cell-wall peptidoglycan. Cells cultured in TSB medium contain anteiso-C\(_{15}:0\) as the predominant cellular fatty acid. Major polar lipids are diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the DNA of the type strain is 51.5 mol%.

The type strain, RCB-08\(^T\) (=KCTC 13222\(^T\)=CECT 7358\(^T\)), was isolated from the gut of an insect, *Diestrammena apicalis*, collected in the Daejeon district, Republic of Korea.
Acknowledgements

We thank Dr J. P. Euzéby for his valuable advice on nomenclature when naming strain RCB-08. This work was supported by a grant from the Korea Research Institute of Bioscience and Biotechnology and the Research Initiative Program (KGS2330911) and the 2IC Frontier Microbial Genomics and Applications Center Program (MGC0900938) of the Korean Ministry of Science and Technology.

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