Paenibacillus barcinonensis sp. nov., a xylanase-producing bacterium isolated from a rice field in the Ebro River delta

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A Gram-positive, endospore-forming, xylanase-producing bacterium isolated from a rice field was studied taxonomically. The strain grows at 10–40 °C and in the presence of lysozyme or 5 % (w/v) NaCl. Chemotaxonomic analysis revealed that MK-7 was the predominant menaquinone of the isolated strain, while the major fatty acid was anteiso-C_{15:0}. Comparison of 16S rRNA gene sequences showed that strain BP-23^T fell within the radiation of the cluster comprising *Paenibacillus* species. The highest 16S rRNA gene sequence similarities were found with *Paenibacillus illinoisensis* (97.4 %), *Paenibacillus pabuli* (97.1 %) and *Paenibacillus amylolyticus* (96.9 %). The DNA–DNA relatedness of strain BP-23^T with respect to these three species was very low (32.7, 31.6 and 23.0 %, respectively). On the basis of phenotypic and genotypic data, strain BP-23^T should be placed in the genus *Paenibacillus* and designated a novel species, for which the name *Paenibacillus barcinonensis* sp. nov. is proposed. The type strain is BP-23^T (=CECT 7022^T = DSM 15478^T).

Members of the genus *Bacillus* are common saprophytic components of soil microbiota (Claus & Berkeley, 1986). Some species are known to secrete a variety of extracellular enzymes, several of which have important industrial applications (Outtrup & Jørgensen, 2002). Genomic analysis of the genus *Bacillus*, using rRNA–DNA sequencing (Ash *et al.*, 1991), has led to its division into distinct genera. One of these is the genus *Paenibacillus* (Ash *et al.*, 1993), which comprises rRNA group 3 and includes several long-established species, such as *Paenibacillus polymyxa*, *Paenibacillus alvei* and *Paenibacillus macerans*, and an increasing number of newly identified species isolated from soil and lignocellulosic materials (Berge *et al.*, 2002). In the present study, the taxonomic status of strain BP-23^T, previously isolated from soil after enrichment culture on rice straw (Blanco & Pastor, 1993) and selected as a xylanase-producer (Blanco *et al.*, 1995), was analysed.

Strain BP-23^T was isolated from soil from a rice field in the delta of the River Ebro (Spain) after enrichment culture at room temperature on rice straw for 2 months (Blanco & Pastor, 1993). A sample was then suspended in water, incubated at 70 °C for 10 min, then poured onto nutrient agar (Scharlau) plates. Several colonies appeared after 2 days incubation; these were tested for xylanase activity on nutrient agar plates containing 0.4 % (w/v) xylan. Of the colonies that formed clear haloes of xylan degradation, strain BP-23^T was selected for further studies (Blanco & Pastor, 1993). The following reference strains were used in the study: *Paenibacillus illinoisensis* DSM 11733^T, *Paenibacillus pabuli* DSM 3036^T and *Paenibacillus amylolyticus* DSM 15211^T. All strains were grown on nutrient broth (Difco) to which 15 g agar l⁻¹ (Difco) had been added if needed.

Cell morphology and the shape of the spores were examined by phase-contrast microscopy (BX40 microscope; Olympus) using cells grown for 2 days at 30 °C on nutrient agar containing 0.1 % (w/v) MnSO₄. The sizes of cells and swollen sporangia were analysed on a Hitachi H600 AB transmission electron microscope at an operating voltage of 100 kV, using cells stained with 2 % (v/v) glutaraldehyde and 1 % (w/v) OsO₄ and 0.8 % (w/v) FeCNK in 0.1 M

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BP-23^T is AJ716019.

Micrographs of sporulating cells of strain BP-23^T and an extended version of the neighbour-joining tree shown in Fig. 1 are available as supplementary material in IJSEM Online.

phosphate buffer. The biochemical properties of strains grown on nutrient agar were determined as described previously (Gordon *et al.*, 1973; Claus & Berkeley, 1986) and by using API 50 CH tests (bioMérieux). Respiratory quinones and polar lipids were analysed as described by Tindall (1990). The cellular fatty acid content was analysed by using the method described by Kucheryava *et al.* (1999).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out as described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using the CEQ DTCS Quick Start kit (Beckman Coulter) according to the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQ 8000 genetic analysis system (Beckman Coulter). The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequence determined in this study against those of representatives of the main bacterial lineages available from the public databases. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969). A dendrogram was reconstructed from a distance matrix by using the treeing algorithm of De Soete (1983) and Felsenstein (1993).

Fully automated ribotyping of *EcoRI*-digested samples was performed with a RiboPrinter system (DuPont Qualicon) as described by Bruce (1996). Images of hybridization were analysed according to Barney *et al.* (2001). DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977).

DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992).

The G+C content of the DNA was determined according to Mesbah *et al.* (1989) and Tamaoka & Komagata (1984). DNA was enzymically hydrolysed and dephosphorylated, and the resultant nucleosides were analysed by HPLC.

In a previous study, strain BP-23^T had been preliminarily assigned to the genus *Bacillus* (Blanco & Pastor, 1993). The strain was selected as a high-level producer of xylanase in media supplemented with rice straw, demonstrating the presence of a complex enzyme system for xylan degradation (Blanco *et al.*, 1995, 1999; Gallardo *et al.*, 2003). Colonies of strain BP-23^T were circular to slightly irregular, pale yellow in colour and 0.5 mm in diameter after growth for 48 h at 30 °C and pH 7.0 in nutrient broth. The cells of strain BP-23^T were rod-shaped, measuring 0.5–1 × 1.5–4.5 µm, and produced ellipsoidal endospores in swollen sporangia at a subterminal position (see the supplementary figure available in IJSEM Online). The phenotypic properties of strain BP-23^T are summarized in Table 1.

Table 1. Phenotypic properties of strain BP-23^T and closely related *Paenibacillus* species

Strains: 1, *P. barcinonensis* BP-23^T; 2, *P. pabuli* DSM 3036^T; 3, *P. illinoisensis* DSM 11733^T; 4, *P. amylolyticus* DSM 15211^T. All of the strains are Gram-positive, catalase-positive, grow in the presence of 2% (w/v) NaCl, hydrolyse gelatin and produce acid from glucose, D-xylose, L-arabinose and mannitol. None of the strains produces acetylmethylcarbinol, utilizes citrate or produces acid from sorbitol. Symbols: +, positive; –, negative; ND, not determined.

Test	1	2	3	4*
Anaerobic growth	+	+	–	+
Oxidase	–	+	+	–
pH in Voges–Proskauer broth	4.7	4.7	4.7	5.2
Maximum growth temp.	40	40	45†	40
Minimum growth temp.	10	ND	ND	10
Growth in the presence of:				
NaCl at 5% (w/v)	+	+	–	–
Lysozyme at 0.001% (w/v)	+	+	–	–
Nitrate reduction	–	+	–	+
Urease	–	–	–	ND
Hydrolysis of:				
Starch	–	+	+	+
Casein	–	–	+	+
Utilization of propionate	–	ND	–	–
Acid from:				
Rhamnose	–	+	+	–
Lactose	+	+	+	–
D-Raffinose	+	+	+	–

*Data from Shida *et al.* (1997b).

†Weak growth.

Chemotaxonomic characterization showed that the major isoprenoid quinone was MK-7, which is the major menaquinone generally found in aerobic endospore-forming rods. The major polar lipids present were diphosphatidylglycerol, phosphatidylethanolamine and two amino-positive phospholipids that could not be identified. Only traces of phosphatidylglycerol could be detected. The cellular fatty acid profile of strain BP-23^T showed anteiso-C_{15:0} to be the predominant fatty acid (37.24%); this is similar to the situation for members of the genus *Paenibacillus* (Kämpfer, 2002). The other major fatty acids were C_{16:1ω11c} (15.22%), iso-C_{15:0} (11.62%) and C_{16:0} (11.45%).

The almost-complete sequence of the 16S rRNA gene of strain BP-23^T was determined: it comprised 1523 nt ranging from position 32 (5') to 1541 (3') according to *Escherichia coli* numbering (Brosius *et al.*, 1978). Comparison of 1150 unambiguous nucleotides between positions 45 and 1390 showed that the strain fell within the radiation of the genus *Paenibacillus* and represented a distinct lineage within the genus (Fig. 1; see also the extended tree available as a supplementary figure in IJSEM

Fig. 1. Neighbour-joining tree showing similarity of the 16S rRNA gene sequence of strain BP-23^T to those of species of the genus *Paenibacillus*. Sequences of members of the *Bacillus/Staphylococcus* group were used to root the dendrogram. The strain designations and GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the reference strains used are indicated. Bar, 5 nucleotide substitutions per 100 nucleotides.

Online). The highest levels of 16S rRNA gene sequence similarity were found with respect to *P. illinoisensis* (GenBank/EMBL/DDBJ accession no. D85397), *P. pabuli* (X60630) and *P. amylolyticus* (D85396), the values being 97.4, 97.1 and 96.9%, respectively. Ribotyping was performed with strain BP-23^T and species with highly similar 16S rRNA gene sequences (Fig. 2). Strain BP-23^T generated a ribofragment pattern that was clearly different from those of closely related species such as *P. illinoisensis*, *P. pabuli* and *P. amylolyticus*. DNA–DNA hybridization was performed to determine the genomic relatedness between strain BP-23^T and *P. illinoisensis*, *P. pabuli* and *P. amylolyticus*, the species with the highest levels of 16S rRNA gene

sequence similarity to the strain. Strain BP-23^T exhibited reassociation values of 32.7, 31.6 and 23.0%, respectively, with respect to the aforementioned species, indicating that it is not related to them at the species level (Wayne *et al.*, 1987). The DNA G+C content of strain BP-23^T was 45.0 mol%, which lies within the range observed for members of the genus *Paenibacillus* (Shida *et al.*, 1997a).

On the basis of the above results, it was concluded that strain BP-23^T represents a novel species of *Paenibacillus*, for which the name *Paenibacillus barcinonensis* sp. nov. is proposed. Strain BP-23^T currently constitutes the only strain of this novel species and is the type strain.

Description of *Paenibacillus barcinonensis* sp. nov.

Paenibacillus barcinonensis (bar.ci.no.nen'sis. L. masc. adj. *barcinonensis* from *Barcino*, the Roman name for Barcelona, the city in Spain where the strain was isolated).

Gram-positive, facultatively anaerobic rods (0.5–1 × 1.5–4.5 µm). Ellipsoidal endospores form in swollen sporangia at a subterminal position. Colonies are circular to slightly irregular, pale yellow in colour and 0.5 mm in diameter after 2 days growth at 30 °C in nutrient broth. Growth occurs at temperatures in the range 10–40 °C. Growth occurs at pH 5.0–10.4. Growth occurs in the presence of 5% (w/v) NaCl and 0.001% (w/v) lysozyme. Catalase-positive. Oxidase- and urease-negative. Nitrate is not reduced to nitrite or nitrogen. Acetylmethylcarbinol is not produced. The pH in Voges–Proskauer broth is 4.7. Gelatin is hydrolysed. Casein and starch are not hydrolysed. Citrate and propionate are not utilized. Acid is produced from glycerol, D-arabinose, L-arabinose, ribose, D-xylose,

Fig. 2. RiboPrint patterns of strain BP-23^T and three *Paenibacillus* type strains generated by digestion of genomic DNA using the restriction enzyme *EcoRI*. Strains: 1, *P. pabuli* DSM 3036^T; 2, *P. amylolyticus* DSM 15211^T; 3, *P. barcinonensis* BP-23^T; 4, *P. illinoisensis* DSM 11733^T.

methyl β -xyloside, galactose, D-glucose, D-fructose, D-mannose, mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, β -gentiobiose, D-turanose and gluconate. Acid is not produced from erythritol, L-xylose, adonitol, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, inulin, melezitose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate. The major fatty acids are anteiso-C_{15:0}, C_{16:1} ω 11c, iso-C_{15:0} and C_{16:0}. The predominant menaquinone is MK-7. The major polar lipids present are diphosphatidylglycerol, phosphatidylethanolamine and two unidentified amino-phospholipids. The DNA G + C content is 45.0 mol%.

Isolated from soil from a rice field in the Ebro River delta, Spain. The type strain is BP-23^T (=CECT 7022^T = DSM 15478^T).

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