Paenibacillus humicus sp. nov., isolated from poultry litter compost

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Two bacterial strains, PC-142 and PC-147^T, isolated from poultry litter compost, were characterized with respect to their phenetic and phylogenetic characteristics. The isolates were endospore-forming rods that were reddish in colour after Gram staining. They were catalase- and oxidase-positive, were able to degrade starch and gelatin and grew at 15–40 °C and pH 5.5–10.0. The predominant fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$, the major respiratory quinone was menaquinone MK-7, the cell-wall peptidoglycan was of the A1γ type and the G+C content of the DNA was 58 mol%. The 16S rRNA gene sequence analysis and phenetic characterization indicated that these organisms belong to the genus *Paenibacillus*, with *Paenibacillus pasadenensis* SAFN-007^T as the closest phylogenetic neighbour (97.5 %). Strains PC-142, PC-147^T and *P. pasadenensis* SAFN-007^T represent a novel lineage within the genus *Paenibacillus*, characterized by a high DNA G+C content (58–63 mol%). The low levels of 16S rRNA gene sequence similarity with respect to other taxa with validly published names and the identification of distinctive phenetic features in the two isolates indicate that strains PC-142 and PC-147^T represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus humicus* sp. nov. is proposed. The type strain is PC-147^T (=DSM 18784^T =NBRC 102415^T =LMG 23886^T).

In a study designed to characterize culturable heterotrophic bacteria in composts, endospore-forming rods affiliated to the genus *Paenibacillus* were isolated from poultry litter compost. This compost was produced from chicken wastes from aviaries, meat meal, bird bones, vegetable ash and grape husks. The material was stored for 5–6 months until it had composted. The composting process was conducted in piles, under a covered area, for a period of 45 days. Two isolates from the compost had a randomly amplified polymorphic DNA (RAPD) profile that was distinct from those of other bacterial isolates (identified as members of the genera *Staphylococcus*, *Bacillus* and *Pseudomonas*; data not shown) recovered from the same compost. On the basis of 16S rRNA gene sequence analysis, the two isolates, designated strains PC-142 and PC-147^T, were presumed to be members of the genus *Paenibacillus* and were further characterized by using a polyphasic approach.

The bacterial isolates were recovered on plate count agar (PCA) at 30 °C, purified by subculturing on the same medium and then cryopreserved at −80 °C in a nutritive broth supplemented with 15 % (v/v) glycerol. PCA was used for culture maintenance, and biochemical and physiological tests were performed in modified Luria–Bertani medium (Tiago *et al.*, 2004) incubated at 30 °C. Colony and cell morphology, Gram-staining, cytochrome *c* oxidase and catalase activities, motility and flagellation were assessed according to the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). The pH range for growth was examined in culture medium containing 12 mM MES (Sigma), to adjust the pH to 5.5, and 12 mM CAPS (Sigma), to adjust the pH to 9.0 and 10.0. The NaCl tolerance and temperature ranges for growth were assayed, respectively, in culture medium supplemented with 1, 3 and 5 % NaCl (w/v) or at 15, 36, 40 and

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PC-142 and PC-147^T are AM411529 and AM411528, respectively.

RAPD patterns and an extended phylogenetic tree for strains PC-142 and PC-147^T and related taxa are available as supplementary figures with the online version of this paper.

Table 1. Distinctive characteristics of strains PC-142 and PC-147^T and *P. pasadenensis* NBRC 101214^T

Strains PC-142, PC-147^T and *P. pasadenensis* NBRC 101214^T tested positive for β-galactosidase, for the hydrolysis of gelatin and aesculin and for the assimilation of the following carbon sources: D-glucose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-fructose, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, D-cellobiose, D-lactose, D-melibiose, sucrose, trehalose, D-melezitose, D-raffinose, starch, glycogen, gentiobiose and D-turanose. All of the strains tested negative for the following: Gram staining, nitrate reduction, arginine dihydrolase, urease, indole production and glucose fermentation. All were unable to assimilate the following carbon sources: caprate, adipate, malate, citrate, phenylacetate, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, inulin, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate and 5-ketogluconate. Strains PC-142 and PC-147^T were negative for anaerobic growth, lysine and ornithine decarboxylase and tryptophan deaminase activities, citrate utilization and H_2S production and for growth at 45 °C, with 5 % NaCl and on Bacillus cereus medium (*P. pasadenensis* NBRC 101214^T not tested). PC-142 and PC-147^T were unable to use the following carbon sources: (fermentation/oxidation) D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, D-melibiose, amygdalin and L-arabinose; (acid production) D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, L-arabinose, D-arabinose, glycerol, erythritol, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, arbutin, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, 2-ketogluconate and 5-ketogluconate (data not shown for *P. pasadenensis* NBRC 101214^T). Strain PC-142 showed weak acid production for D-lactose, D-melibiose and D-turanose; strain PC-147^T was unable to produce acids from these carbon sources (data not shown for *P. pasadenensis* NBRC 101214^T).

Characteristic	Strains PC-142 and PC-147^T	*P. pasadenensis* NBRC 101214^T
Growth with 3 % NaCl	+	−
Assimilation of:		
L-Arabinose	−	+
Glycerol	−	+
Malate	−	+
L-Rhamnose	−	+
Acid production from:		
L-Arabinose	−	+
D-Fructose	−	+
D-Galactose	−	+
D-Glucose	−	+
Glycogen	−	+
D-Mannose	−	+
D-Mannitol	−	+
D-Melezitose	−	+
D-Raffinose	−	+
Starch	−	+
D-Xylose	−	+
DNA G+C content (mol%)	58	63
Isolation source	Poultry litter compost	Floor of clean-room entrance

45 °C. The ability of the strains to grow in the presence of 0.001 % (w/v) lysozyme was tested in tryptic casein soy broth medium. Anaerobic growth was tested under a N_2-saturated atmosphere in the presence of 0.1 % KNO_3 (w/v). The same medium, under aerobic conditions, was used to assay nitrate reduction. The production of extracellular amylases, gelatinases and tweenases (Tween 80) was tested as described previously (Tiago *et al.*, 2004). Other biochemical tests were performed using API 20E and API 20NE galleries, and the nutritional pattern was determined using API 50 CH and API 20NE kits, according to the instructions of the manufacturer (bioMérieux) at 30 °C. The API 50 CH carbon-source utilization kit was used both with the medium recommended for testing acid production (50 CHB/E; bioMérieux) and with mineral medium B (Barreiros *et al.*, 2003) supplemented with 4 mM $(NH_4)_2SO_4$, vitamins (*p*-aminobenzoic acid, folic acid, nicotinic acid, pantothenic acid, biotin, cyanocobalamin, inositol, riboflavin, pyridoxine, thiamine) (40 μg l^{-1}) and nitrogenated bases (adenine, cytosine,

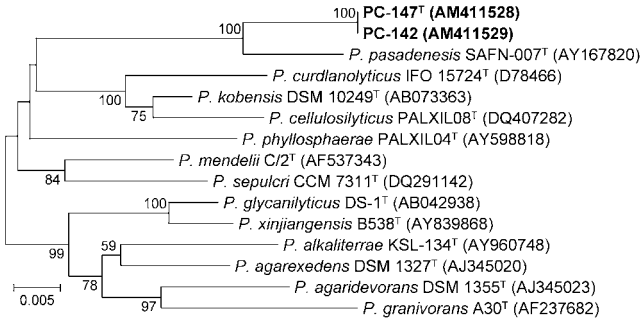

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the nearest neighbours of strain PC-142 and PC-147^T. Bootstrap percentages were generated from 1000 resamplings; only values greater than 50 % are shown. Bar, 1 substitution per 200 nucleotide positions. An extended version of this tree is available as Supplementary Fig. S2 in IJSEM Online.

inosine, thymine and uracil) and amino acids (methionine, phenylalanine, tryptophan, arginine, histidine, alanine, glycine, proline and tyrosine) (5 mg l^{-1}).

The DNA G+C content, respiratory quinones and fatty acid methyl ester composition were determined as described previously (Vaz-Moreira *et al.*, 2007) using the methods of Mesbah *et al.* (1989), Tindall (1989) and Kuykendall *et al.* (1988), respectively. The diaminopimelic acid isomer was determined by one-dimensional TLC on cellulose plates (Merck) by using the solvent system of Rhuland *et al.* (1955).

The sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracts as described previously (Rainey *et al.*, 1996). The 16S rRNA gene sequence was compared with others available in the GenBank/EMBL/DDBJ database by using BLASTN (NCBI) and FASTA (EMBL-EBI) and was aligned with reference sequences. Phylogenetic analysis was conducted using MEGA, version 3.1 (Kumar *et al.*, 2004). Levels of sequence relatedness were estimated using the model of Jukes & Cantor (1969), and the phylogenetic tree was created using the neighbour-joining method. Other methods (minimum evolution and maximum parsimony) were used to assess the tree stability. A total of 1322 nucleotide positions in each 16S rRNA gene were included in the analysis. Non-homologous and ambiguous nucleotide positions were excluded from the calculations.

After 24 h incubation at 30 °C on PCA, isolates PC-142 and PC-147^T formed flat, very smooth, translucent colonies that tended to produce a swarming effect. These strains were unable to produce growth on Bacillus cereus medium (Pronadisa) agar after 7 days incubation. They were able to produce acid from, and assimilate, several sugars (see Table 1 and the species description). The major fatty acid methyl esters were anteiso-$C_{15:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$, which accounted for more than 60 % of the total.

Table 2. Fatty acid methyl ester compositions of strains PC-142 and PC-147^T and *P. pasadenensis* NBRC 101214^T

Fatty acid methyl ester	PC-142	PC-147^T	*P. pasadenensis* NBRC 101214^T
iso-$C_{14:0}$	1.7	1.5	1.2
$C_{14:0}$	1.2	1.5	–
iso-$C_{15:0}$	21.1	16.9	16.4
anteiso-$C_{15:0}$	39.2	41.9	38.2
$C_{15:0}$	2.6	1.6	4.1
iso-$C_{16:0}$	13.2	10.6	12.3
$C_{16:0}$	6.8	8.0	5.1
$C_{16:1}$	–	–	3.3
iso-$C_{17:0}$	5.8	4.4	7.3
anteiso-$C_{17:0}$	5.7	6.1	8.3
$C_{18:0}$	1.4	1.6	–
Others*	1.4	5.9	3.9

*Includes anteiso-$C_{13:0}$, anteiso-$C_{14:0}$, $C_{16:1}\omega7c$ alcohol, anteiso-$C_{17:1}\omega9c$, $C_{18:1}\omega9c$ and summed feature 10 (unidentified).

Other minor components (representing more than 4 %) were iso-$C_{17:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$. The DNA G+C content for PC-147^T and PC-142 was, respectively, 58.3 ± 0.3 and 58.1 ± 0.2 mol%. Menaquinone MK-7 was the major respiratory quinone in both strains. The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid, suggesting the presence of peptidoglycan type A1γ (Schleifer & Kandler, 1972). Strains PC-142 and PC-147^T differed only in the ability to produce acid from three carbon sources (D-lactose, D-melibiose and D-turanose); they had similar fatty acid methyl ester compositions and DNA G+C contents and their 16S rRNA gene sequences were identical. These findings were confirmed by the similarity of the RAPD patterns obtained for isolates PC-142 and PC-147^T (see Supplementary Fig. S1, available in IJSEM Online).

The diagnostic diamino acid of the peptidoglycan, the menaquinone and the fatty acid methyl ester composition of isolates PC-142 and PC-147^T confirm the results of the 16S rRNA gene sequence comparative analysis, identifying these organisms as members of the genus *Paenibacillus*. On the basis of the 16S rRNA gene sequence analysis, the closest phylogenetic neighbours of isolates PC-142 and PC-147^T are *Paenibacillus pasadenensis* SAFN-007^T (97.5 %), *Paenibacillus kobensis* DSM 10249^T (94.3 %) and *Paenibacillus mendelli* C/2^T (93.8 %) (Fig. 1; an extended version of this tree is available as Supplementary Fig. S2). In the light of these results, the nutritional and chemotaxonomic properties of strains PC-142 and PC-147^T were compared with those of *P. pasadenensis* NBRC 101214^T (Osman *et al.*, 2006) (Tables 1 and 2). The fatty acid methyl ester patterns obtained for PC-142, PC-147^T and *P. pasadenensis* NBRC 101214^T confirm the taxonomic relatedness of these organisms and their affiliation to the same genus (Table 2). However, the DNA G+C contents of these

three organisms (58.3 ± 0.3 mol% for PC-147^T, 58.1 ± 0.2 mol% for PC-142 and 63.4 ± 0.6 mol% for *P. pasadenensis* NBRC 101214^T) demonstrate the taxonomic distinctiveness of the two isolates. Amongst the highest DNA G+C contents reported for this genus were those obtained for *Paenibacillus validus* (54–55 mol%; buoyant density method) (Heyndrickx *et al.*, 1995) and *Paenibacillus panacisoli* (Ten *et al.*, 2006). Strains PC-142 and PC-147^T and *P. pasadenensis* NBRC 101214^T form a high-G+C lineage within the genus *Paenibacillus*.

As strains PC-142 and PC-147^T show less than 98.5 % 16S rRNA gene sequence similarity with respect to their closest phylogenetic neighbour, *P. pasadenensis,* DNA–DNA hybridizations to demonstrate their separate species status were considered unnecessary in view of the results of recent investigations (Stackebrandt & Ebers, 2006). Characteristics that serve to distinguish PC-142 and PC-147^T from their closest phylogenetic neighbour, *P. pasadenensis* NBRC 101214^T, are presented in Table 1. On the basis of the data presented, strains PC-142 and PC-147^T represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus humicus* sp. nov. is proposed.

Description of *Paenibacillus humicus* sp. nov.

Paenibacillus humicus (hu′mi.cus, L. n. *humus* earth, soil and, in earth sciences or agriculture, humus; L. suff. *-icus -a -um* suffix used with the sense of belonging to; N.L. masc. adj. *humicus* pertaining to humus).

Cells are motile rods (2.0 μm long and 0.5 μm wide) with peritrichous flagella. Gram staining is negative. Terminal, ellipsoidal endospores are produced in swollen sporangia. On nutritive medium, such as PCA or Luria–Bertani agar, colonies are white/translucent, very smooth and flat. Swarming is observed. Catalase- and oxidase-positive. Growth occurs in the presence of 3 % NaCl and at 15–40 °C and pH 5.5–10. No growth occurs under anaerobic conditions, at 45 °C or in the presence of 5 % NaCl. Nitrate is not reduced. Tests positively for β-galactosidase, Voges–Proskauer (API 20NE) and growth in the presence of 0.001 % lysozyme. Starch, gelatin, Tween 80 and aesculin are hydrolysed. The following carbon sources are assimilated: starch, amygdalin, arbutin, D-cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, glycogen, D-lactose, maltose, D-mannitol, D-melezitose, D-melibiose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, *N*-acetyl-glucosamine, potassium gluconate, D-raffinose, salicin, sucrose, trehalose, D-turanose and D-xylose. Acid is produced from amygdalin, D-cellobiose, maltose, sucrose and trehalose. The peptidoglycan cell wall is of the A1γ type. The major cellular fatty acid components are anteiso-$C_{15:0}$ (approx. 40 %), iso-$C_{15:0}$ (17–21 %) and iso-$C_{16:0}$ (11–13 %). The major respiratory quinone is MK-7 and the DNA G+C content is 58 mol%.

The type strain, PC-147^T (=DSM 18784^T =NBRC 102415^T =LMG 23886^T), was isolated from final compost produced from poultry litter.

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