Paenibacillus residui sp. nov., isolated from urban waste compost

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Two bacterial strains, MC-246^T and MC-247, were isolated from municipal urban waste compost and characterized by a polyphasic approach. Both isolates were Gram-stain-variable, endospore-forming rods that were catalase-, oxidase- and β -galactosidase-positive, and able to grow at 25–50 °C and pH 7.0–9.0, with optimum growth at 37 °C and pH 7. The predominant cellular fatty acids were anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{17:0}; the major respiratory quinone was menaquinone MK-7; the cell wall peptidoglycan was of type A1 γ ; and the DNA G+C content was 49 mol%. These characteristics, as well as data from 16S rRNA gene sequence analysis, showed that these strains were affiliated with the genus *Paenibacillus*; the type strains of *Paenibacillus ginsengarvi* and *Paenibacillus hodogayensis* were among their closest neighbours (<94.2% sequence similarity). Nevertheless, the hypothesis that strains MC-246^T and MC-247 could represent a novel species was supported by the low 16S rRNA gene sequence similarity values shared with other members of the genus *Paenibacillus* and by the observation of distinct biochemical and physiological traits. Strains MC-246^T and MC-247 shared 99.6% 16S rRNA gene sequence similarity and showed almost identical MALDI-TOF mass spectra, but could be distinguished at the phenotypic and genotypic level. However, DNA–DNA hybridization between strains MC-246^T and MC-247 resulted in values above 70% indicating that both organisms represent a single species, for which the name *Paenibacillus residui* sp. nov. is proposed; the type strain is MC-246^T (=DSMZ 22072^T =CCUG 57263^T).

Composting is a process of biological decomposition, nowadays considered the best process for the treatment of the organic fraction of urban waste (Fehr, 2007). At the end of the treatment, the organic matter is converted into a stabilized humus-like product (compost) that is frequently used in gardening and agriculture. In this study, two bacterial strains isolated from municipal urban waste compost were characterized and identified as members of the genus *Paenibacillus* (Vaz-Moreira *et al.*, 2008). At the time of writing, the genus *Paenibacillus* proposed by Ash

et al. (1993) comprises more than 100 species with validly published names (Euzéby, 1997) and includes isolates from a wide variety of habitats, such as soil, sediments and compost, water, plant rhizosphere, food, diseased insect larvae, faeces and human fluids. Members of the genus *Paenibacillus* are facultatively anaerobic or strictly aerobic, rod-shaped, Gram-positive, -negative or -variable, motile, endospore-forming bacteria with swollen sporangia (Shida *et al.*, 1997). Relevant chemotaxonomic markers of this genus are a genomic DNA G+C content of 39.0–55.8 mol%, the presence of menaquinone MK-7 and peptidoglycan of type A1 γ (Shida *et al.*, 1997).

Strains MC-246^T and MC-247 were isolated from municipal urban waste compost produced from undifferentiated urban waste (Vaz-Moreira *et al.*, 2008) and purified by subculturing on plate count agar [PCA (Pronadisa), containing (g l⁻¹): tryptone, 5; yeast extract, 2.5; glucose, 1; agar, 15]. Cultures were incubated at 30 °C and preserved at –80 °C in nutrient broth with 15% (v/v) glycerol. The strains under study were characterized for

Abbreviation: MALDI-TOF, matrix-assisted laser-desorption/ionization time-of-flight.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MC-246^T and MC-247 are FN293173 and FN293174, respectively.

A phylogenetic tree based on 16S rRNA gene sequences, *Eco*RI RiboPrints, RAPD profiles and a dendrogram generated by the BioTyper software showing MALDI-TOF MS similarities of strains MC-246^T and MC-247 and related strains are available with the online version of this paper.

their phenotypic and chemotaxonomic features. The type strains of closely related species (*Paenibacillus hodogayensis* DSM 22253^T and *Paenibacillus ginsengarvi* DSM 18677^T) were assayed in parallel to minimize differences due to experimental conditions. Colony and cell morphology, Gram-stain reaction, cytochrome *c* oxidase and catalase activities, endospore production and motility were analysed based on the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). Unless otherwise stated, all biochemical and physiological tests were performed as described previously (Vaz-Moreira *et al.*, 2007). Biochemical and nutritional tests were assayed using API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) following the manufacturer's instructions. The Voges-Proskauer test was also assayed in methyl red/Voges-Proskauer medium (Oxoid) incubated at 30 °C for 48 h. Hydrolysis of Tween 80 and starch was performed as described by Tiago *et al.* (2004). Casein hydrolysis and urease activity were assayed as described by Smibert & Krieg (1994). Antimicrobial susceptibility and RAPD profiles (with primers M13 and OPA3) were assayed as described previously (Ferreira da Silva *et al.*, 2006).

Determination of the genomic DNA G+C content and analysis of respiratory quinones were performed as described previously (Vaz-Moreira *et al.*, 2007) using the methods of Mesbah *et al.* (1989) and Tindall (1989), respectively. The cellular fatty acid composition was analysed as described by Kämpfer & Kroppenstedt (1996). All strains were cultivated for 2 days on tryptic soy agar at 30 °C.

Purified peptidoglycan preparations were obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion (Schleifer & Seidl, 1985). The amino acid and peptide compositions of peptidoglycan hydrolysates (4 M HCl, 100 °C for 16 h and 0.75 h, respectively) were analysed by one- and two-dimensional TLC on cellulose plates using previously described solvent systems (Rhuland *et al.*, 1955; Schleifer & Kandler, 1972).

The nucleotide sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracts as described previously (Ferreira da Silva *et al.*, 2007). The 16S rRNA gene sequence was compared with others available in GenBank/EMBL/DDBJ using the FASTA package from EMBL-EBI (<http://www.ebi.ac.uk>). Phylogenetic analysis was conducted using the software BioNumerics (version 5.1; Applied Maths). Sequence relatedness was estimated based on the model of Jukes & Cantor (1969) and the dendrogram was recreated using the neighbour-joining method. Other methods, namely maximum-parsimony and maximum-likelihood, were applied to assess the tree stability. Non-homologous and ambiguous nucleotide positions were excluded from the calculations. For spectroscopic DNA-DNA hybridization, 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 hybridizations were carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). Fully automated ribotyping of *Eco*RI-digested samples was performed with a RiboPrinter system (DuPont Qualicon) as described by Bruce (1996). Analysis by matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) mass spectra of strains MC-246^T and MC-247 and close phylogenetic relatives was performed according to the method described by Tóth *et al.* (2008).

The 16S rRNA gene sequence analysis revealed that strains MC-246^T and MC-247 had a similarity of 99.6% and shared 93.7% similarity with the type strain of *P. hodogayensis* and 94.2 and 93.8% similarities with the type strain of *P. ginsengarvi*, respectively (Fig. 1, Supplementary Fig. S1, available in IJSEM Online). Results of the phenotypic characterization of strains MC-246^T, MC-247, *P. hodogayensis* DSM 22253^T and *P. ginsengarvi* DSM 18677^T are presented in

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence analysis showing the relationship of strains MC-246^T and MC-247 with members of the genus *Paenibacillus*. The tree was generated by the neighbour-joining method and bootstrap values ($\geq 50\%$) generated from 1000 resamplings are indicated at branch points. Filled circles indicate branches also recovered by the maximum-likelihood method. Bar, 1 substitution per 100 nt positions.

Table 1 and in the species description. The major cellular fatty acid methyl esters of strains MC-246^T and MC-247 and of the reference strains tested were anteiso-C_{17:0}, anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and iso-C_{15:0} (Table 2). The DNA G+C contents determined for strains MC-246^T and MC-247 were 49.3 ± 0.6 and 49.0 ± 0.3 mol%, respectively; these values are within the range observed for members of the genus *Paenibacillus* (39.0–55.8 mol%) (Takeda *et al.*, 2002). Menaquinone MK-7 was the major respiratory quinone in both strains and the peptidoglycan was of type A1γ (based on directly cross-linked *meso*-diaminopimelic

acid). Chemotaxonomic characterization and the 16S rRNA gene-based phylogenetic analysis confirm the affiliation of strains MC-246^T and MC-247 to the genus *Paenibacillus*. However, the low 16S rRNA sequence similarity with other species of this genus (≤ 94 %) and the observation of several differences in the nutritional pattern and the production of extracellular enzymes support the exclusion of strains MC-246^T and MC-247 from any species with validly published names. Strains MC-246^T and MC-247 share a 16S rRNA gene sequence similarity of 99.6 % and are distinguishable at the phenotypic (differences in nutritional pattern and the

Table 1. Distinctive characteristics of strains MC-246^T and MC-247 and the closely related species *P. hodogayensis* DSM 22253^T and *P. ginsengarvi* DSM 18677^T

Strains: 1, MC-246^T; 2, MC-247; 3, *P. hodogayensis* DSM 22253^T; 4, *P. ginsengarvi* DSM 18677^T. All strains tested in parallel in the present study. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4
Assimilation of:				
D-Mannitol	–	+	+	–
N-Acetylglucosamine	–	–	+	+
Potassium gluconate	–	–	+	+
Acid production from:				
D-Arabinose	–	–	–	+
D-Ribose	+	+	–	+
D-Xylose	+	–	–	+
Adonitol	–	+	–	–
D-Glucose	+	–	–	+
D-Fructose	w	+	–	–
Dulcitol	+	+	–	–
D-Mannitol	–	+	+	–
D-Sorbitol	–	+	–	–
Methyl α-D-glucopyranoside	+	–	–	+
Amygdalin	+	+	–	+
Arbutin	+	–	–	+
Salicin	+	+	–	+
Lactose	–	+	+	+
Melibiose	–	–	+	+
Sucrose	–	–	+	+
Raffinose	–	–	–	+
Turanose	–	+	–	+
D-Tagatose	–	+	–	–
D-Arabitol	–	+	–	–
Enzymes produced				
Alkaline phosphatase	+	+	–	–
Acid phosphatase	+	+	–	+
Esterase C4	+	+	–	–
Esterase lipase C8	+	+	–	–
Valine arylamidase	+	+	–	–
Trypsin	+	+	+	–
α-Chymotrypsin	+	+	–	–
α-Glucosidase	+	+	–	+
β-Glucosidase	+	+	–	–
N-Acetyl-β-glucosaminidase	+	+	–	+
α-Fucosidase	+	+	–	–
Urease	+	–	–	–
Isolation source	Urban waste compost	Urban waste compost	Damp humus	Ginseng field soil

Table 2. Cellular fatty acid methyl ester composition (%) of strains MC-246^T, MC-247, *P. hodogayensis* DSM 22253^T and *P. ginsengarvi* DSM 18677^T

Strain: 1, MC-246^T; 2, MC-247; 3, *P. hodogayensis* DSM 22253^T; 4, *P. ginsengarvi* DSM 18677^T. —, Not detected. All strains tested in parallel in the present study.

Fatty acid	1	2	3	4
C _{14:0}	0.30	0.41	1.21	0.49
C _{15:0}	2.61	2.62	—	—
C _{16:0}	6.83	6.97	10.54	4.07
C _{17:0}	1.43	1.11	1.39	1.40
iso-C _{14:0}	0.77	1.05	4.86	5.55
iso-C _{15:0}	9.44	11.19	18.90	20.34
iso-C _{16:0}	15.75	19.44	21.12	28.94
iso-C _{17:0}	11.05	11.11	8.23	8.30
iso-C _{18:0}	0.30	0.32	—	—
anteiso-C _{11:0}	—	0.11	—	—
anteiso-C _{15:0}	36.65	33.50	29.30	27.66
anteiso-C _{17:0}	14.86	12.18	3.98	3.25
C _{16:1ω7c}	—	—	0.46	—

ability to produce urease; Table 1) and genotypic levels (*Eco*RI RiboPrints and RAPD genotyping; Supplementary Figs S2 and S3). Nevertheless, the identical MALDI-TOF mass spectra (Supplementary Fig. S4) and DNA–DNA hybridization analysis, which gave values of 76–88 %, values that are above the threshold of 70 % established for the definition of bacterial species (Wayne *et al.*, 1987), support the inclusion of strains MC-246^T and MC-247 in the same species, for which the name *Paenibacillus residui* sp. nov. is proposed; strain MC-246^T is the type strain.

Description of *Paenibacillus residui* sp. nov.

Paenibacillus residui (re.si'du.i. L. n. *residuum* leavings, scraps, residues; L. gen. n. *residui* of leavings, scraps, residues).

Cells may stain Gram-positive or Gram-negative and are motile rods (1.4–1.6 µm long and 0.5 µm wide). Polar flagella may be observed and oval spores are positioned centrally in swollen sporangia. Colonies are translucent and irregular on PCA after 48 h of incubation. Catalase- and oxidase-positive. Growth occurs at 25–50 °C, at pH 7–9 and in up to 2 % NaCl, with optimal growth at about 37 °C, pH 7 and 1 % NaCl. Growth does not occur in an anaerobic atmosphere and nitrate is not reduced. Citrate is not used, and H₂S, indole and acetoin are not produced. Urease activity is variable. Glucose is not fermented. Gelatin, starch, Tween 80 and casein are not hydrolysed. Aesculin is hydrolysed. Acid and alkaline phosphatase, esterase C₄, esterase lipase C₈, leucine and valine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase are produced. Arginine dihydrolase, lysine and ornithine

decarboxylase, tryptophan deaminase, lipase C₁₄, cystine arylamidase, α-galactosidase, β-glucuronidase and α-mannosidase are not produced. The sole carbon sources assimilated are D-glucose, maltose, D-ribose, D-fructose, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, cellobiose, trehalose, gentiobiose, turanose and D-tagatose. The following are not assimilated: L-arabinose, D-mannose, *N*-acetylglucosamine, potassium gluconate, caprate, adipate, malate, citrate, phenylacetate, glycerol, D-arabinose, L-arabinose, D-xylose, L-xylose, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, inositol, D-sorbitol, methyl α-D-mannopyranoside, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, D-lyxose, D-fucose, L-fucose, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate. Acid is produced from glycerol, D-fructose, D-ribose, dulcitol, amygdalin, salicin, cellobiose, maltose, trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, L-arabinose, L-xylose, methyl β-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, inositol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, D-lyxose, D-fucose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Variable for the assimilation of D-mannitol, erythritol, adonitol, dulcitol, sucrose and D-arabitol, and for acid production from D-xylose, adonitol, D-glucose, D-mannitol, D-sorbitol, methyl α-D-glucopyranoside, arbutin, lactose, turanose, D-tagatose and D-arabitol. Resistant to cephalothin (30 µg), ceftazidime (30 µg), colistin sulfate (50 µg) and streptomycin (10 µg). Susceptible to meropenem (10 µg), amoxicillin (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), SXT (sulfamethoxazole/trimethoprim, 23.75/1.25 µg), tetracycline (30 µg), ticarcillin (75 µg) and sulfamethoxazole (25 µg). The predominant cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{17:0} and the major respiratory quinone is MK-7. The cell wall peptidoglycan is of the A1γ type (directly cross-linked *meso*-diaminopimelic acid). The DNA G + C content of the type strain is 49 mol%.

The type strain is MC-246^T (=DSM 22072^T =CCUG 57263^T), isolated from municipal urban waste compost.

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References

- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- Bruce, J. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Technol* **50**, 77–81.

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Euzéby, J. P. (1997). List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592. <http://www.bacterio.net>
- Fehr, M. (2007). Confirming decentralised composting as a definite option in urban waste management. *Int J Environ Technol Manag* **7**, 274–285.
- Ferreira da Silva, M., Tiago, I., Veríssimo, A., Boaventura, A. R., Nunes, O. C. & Manaia, C. M. (2006). Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. *FEMS Microbiol Ecol* **55**, 322–329.
- Ferreira da Silva, M., Vaz-Moreira, I., Gonzalez-Pajuelo, M., Nunes, O. C. & Manaia, C. M. (2007). Antimicrobial resistance patterns in *Enterobacteriaceae* isolated from an urban wastewater treatment plant. *FEMS Microbiol Ecol* **60**, 166–176.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Murray, R. G. E., Doetsch, R. N. & Robinow, F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behavior of the isomers of α , ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp. 201–215. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Takeda, M., Kamagata, Y., Shinmaru, S., Nishiyama, T. & Koizumi, J. (2002). *Paenibacillus koleovorans* sp. nov., able to grow on the sheath of *Sphaerotilus natans*. *Int J Syst Evol Microbiol* **52**, 1597–1601.
- Tiago, I., Teixeira, I., Silva, S., Chung, P., Veríssimo, A. & Manaia, C. M. (2004). Metabolic and genetic diversity of mesophilic and thermophilic bacteria isolated from composted municipal sludge on poly- ϵ -caprolactones. *Curr Microbiol* **49**, 407–414.
- Tindall, B. J. (1989). Fully saturated menaquinones in the archaeobacterium *Pyrobaculum islandicum*. *FEMS Microbiol Lett* **60**, 251–254.
- Tóth, E. M., Schumann, P., Borsodi, A. K., Kéki, Z., Kovács, A. L. & Márialigeti, K. (2008). *Wohlfahrtiimonas chitiniclastica* gen. nov., sp. nov., a new gammaproteobacterium isolated from *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). *Int J Syst Evol Microbiol* **58**, 976–981.
- Vaz-Moreira, I., Nobre, M. F., Nunes, O. C. & Manaia, C. M. (2007). *Gulbenkiania mobilis* gen. nov., sp. nov., isolated from treated municipal wastewater. *Int J Syst Evol Microbiol* **57**, 1108–1112.
- Vaz-Moreira, I., Silva, E., Manaia, C. M. & Nunes, O. C. (2008). Diversity of bacterial isolates from commercial and homemade composts. *Microb Ecol* **55**, 714–722.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.