Saccharibacillus sacchari gen. nov., sp. nov., isolated from sugar cane

Raúl Rivas,¹ Paula García-Fraile,¹ José Luis Zurdo-Piñeiro,¹ Pedro F. Mateos,¹ Eustoquio Martínez-Molina,¹ Eulogio J. Bedmar,² Juan Sánchez-Raya² and Encarna Velázquez¹

Correspondence
Encarna Velázquez
evp@usal.es

¹Departamento de Microbiología y Genética, Lab 209, Edificio Departamental de Biología Campus Miguel de Unamuno, Universidad de Salamanca, Spain

²Estación Experimental del Zaidín, CSIC, Granada, Spain

A bacterial strain designated GR21^T was isolated from apoplastic fluid of *Saccharum officinarum* (sugar cane). Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate forms a separate branch within the family 'Paenibacillaceae', with *Paenibacillus* as the closest related genus. Within this genus, the closest related species is *Paenibacillus xylanilyticus*, with 93.4 % similarity to the sequence of the type strain. The isolate has Gram-variable, facultatively anaerobic, rod-shaped cells, motile by polar and subpolar flagella. Round, non-ornamented, central or subterminal spores are formed in unswollen sporangia. The strain is catalase-positive and oxidase-negative on nutrient agar medium. Cellulose and aesculin were hydrolysed, whereas xylan, starch and gelatin were not. Growth was supported by many carbohydrates as carbon sources. Strain GR21^T displayed a lipid profile consisting of diphosphatidylglycerol, phosphatidylglycerol, an unknown aminophospholipid, two unknown glycolipids and an unknown phosphoglycolipid. MK-7 was the predominant menaquinone and anteiso-C_{15:0} was the major fatty acid. The DNA G + C content was 57.8 mol%. Phylogenetic and phenotypic analyses, including assimilation of carbon sources and exoenzyme production commonly used for classification within the family 'Paenibacillaceae', showed that strain GR21^T belongs to a new genus within this family, for which the name *Saccharibacillus sacchari* gen. nov., sp. nov. is proposed. The type strain of *Saccharibacillus sacchari* is GR21^T (=LMG 24085^T =DSM 19268^T).

The family 'Paenibacillaceae' currently contains eight genera of Gram-positive, -variable or -negative sporulating bacilli. The description of the most recently described genus of the family, *Cohnella* (Kämpfer *et al.*, 2006), was based on its phylogenetic relationship with respect to the remaining genera of the family as well as on its polar lipid profile. Some species of this family, mainly belonging to the genus *Paenibacillus*, have been isolated from the plant rhizosphere, but only *Paenibacillus polymyxa* has been reported as a plant endophyte (Cho *et al.*, 2006). In the present work, we describe the isolation and identification of a novel bacterium isolated from apoplastic sap of sugar cane (*Saccharum officinarum*) in Granada (Spain).

For the isolation of strain GR21^T, stem pieces were washed with sterile tap water and soaked in 5 % chloramine T solution for 2 min. After removal of the rind with a sterile

sharp knife, the cylinder of parenchyma tissue was rinsed with 70 % ethanol and flame-sterilized. Apoplastic sap from the internodes was collected by centrifugation according to Dong *et al.* (1994), and serial dilutions were streaked on tryptone soybean agar (TSA) and incubated at 30 °C for 7–10 days. After flaming, no contamination was observed when several stem pieces were incubated on TSA plates.

Strain GR21^T was grown on nutrient agar for 48 h at 22 °C to check for motility by phase-contrast microscopy using the hanging drop method. Gram staining was carried out by the procedure described by Doetsch (1981). The flagellation type was determined by electron microscopy after 48 h incubation on nutrient agar at 22 °C. Cells were gently suspended in sterile water and then stained with 0.2 % uranyl acetate and examined at 80 kV with a Zeiss EM 209 transmission electron microscope. For scanning microscopy, cells were fixed overnight in phosphate buffer (pH 7.0) which contained 2 % paraformaldehyde and 0.2 % glutaraldehyde, dehydrated through a graded ethanol series, critical-point dried and sputter-coated with gold.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GR21^T is EU014873.

Electron micrographs of cells of strain GR21^T, an extended neighbour-joining tree and results of 2D TLC of polar lipids of strain GR21^T are available as supplementary material with the online version of this paper.

Samples were observed under a Zeiss DSM 490 electron microscope (Germany). Strain GR21^T was Gram-variable and motile by means of polar and subpolar flagella (Supplementary Fig. S1, available in IJSEM Online). Round, non-ornamented central or subterminal spores are formed in unswollen sporangia. In the genus *Paenibacillus*, the phylogenetically closest related genus of strain GR21^T (see below), the spores are commonly ornamented and are formed in swollen sporangia.

Amplification of the 16S rRNA gene and its sequencing was performed according to the method of Rivas *et al.* (2007). A nearly complete 16S rRNA gene sequence was obtained and compared with those deposited in GenBank using the BLASTN (Altschul *et al.*, 1990) and EzTaxon (Chun *et al.*, 2007) programs. Sequences were aligned using the CLUSTAL_X software (Thompson *et al.*, 1997). Distances were calculated according to Kimura's two-parameter method (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and minimum-evolution (Rzhetsky & Nei, 1992) methods. Bootstrap analysis was based on 1000 resamplings. The MEGA2 package (Kumar *et al.*, 2001) was used for all analyses. Comparison of the nearly complete 16S rRNA

gene sequence of strain GR21^T against sequences held in GenBank indicated that the organism is phylogenetically related to members of the family 'Paenibacillaceae', with *Paenibacillus* as the closest related genus (Fig. 1). According to the results obtained with the EzTaxon program, the most closely related type strains of species of this genus with validly published names were those of *Paenibacillus xylanilyticus*, *P. massiliensis*, *P. chitinolyticus* and *P. sepulcri*, with identities ranging from 93.4 to 93.0 %. The BLASTN program showed similar results, but this comparison showed that the strain HR1, incorrectly named *Paenibacillus xinjiangensis*, a name that has already been validly published within the genus *Paenibacillus*, has 98 % similarity in its 16S rRNA gene with respect to strain GR21^T. Although strain HR1 could belong to the new genus described below, it was not included in this work because only its 16S rRNA gene sequence is currently known (nevertheless, this sequence was included in Supplementary Fig. S2 to show the phylogenetic location of strain HR1). Fig. 1 shows the phylogenetic tree obtained with the neighbour-joining method (an expanded tree is shown in Supplementary Fig. S2). Similar results were obtained when the sequences were analysed using the minimum-evolution method (not shown). The phylogenetic analysis showed the

Fig. 1. Comparative sequence analysis of the 16S rRNA gene from strain GR21^T (*Saccharibacillus sacchari* gen. nov., sp. nov.) and representative species of related genera from the family 'Paenibacillaceae' using the neighbour-joining method. The significance of each branch is indicated by a bootstrap percentage calculated for 1000 subsets. Bar, 2 substitutions per 100 nucleotide positions. An extended version of this tree is available as Supplementary Fig. S2.

location of strain GR21^T on an independent branch clearly separated from all genera of the family 'Paenibacillaceae'. This result supports the classification of this strain in a new genus within this family, with the genus *Paenibacillus* as the closest related genus, the most closely related species of which is *P. xylanilyticus*, with 93.4 % similarity to the type strain. The genera *Thermobacillus* and *Cohnella* were the next most closely related genera, with a maximum of 89.8 and 90.2 % similarity, respectively.

Chemotaxonomic analyses were performed by the Identification Service of the DSMZ according to Tindall (1990) in the case of respiratory quinones and polar lipids and Kämpfer & Kroppenstedt (1996) in the case of fatty acids. To perform the analysis of peptidoglycan, whole cells of strain GR21^T were hydrolysed with HCl at 100 °C for 15 h. The hydrolysates were subjected to TLC on cellulose plates using the solvent system of Rhuland *et al.* (1955).

As is found in the case of the genus *Paenibacillus* (Shida *et al.*, 1997), *meso*-diaminopimelic acid was detected in the peptidoglycan of strain GR21^T. No data on peptidoglycan composition have been reported for the remaining genera of the family 'Paenibacillaceae'. MK-7 was the predominant isoprenoid quinone found in strain GR21^T, and only a small amount of MK-6 was detected (1.5 %). An unidentified menaquinone was also found (2 %). Respiratory quinones have low resolution within the family 'Paenibacillaceae', since MK-7 is the major quinone in most members of the family. The presence of an unidentified quinone may be a differentiating character of the new genus, but this hypothesis should be confirmed when more species of this genus are described.

The major cellular fatty acid of the strain was anteiso-C_{15:0} (45.2 %). C_{16:0} represented 25.2 %, and other fatty acids were present in amounts of less than 10 % (Table 1). Although anteiso-C_{15:0} is the dominant cellular fatty acid in all members of the genus *Paenibacillus* (Shida *et al.*, 1997), the profile of strain GR21^T differs from those of members of the genus *Paenibacillus* in the amounts of the fatty acids C_{14:0} and anteiso-C_{17:0}. More differences were found in the case of the genus *Cohnella* (Kämpfer *et al.*, 2006) and particularly in the case of the genus *Thermobacillus* (Touzel *et al.*, 2000).

Strain GR21^T displayed a lipid profile consisting of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), an aminophospholipid (PN), two unknown glycolipids (GL1 and GL2) and an unknown phosphoglycolipid (PGL). DPG was the major polar lipid, as is the case for other genera of the family 'Paenibacillaceae' (Kämpfer *et al.*, 2006). PG is also present in those genera that contain glycolipids GL1 or GL2. In strain GR21^T, both unknown glycolipids were present. Strain GR21^T contains PN instead of phosphatidylethanolamine, which is found in other genera of the family 'Paenibacillaceae'. PGL is present in small amounts in strain GR21^T and has not been reported in the other genera of the family 'Paenibacillaceae'. Therefore, the polar lipid profile of strain GR21^T

Table 1. Fatty acid contents of strain GR21^T (*Saccharibacillus sacchari* gen. nov., sp. nov.) and closely related genera of the family 'Paenibacillaceae'

Values are percentages of total fatty acids. Data for reference genera were compiled from Kämpfer *et al.* (2006) and Touzel *et al.* (2000) (*Paenibacillus*), Kämpfer *et al.* (2006) and Cho *et al.* (2007) (*Cohnella*) and Touzel *et al.* (2000) and Watanabe *et al.* (2007) (*Thermobacillus*). ND, Not detected; NR, not reported.

Fatty acid	Strain GR21 ^T	<i>Paenibacillus</i>	<i>Cohnella</i>	<i>Thermobacillus</i>
Saturated acids				
Straight-chain				
C _{12:0}	0.7	NR	NR	NR
C _{14:0}	7.6	1.2–3.3	1–5	0.5–1.2
C _{15:0}	ND	0.6–6.7	1.4–8	1.8–4.2
C _{16:0}	25.2	8.1–28.0	6.6–25.3	2.9–21.4
C _{17:0}	ND	NR	1.2	0.3–0.9
C _{18:0}	ND	NR	NR	0.7
iso-branched				
C _{14:0}	7.7	0.6–7.2	2.1–3.9	1–4.4
C _{15:0}	3.0	1.0–12.0	3.2–11.7	0.9–4.6
C _{16:0}	6.3	0.5–6.0	11.9–45.5	39.0–48.0
C _{17:0}	0.8	1–6.9	2.8	1.4–1.8
C _{18:0}	ND	NR	NR	1.1
anteiso-branched				
C _{13:0}	0.2	NR	0.8	0.5
C _{15:0}	45.2	28.2–80.0	22.0–31.2	8.7–33.3
C _{17:0}	2.3	6.0–7.0	2.6–6.7	7.4–12.4
Unsaturated acids				
anteiso-C _{17:1}	ND	NR	1.1–1.9	NR
C _{16:1} ω11 <i>c</i>	0.8	NR	0.9	NR
C _{17:1} ω6 <i>c</i>	ND	NR	1	NR
C _{18:1} ω7 <i>c</i>	ND	NR	4	NR

(Supplementary Fig. S3) is clearly different from those of members of other genera of the family 'Paenibacillaceae' reported by Kämpfer *et al.* (2006), supporting the affiliation of strain GR21^T to a new genus within the family 'Paenibacillaceae'.

DNA for determination of the base composition was prepared according to Chun & Goodfellow (1995). The G+C content of the DNA was determined using the thermal denaturation method (Mandel & Marmur, 1968). The G+C content of strain GR21^T was 57.8 mol%.

The phenotypic characterization was performed according to the standard methods described by Claus & Berkeley (1986) and Logan & Berkeley (1984) and by using the API 20NE and API 20E systems (bioMérieux) according to the manufacturer's instructions. Amylases and cellulases were analysed as described previously (Rivas *et al.*, 2003). Growth was determined at temperatures ranging from 4 to 45 °C in YED medium (0.5 % yeast extract, 0.7 % glucose and 2 % agar). Growth at pH 5.7 and 6.8 was tested as described by Claus & Berkeley (1986), growth at pH 7.0–8.0 was tested in YED medium containing 100 mM

Na₂HPO₄/NaH₂PO₄ and growth at pH 9.0–10.0 was tested in YED medium containing 100 mM NaHCO₃/Na₂CO₃. The phenotypic characteristics are given in the genus and species descriptions. Differential characteristics with respect to the closest related genera are shown in Table 2.

On the basis of phylogenetic, chemotaxonomic and phenotypic data, we propose that isolate GR21^T belongs to a new genus and species within the family 'Paenibacillaceae', for which the name *Saccharibacillus sacchari* gen. nov., sp. nov. is proposed.

Description of *Saccharibacillus* gen. nov.

Saccharibacillus (Sac.cha.ri.ba.cil'lus. N.L. n. *Saccharum* a botanical genus name; L. masc. n. *bacillus* a small staff or rod; N.L. masc. n. *Saccharibacillus* a small rod isolated from *Saccharum officinarum*, sugar cane).

Gram-variable, motile, rod-shaped bacteria. Round, non-ornamented spores are formed in unswollen sporangia. Catalase- and oxidase-negative. Main menaquinone is MK-7. Predominant lipids are diphosphatidylglycerol, phosphatidylglycerol and an unknown aminophospholipid. In addition, two unknown glycolipids and an unknown phosphoglycolipid are present. The major fatty acids are

anteiso-C_{15:0} and C_{16:0}. The type species is *Saccharibacillus sacchari*.

Description of *Saccharibacillus sacchari* sp. nov.

Saccharibacillus sacchari (sac'cha.ri. L. gen. n. *sacchari* of sugar, referring to the isolation source of the type strain, inner tissues of sugar cane).

Cells are 0.8–1 µm wide and 3–4 µm long. Motile by means of polar or subpolar flagella. Spores are formed in a central or subterminal position in cells. Aerobic or facultatively anaerobic and chemo-organotrophic. Colonies on TSA are circular with irregular borders, pale pink, not mucoid, opaque and usually 1–2 mm in diameter within 48 h at 28 °C. Growth occurs at 8–40 °C; optimal growth at 28 °C and pH 7. Acetoin is produced. Grows in the presence of 2 % NaCl. Nitrate is reduced to nitrite. Cellulases, xylanases and β-galactosidase are produced actively, but amylases, gelatinase, caseinase, arginine dihydrolase, indole, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, phenylalanine deaminase and hydrogen sulfide are not produced. Aesculin is hydrolysed. Acid but not gas is produced from D-glucose. D-Glucose, mannose, maltose, mannitol and gluconate are used as carbon sources.

Table 2. Differential characteristics between strain GR21^T (*Saccharibacillus sacchari* gen. nov., sp. nov.) and phylogenetically related genera from the family 'Paenibacillaceae'

Data for *Paenibacillus* were compiled in this study from those recorded for the closest species to strain GR21^T, *P. xylanilyticus*, *P. sepulcri*, *P. massiliensis* and *P. chitinolyticus*, which are basically in agreement with those reported for the genus *Paenibacillus* by Shida *et al.* (1997). Data for *Cohnella* were taken from Teng *et al.* (2003), Kämpfer *et al.* (2006) and Cho *et al.* (2007) and data for *Thermobacillus* were taken from Touzel *et al.* (2000) and Watanabe *et al.* (2007). +, Positive; –, negative; v, variable; ND, no data available.

Characteristic	<i>Saccharibacillus</i>	<i>Paenibacillus</i>	<i>Cohnella</i>	<i>Thermobacillus</i>
Spore shape	Round	Oval	Round or oval	Oval ^{a*}
Sporangia	Not swollen	Swollen	ND	Swollen ^a
Anaerobic growth	+	v	–	–
Catalase	–	+	v	+
Oxidase	–	v	v	v
Nitrate reduction to nitrite	+	v	+ ^b	– ^a
Gelatinase	–	v	– ^c	– ^a
Caseinase	–	v	– ^b	– ^a
Amylase	–	v	ND	+
Acetoin production	+	v	– ^b	ND
Growth at pH 5.7	+	–	+ ^c	v
Growth at 50 °C	–	–	+	+
Growth in presence of 2 % NaCl	+	+	– ^c	+
Acid from:				
D-Glucose	+	+	v	– ^d
D-Xylose	–	v	v	ND
L-Arabinose	–	v	v	ND
Mannitol	–	v	v	ND
Major polar lipids	DPG, PG	DPG, PE	DPG, PG, PE	ND
Major fatty acid(s) (>25 %)	ai-C _{15:0} , C _{16:0}	ai-C _{15:0}	ai-C _{15:0} , i-C _{16:0} , C _{16:0}	i-C _{16:0} , ai-C _{15:0}

*Data available for the following species only: a, *Thermobacillus xylanilyticus*; b, *Cohnella hongkongensis*; c, *Cohnella laeviribosi*; d, *Thermobacillus composti*.

Assimilation of L-arabinose, D-xylose, N-acetylglucosamine, caprate, adipate, malate, citrate and phenylacetate is negative. The DNA G+C content of the type strain is 57.8 mol%.

The type strain, GR21^T (=LMG 24085^T =DSM 19268^T), was isolated from inner tissues of *Saccharum officinarum* in Granada (Spain).

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References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Cho, K. M., Hong, S. Y., Lee, S. M., Kim, Y. H., Kahng, G. G., Kim, H. & Yun, H. D. (2006). A cel44C-man26A gene of endophytic *Paenibacillus polymyxa* GS01 has multi-glycosyl hydrolases in two catalytic domains. *Appl Microbiol Biotechnol* **73**, 618–630.
- Cho, E. A., Lee, J. S., Lee, K. C., Jung, H. C., Pan, J. G. & Pyun, Y. R. (2007). *Cohnella laeviribosi* sp. nov., isolated from a volcanic pond. *Int J Syst Evol Microbiol* **57**, 2902–2907.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J. H., Jung, Y. J., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Dong, Z., Canny, M. J., McCully, M. E., Roboredo, M. R., Fernandez Cabadilla, C., Ortega, E. & Rodés, R. (1994). A nitrogen-fixing endophyte of sugarcane stems. A new role for the apoplast. *Plant Physiol* **105**, 1139–1147.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H.-J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov., and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 781–786.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001). Molecular evolutionary genetics analysis software. Tempe, AZ: Arizona State University.
- Logan, N. A. & Berkeley, R. C. W. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* **130**, 1871–1882.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behaviour of the isomers of α,ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.
- Rivas, R., Sánchez, M., Trujillo, M. E., Zurdo-Piñeiro, J. L., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2003). *Xylanimonas cellulosilytica* gen. nov., sp. nov., a xylanolytic bacterium isolated from a decayed tree (*Ulmus nigra*). *Int J Syst Evol Microbiol* **53**, 99–103.
- Rivas, R., García-Fraile, P., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2007). Characterization of xylanolytic bacteria present in the bract phyllosphere of the date palm *Phoenix dactylifera*. *Lett Appl Microbiol* **44**, 181–187.
- Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- 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.
- Teng, J. L., Woo, P. C., Leung, K. W., Lau, S. K., Wong, M. K. & Yuen, K. Y. (2003). Pseudobacteraemia in a patient with neutropenic fever caused by a novel paenibacillus species: *Paenibacillus hongkongensis* sp. nov. *Mol Pathol* **56**, 29–35.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Touzel, J. P., O'Donohue, M., Debeire, P., Samain, E. & Breton, C. (2000). *Thermobacillus xylanilyticus* gen. nov., sp. nov., a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil. *Int J Syst Evol Microbiol* **50**, 315–320.
- Watanabe, K., Nagao, N., Yamamoto, S., Toda, T. & Kurosawa, N. (2007). *Thermobacillus composti* sp. nov., a moderately thermophilic bacterium isolated from a composting reactor. *Int J Syst Evol Microbiol* **57**, 1473–1477.