Paenibacillus riograndensis sp. nov., a nitrogen-fixing species isolated from the rhizosphere of *Triticum aestivum*

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A bacterial strain designated SBR5^T was isolated from the rhizosphere of *Triticum aestivum*. A phylogenetic analysis based on the 16S rRNA gene sequence placed the isolate within the genus *Paenibacillus*, being most closely related to *Paenibacillus graminis* RSA19^T (98.1 % similarity). The isolate was a Gram-reaction-variable, motile, facultatively anaerobic bacterium, with spores in a terminal position in cells. Starch was utilized and dihydroxyacetone and catalase were produced. Strain SBR5^T displayed plant-growth-promoting rhizobacteria characteristics: the ability to fix nitrogen and to produce siderophores and indole-3-acetic acid. The DNA G + C content was 55.1 mol%. Chemotaxonomic analysis of the isolated strain revealed that MK-7 was the predominant menaquinone, while the major fatty acid was anteiso-C_{15:0}. DNA–DNA hybridization values between strain SBR5^T and *P. graminis* RSA19^T, *Paenibacillus odorifer* TOD45^T and *Paenibacillus borealis* KK19^T were 43, 35 and 28 %, respectively. These DNA relatedness data and the results of phylogenetic and phenotypic analyses showed that strain SBR5^T should be considered as the nitrogen-fixing type strain of a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus riograndensis* sp. nov. is proposed. The type strain is SBR5^T (=CCGB 1313^T =CECT 7330^T).

The bacilli are a wide group of micro-organisms that are characterized by endospore formation. Currently, this group includes several families and genera, many of which formerly belonged to the genus *Bacillus*, which has been separated into several novel genera that belong to several families. This is the case for the genus *Paenibacillus*, which was proposed by Ash *et al.* (1994) and belongs to the family 'Paenibacillaceae'. Bacteria belonging to the genus *Paenibacillus* are among the most widely distributed micro-organisms and play significant roles in microbial communities (Reva *et al.*, 1995). They can be found associated with plants or freely in soils and have potential applications in different fields of agricultural biotechnology as inoculants for crop production.

Nitrogen fixation has been described in several species of the genus *Paenibacillus*, such as *P. polymyxa* (Grau & Wilson, 1962), *P. macerans*, *P. durus* (*P. azotofixans*), *P. peoriae* (Montefusco *et al.*, 1993), *P. borealis* (Elo *et al.*,

2001), *P. graminis*, *P. odorifer* (Berge *et al.*, 2002), *P. brasiliensis* (von der Weid *et al.*, 2002), *P. massiliensis* (Roux & Raoult, 2004), *P. wynnii* (Rodríguez-Díaz *et al.*, 2005), *P. sabinae* (Ma *et al.*, 2007a), *P. zanthoxyli* (Ma *et al.*, 2007b), '*P. donghaensis*' (Choi *et al.*, 2008) and *P. forsythiae* (Ma & Chen, 2008). Some of these bacteria are promising candidates for crop inoculation, not only for their nitrogen-fixing ability, but also for their capacity to promote plant growth through the production of phytohormones (auxins and cytokinins) and antimicrobial substances (Rosado *et al.*, 1996). Although several species of plant-growth-promoting rhizobacteria (PGPR) have already been described (Lebuhn *et al.*, 1997; Timmusk & Wagner, 1999; Timmusk *et al.*, 1999; Helbig, 2001; von der Weid *et al.*, 2003), the vast majority of rhizospheric bacterial species present in many soils remain unknown, and their identification could be useful in the formulation of new inoculants to improve crop production.

In the present report, we describe the morphological, phylogenetic and physiological characteristics of a novel PGPR, strain SBR5^T, isolated from the rhizosphere of *Triticum aestivum* cultivated in Rio Grande do Sul State, Brazil.

Abbreviation: PGPR, plant-growth-promoting rhizobacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SBR5^T is EU257201.

Aliquots of serially diluted pasteurized (10 min, 80 °C) rhizosphere suspensions of wheat (*Triticum aestivum*) were inoculated onto thiamine-biotin agar (TB, nitrogen-free medium; Seldin *et al.*, 1983) and incubated in anaerobic jars (Permution) for 7 days at 28 °C. Anaerobic bacilli colonies were transferred to fresh TB agar plates for another period of anaerobic incubation. Single colonies were then transferred to aerobic GB broth (Seldin *et al.*, 1983). A bacterial strain, designated SBR5^T, was isolated and a pure culture was maintained in a glycerol suspension (20 %) at –20 °C.

The morphology of cells was examined by phase-contrast microscopy. Flagellum and spore types were examined with a scanning electron microscope (XL-30, Philips) using cells cultured for 48 h in GB broth. Cells were fixed according to Borges *et al.* (2004). Gram behaviour was ascertained by staining (Doetsch, 1981). Motility was verified by the SIM (hydrogen_sulfide, indole, motility; MacFaddin, 2000) test.

The SBR5^T isolate was also evaluated for the presence of desirable PGPR characteristics. To assess indole-3-acetic acid, indolepyruvic acid, and indoleacetamide production (collectively referred to as indolic compounds), the method described by Glickmann & Dessaux (1995) was used. To determine the nitrogen-fixing capability of this strain, an assay based on sulfur digestion and distillation with 10 mol NaOH l⁻¹, as described by Keeney & Bremner (1966), was performed together with PCR amplification of a 360 bp fragment of the *nifH* gene using the universal degenerate primers PolF and PolR, described by Poly *et al.* (2001). Strain SBR5^T was also analysed for its siderophore production capacity in Petri dishes containing King B medium (Glickmann & Dessaux, 1995) supplemented with a chrome azurol S complex [CAS/iron(III)/hexadecyltrimethyl ammonium bromide], as described by Schwyn & Neilands (1987).

Chromosomal DNA was extracted and purified according to standard methods (Sambrook & Russell, 2001). The 16S rRNA gene sequence of strain SBR5^T was amplified using primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGATGATCCAGCC-3' under conditions described by Rivas *et al.* (2003). The corresponding positions in the *Escherichia coli* small-subunit rRNA gene sequence for these primers are 8–27 and 1498–1522, respectively. The 16S rRNA gene sequence was determined in both the forward and reverse directions using the primers described above and an ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems) at the ACTGene Laboratory (Centro de Biotecnologia, UFRGS, RS, Brazil). An almost-complete (1506 bp) 16S rRNA gene sequence was obtained and compared with those deposited in public databases. Sequences were aligned using the CLUSTAL_X software (Thompson *et al.*, 1997). Evolutionary distances were calculated using the method of Kimura (1980). Phylogenetic trees were inferred using neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1993) and maximum-parsimony (Felsenstein, 1983)

methods. Bootstrap analysis was based on 1000 resamplings. The MEGA 2.1.0 package (Kumar *et al.*, 2001) was used for all analyses. As no significant topological differences were found among the phylogenetic trees obtained by the three methods used, only the tree constructed by using the neighbour-joining method is shown (Fig. 1). Calculations of pair-wise 16S rRNA gene sequence similarities were performed using the EzTaxon software (assessed in October 2007; Chun *et al.*, 2007). The results obtained were confirmed by Megablast analysis (<http://www.ncbi.nlm.nih.gov/>).

DNA–DNA hybridization and DNA G+C content were determined as described by De Ley *et al.* (1970).

Analysis of respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ, Braunschweig, Germany. Determination of the peptidoglycan structure was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was used instead of paper chromatography. Quantitative analysis of amino acids was performed, after derivatization by gas chromatography, with 2 ml of 4 M alcoholic methanol and heating at 70 °C for 2 h according to MacKenzie (1987).

The predominant fatty acids were analysed by GLC as described in the MIS operating manual (MIDI, 2001). The results shown in Table 1 were determined under the same conditions for all strains used for comparison.

Phenotypic characterization was performed according to the standard methods described by Claus & Berkeley (1986).

Cells of strain SBR5^T were Gram-reaction-variable, rod-shaped, sporulating and motile. The isolate produced ellipsoidal spores with a regular stripe pattern (Fig. 2). Regarding PGPR abilities, the SBR5^T isolate produced 213.7 and 269.4 µg indolic compounds ml⁻¹ after 72 and 144 h of incubation, respectively, and was able to fix 8 µg N ml⁻¹. Strain SBR5^T produced a yellow halo in the blue-green media, which indicated its ability to produce siderophores.

Strain SBR5^T is phylogenetically related to members of the genus *Paenibacillus*. As shown in Fig. 1, the most closely related recognized type strains were *P. graminis* RSA19^T (98.1 % similarity), *P. odorifer* TOD45^T (95.8 %) and *P. borealis* KK19^T (96.3 %). Phylogenetic analysis based on *nifH* sequences revealed that the SBR5^T strain also clustered together with species of the genus *Paenibacillus* (Fig. 3). The novel strain showed high levels of *nifH* gene sequence similarity with *P. graminis* (78 %), *P. wynnii* (79 %), *P. odorifer* (77 %) and *P. borealis* (74 %).

The values for DNA–DNA hybridization between strain SBR5^T and *P. graminis* RSA19^T, *P. odorifer* TOD45^T and *P. borealis* KK19^T were 43, 35 and 28 %, respectively. In terms of DNA–DNA hybridization, the threshold value for the

Fig. 1. Rooted phylogenetic tree based on 16S rRNA sequence comparisons of strain SBR5^T and selected members of the genus *Paenibacillus*. GenBank accession numbers are given in parentheses. Sequence of *Lactobacillus delbrueckii* subsp. *lactis* was used to root the dendrogram. Bootstrap analyses were based on 1000 replications.

definition of a species is considered to be 70% (Wayne *et al.*, 1987); consequently, our results indicate that the strain isolated in this study does not belong to any of the known species of the genus *Paenibacillus*. The DNA G + C content of strain SBR5^T was 55.1 mol%. Although this is higher than those described for the majority of species of the genus *Paenibacillus* (Shida *et al.*, 1997), it is similar to that obtained for *Paenibacillus stellifer* (55.6 mol%, Suominen *et al.*, 2003), another nitrogen-fixing member of the genus.

Unsaturated menaquinone with seven isoprene units (MK-7) was the predominant isoprenoid quinone found in strain SBR5^T. The major polar lipids present were diphosphatidylglycerol, phosphatidylglycerol and one unknown phospholipid that could not be identified. The total hydrolysate (4 M HCl, 16 h, 100 °C) of the peptidoglycan contained the amino acids Lys, Glu and Ala in a molar ratio of approximately 1.0:1.0:3.3. The partial hydrolysate (4 M HCl, 0.75 h, 100 °C) of the peptidoglycan contained (in addition to the amino acids Lys, Glu and Ala) the peptides L-Ala→D-Glu, L-Ala→L-Lys, L-Ala→L-Ala→L-Lys, L-Lys→D-Ala, L-Ala→L-Lys→D-Ala, L-Ala→L-Ala→L-Lys→D-Ala and L-Ala→D-Ala. From these data it was concluded that strain SBR5^T shows the peptidoglycan type A3α L-Lys←L-Ala←L-Ala (type A11.5 according to <http://www.dsmz.de>). The predominant fatty acids in strain SBR5^T were anteiso-C_{15:0} and C_{16:0}, comprising 45.7 and

sphatidylglycerol, phosphatidylglycerol and one unknown phospholipid that could not be identified. The total hydrolysate (4 M HCl, 16 h, 100 °C) of the peptidoglycan contained the amino acids Lys, Glu and Ala in a molar ratio of approximately 1.0:1.0:3.3. The partial hydrolysate (4 M HCl, 0.75 h, 100 °C) of the peptidoglycan contained (in addition to the amino acids Lys, Glu and Ala) the peptides L-Ala→D-Glu, L-Ala→L-Lys, L-Ala→L-Ala→L-Lys, L-Lys→D-Ala, L-Ala→L-Lys→D-Ala, L-Ala→L-Ala→L-Lys→D-Ala and L-Ala→D-Ala. From these data it was concluded that strain SBR5^T shows the peptidoglycan type A3α L-Lys←L-Ala←L-Ala (type A11.5 according to <http://www.dsmz.de>). The predominant fatty acids in strain SBR5^T were anteiso-C_{15:0} and C_{16:0}, comprising 45.7 and

Table 1. Cellular fatty acid composition of strain SBR5^T and phylogenetically related species of the genus *Paenibacillus*

Strains: 1, *Paenibacillus riograndensis* sp. nov. SBR5^T; 2, *P. graminis* RSA19^T; 3, *P. odorifer* TOD45^T; 4, *P. wynnii* LMG 22176^T; 5, *P. borealis* KK19^T. Data were from this study and are in agreement with those described by Rodríguez-Díaz *et al.* (2005). Values are percentages of total fatty acids. ND, Not determined.

Fatty acid	1	2	3	4	5
Straight-chain					
C _{14:0}	8.0	10.3	4.7	6.0	18.8
C _{16:0}	17.6	28.5	14.7	32.2	12.5
Iso-branched					
C _{14:0}	3.9	ND	4.3	5.3	4.6
C _{15:0}	10.3	6.4	14.4	5.7	13.2
C _{16:0}	9.0	11.5	5.5	4.9	9.0
C _{17:0}	2.4	ND	2.9	1.3	2.4
Anteiso-branched					
C _{15:0}	45.7	43.3	49.3	33.1	37.0
C _{17:0}	3.16	ND	2.3	>1	2.3

17.6 % of the total, respectively. According to these results, the fatty acid composition of strain SBR5^T is similar to those reported for species of the genus *Paenibacillus* (Shida *et al.*, 1997).

Details of phenotypic characteristics that differentiate strain SBR5^T and phylogenetically related species are given in Table 2. Other characteristics determined are given in the species description. Strain SBR5^T differed from *P. graminis* with respect to growth at 40 °C, gas production from D-glucose and nitrate reduction, from *P. odorifer* with respect to nitrate reduction and acid production from D-mannitol, from *P. wynnii* with respect to spore position and nitrate reduction, and from *P. borealis* with respect to

Fig. 2. Scanning electron microscopy of vegetative cells of strain SBR5^T and spores showing a regular pattern of stripes. Bar, 2μm.

casein hydrolysis and growth at pH 10. Strain SBR5^T differed from all of these species with respect to aesculin hydrolysis.

On the basis of the phylogenetic and phenotypic data, we propose that isolate SBR5^T (=CCGB 1313^T =CECT 7330^T) represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus riograndensis* sp. nov. is proposed.

Description of *Paenibacillus riograndensis* sp. nov.

Paenibacillus riograndensis (ri.o.gran.den'sis. N. L. masc. adj. *riograndensis* referring to Rio Grande do Sul, the state located in Southern Brazil, where the strain was isolated).

Cells are rod-shaped, 0.65–0.8 μm by 3.8–4.5 μm, Gram-reaction-variable, motile and facultatively anaerobic. Spores are in a terminal position in cells. Colonies on GB medium are circular, convex, white and translucent,

Fig. 3. Phylogenetic tree based on partial *nifH* sequences, showing the position of strain SBR5^T in relation to other nitrogen-fixing species of the genus *Paenibacillus*. GenBank accession numbers are given in parentheses. Sequence of *Mesorhizobium ciceri* was used to root the dendrogram. Bootstrap analyses were performed with 1000 cycles. Bar, 0.1 substitutions per nucleotide position.

Table 2. Phenotypic characteristics that differentiate *Paenibacillus riograndensis* SBR5^T from its closest relatives in the genus *Paenibacillus*

Strains: 1, *Paenibacillus riograndensis* SBR5^T (data from this study); 2, *Paenibacillus graminis* RSA19^T (Berge *et al.*, 2002; Ma *et al.*, 2007a); 3, *Paenibacillus odorifer* TOD45^T (Berge *et al.*, 2002; Ma *et al.*, 2007a); 4, *Paenibacillus wynnii* LMG 22176^T (Rodríguez-Díaz *et al.*, 2005); 5, *Paenibacillus borealis* KK19^T (Elo *et al.*, 2001). +, Positive reaction; –, negative reaction; ND, not determined; v, variable reaction. All strains are rod-shaped with ellipsoidal spores, motile, grow anaerobically and are positive for starch hydrolysis, catalase and acid production from D-glucose, fructose, galactose, lactose, maltose, sucrose, D-xylose and trehalose. All strains are negative for growth at 50 °C, acid production from dulcitol, use of citrate as a carbon source, oxidase activity, gelatin hydrolysis, production of hydrogen sulphide and Voges–Proskauer and indole tests. c, Central or paracentral; s, subterminal; T, terminal.

Characteristic	1	2	3	4	5
Spore position	T	T	T	c or s	s or T
Casein hydrolysis	–	–	–	–	+
Aesculin hydrolysis	–	+	+	+	+
Nitrate reduction	–	+	+	+	–
Acid production from:					
L-Arabinose	+	+	+	v	+
Glycerol	+	+	v	v	+
D-Mannitol	+	+	–	+	+
Mannose	+	+	v	+	+
Raffinose	+	+	+	+	–
Gas production from glucose	–	+	–	–	–
Growth in the presence of 5% NaCl	–	–	ND	–	–
Growth at 40 °C	–	+	–	–	–
Growth at pH 10	+	ND	ND	+	–
Urease activity	–	ND	ND	–	ND

typically 1–2 mm in diameter within 24 h at 28 °C. Optimal growth at 28 °C and pH 7. Cannot grow in the presence of 5% NaCl. Catalase-positive and oxidase-negative. DNA G+C content of the type strain is 55.1 mol%. The main fatty acid is anteiso-C_{15:0} and the predominant menaquinone is MK-7. Gas is not produced from D-glucose. Acid is produced from D-glucose, sucrose, D-mannose, lactose, raffinose, maltose, D-xylose, mannitol, L-arabinose, galactose, glycerol, D-fructose and trehalose, but not from dulcitol or *myo*-inositol. Does not utilize citrate as a carbon source for growth. Starch is hydrolysed. Casein and aesculin are not hydrolysed and acetoin is not produced. Gelatinase, urease, phenylalanine deaminase, indole, hydrogen sulphide and acetoin (in Voges–Proskauer medium) are not produced. Nitrate is not reduced to nitrite. Displays PGPR characteristics: able to fix nitrogen, produces siderophores and indole-3-acetic acid.

The type strain, SBR5^T (=CCGB 1313^T =CECT 7330^T), was isolated from the rhizosphere of wheat (*Triticum aestivum*) in Rio Grande do Sul State, Southern Brazil.

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References

- Ash, C., Priest, F. G. & Collins, M. D. (1994). *Paenibacillus* gen. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 51. *Int J Syst Bacteriol* **44**, 852.
- Berge, O., Guinebretière, M., Achouak, W., Normand, P. & Heulin, T. (2002). *Paenibacillus graminis* sp. nov. and *Paenibacillus odorifer* sp. nov., isolated from plant roots, soil and food. *Int J Syst Evol Microbiol* **52**, 607–616.
- Borges, F. P., Wiltuschnig, R. C., Tasca, T. & de Carli, G. A. (2004). Scanning electron microscopy study of *Tritrichomonas augusta*. *Parasitol Res* **94**, 158–161.
- Choi, J. H., Im, W. T., Yoo, J. S., Lee, S. M., Moon, D. S., Kim, H. J., Rhee, S. K. & Roh, D. H. (2008). *Paenibacillus donghaensis* sp. nov., a xylan-degrading and nitrogen-fixing bacterium isolated from East Sea sediment. *J Microbiol Biotechnol* **18**, 189–193.
- Chun, J., Lee, J. H., Jung, Y., 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*, 2nd edn, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- 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.
- Elo, S., Suominen, I., Kämpfer, P., Juhanoja, J., Salkinoja-Salonen, M. & Haahtela, K. (2001). *Paenibacillus borealis* sp. nov., a nitrogen-fixing species isolated from spruce forest humus in Finland. *Int J Syst Evol Microbiol* **51**, 535–545.
- Felsenstein, J. (1983). Parsimony in systematics: biological and statistical issues. *Annu Rev Ecol Syst* **14**, 313–333.
- Glickmann, E. & Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl Environ Microbiol* **61**, 793–796.
- Grau, F. H. & Wilson, P. W. (1962). Physiology of nitrogen-fixation by *Bacillus polymyxa*. *J Bacteriol* **83**, 490–496.
- Helbig, J. (2001). Biological control of *Botrytis cinerea* Pers. ex Fr. in strawberry by *Paenibacillus polymyxa* (isolate 18191). *J Phytopathol* **149**, 265–273.
- Keeney, D. R. & Bremner, J. M. (1966). A chemical index of soil nitrogen availability. *Nature* **211**, 892–893.
- 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). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

- Lebuhn, M., Heulin, T. & Hartmann, A. (1997). Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. *FEMS Microbiol Ecol* **22**, 325–334.
- Ma, Y. C. & Chen, S. F. (2008). *Paenibacillus forsythiae* sp. nov., a nitrogen-fixing species isolated from rhizosphere soil of *Forsythia mira*. *Int J Syst Evol Microbiol* **58**, 319–323.
- Ma, Y., Xia, Z., Liu, X. & Chen, S. (2007a). *Paenibacillus sabinae* sp. nov., a nitrogen-fixing species isolated from the rhizosphere soils of shrubs. *Int J Syst Evol Microbiol* **57**, 6–11.
- Ma, Y., Zhang, J. & Chen, S. (2007b). *Paenibacillus zanthoxyli* sp. nov., a novel nitrogen-fixing species isolated from the rhizosphere of *Zanthoxylum simulans*. *Int J Syst Evol Microbiol* **57**, 873–877.
- MacFaddin, J. F. (2000). *Biochemical Tests for Identification of Medical Bacteria*, 3rd edn. Philadelphia, PA: Lippincott, Williams & Wilkins.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- MIDI (2001). *Sherlock Microbial Identification System Operating Manual*, version 4.0. Newark, DE: MIDI, Inc.
- Montefusco, A., Nakamura, L. K. & Labeda, D. P. (1993). *Bacillus peoriae* sp. nov. *Int J Syst Bacteriol* **43**, 388–390.
- Poly, F., Monrozier, L. J. & Bally, R. (2001). Improvement in the RFLP procedure for studying the diversity of *nifH* genes in communities of nitrogen fixers in soil. *Res Microbiol* **152**, 95–103.
- Reva, O. N., Vyunitskaya, V. A., Reznik, S. R., Kozachko, I. A. & Smirnov, V. V. (1995). Antibiotic susceptibility as a taxonomic characteristic of the genus *Bacillus*. *Int J Syst Evol Microbiol* **45**, 409–411.
- 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.
- Rodríguez-Díaz, M., Lebbe, L., Rodelas, B., Heyrman, J., de Vos, P. & Logan, N. A. (2005). *Paenibacillus wynnii* sp. nov., a novel species harbouring the *nifH* gene, isolated from Alexander Island, Antarctica. *Int J Syst Evol Microbiol* **55**, 2093–2099.
- Rosado, A. S., Seldin, L., Wolters, A. C. & van Elsas, J. D. (1996). Quantitative 16S rDNA-targeted polymerase chain reaction and oligonucleotide hybridization for the detection of *Paenibacillus azotofixans* in soil and the wheat rhizosphere. *FEMS Microbiol Ecol* **19**, 153–164.
- Roux, V. & Raoult, D. (2004). *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. *Int J Syst Evol Microbiol* **54**, 1049–1054.
- Rzhetsky, A. & Nei, M. (1993). Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* **10**, 1073–1095.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schwyn, B. & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* **160**, 47–56.
- Seldin, L., van Elsas, J. D. & Penido, E. G. C. (1983). *Bacillus* nitrogen fixers from Brazilian soils. *Plant Soil* **70**, 243–255.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Emended description of *Paenibacillus amylolyticus* and description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov. *Int J Syst Bacteriol* **47**, 299–306.
- Suominen, I., Spröer, C., Kämpfer, P., Rainey, F. A., Lounatmaa, K. & Salkinoja-Salonen, M. (2003). *Paenibacillus stellifer* sp. nov., a cyclodextrin-producing species isolated from paperboard. *Int J Syst Evol Microbiol* **53**, 1369–1374.
- 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.
- Timmusk, S. & Wagner, E. G. (1999). The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. *Mol Plant Microbe Interact* **12**, 951–959.
- Timmusk, S., Nicander, B., Granhall, U. & Tillberg, E. (1999). Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol Biochem* **31**, 1847–1852.
- von der Weid, I., Duarte, G. F., van Elsas, J. D. & Seldin, L. (2002). *Paenibacillus brasiliensis* sp. nov., a new nitrogen-fixing species isolated from the maize rhizosphere in Brazil. *Int J Syst Evol Microbiol* **52**, 2147–2153.
- von der Weid, I., Alviano, D. S., Santos, A. L., Soares, R. M., Alviano, C. S. & Seldin, L. (2003). Antimicrobial activity of *Paenibacillus peoriae* strain NRRL BD-62 against a broad spectrum of phytopathogenic bacteria and fungi. *J Appl Microbiol* **95**, 1143–1151.
- 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.