Paucisalibacillus globulus gen. nov., sp. nov., a Gram-positive bacterium isolated from potting soil

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A Gram-positive bacterium, designated B22^T, was isolated from potting soil produced in Portugal. This organism is a catalase-positive, oxidase-negative, motile, spore-forming, aerobic rod that grows optimally at 37 °C and pH 8.0–8.5. Optimal growth occurs in media containing 1 % (w/v) NaCl, although the organism can grow in 0–8 % NaCl. The cell wall peptidoglycan is of the A4 α type with a cross-linkage containing D-Asp. The major respiratory quinone is menaquinone 7 and the major fatty acids are anteiso-15 : 0, anteiso-17 : 0 and iso-15 : 0. The DNA G + C content is 37.9 mol%. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain B22^T formed a new branch within the family *Bacillaceae*. The novel isolate is phylogenetically closely related to members of genera of moderately halophilic bacilli and formed a coherent cluster with species of the genera *Salinibacillus*, *Virgibacillus*, *Oceanobacillus* and *Lentibacillus*, supported by bootstrap analysis at a confidence level of 71 %. Strain B22^T exhibited 16S rRNA gene pairwise sequence similarity values of 94.7–94.3 % with members of the genus *Salinibacillus*, 95.1–92.8 % with members of the genus *Virgibacillus*, 94.7–93.2 % with members of the genus *Oceanobacillus* and 93.1–92.3 % with members of the genus *Lentibacillus*. On the basis of phylogenetic analysis and physiological and biochemical characteristics, it is proposed that strain B22^T represents a novel species in a new genus, *Paucisalibacillus globulus* gen. nov., sp. nov. Strain B22^T (= LMG 23148^T = CIP 108857^T) is the type strain of *Paucisalibacillus globulus*.

Gram-positive, rod-shaped, aerobic or facultatively anaerobic, spore-forming bacteria that grow optimally in media containing NaCl are assigned to 18 genera within the family *Bacillaceae* that have similar characteristics and some close phylogenetic relationships. Four of these genera, namely *Salinibacillus* (Ren & Zhou, 2005), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Oceanobacillus* (Lu *et al.*, 2001) and *Lentibacillus* (Yoon *et al.*, 2002), form a coherent phylogenetic cluster. During an investigation of the presence of *Legionella* in composts and potting soils used in Portugal, several slightly halophilic Gram-positive bacteria were isolated and further characterized. One of these isolates shared many physiological and biochemical characteristics with species of the genera mentioned above, but had a distinctly lower NaCl requirement for optimal growth and a distinctive peptidoglycan composition. Furthermore, 16S rRNA gene sequence analysis showed that this organism represents a distinct phylogenetic lineage within the family *Bacillaceae*. In this study, the morphological, physiological, chemotaxonomic and phylogenetic characteristics of strain B22^T are described.

Strain B22^T was isolated from potting soil on buffered charcoal yeast extract (BCYE) medium (Edelstein, 1981), which is normally used for the isolation and growth of *Legionella* spp. Cultures were purified by subculturing and preserved at –70 °C in 5 % yeast extract medium with 15 % glycerol. Despite the organism having been isolated on BCYE, the strain was routinely cultured at 37 °C in alkaline buffered medium 2 (ABM2) with 1 % NaCl, adjusted to pH 8.0 (Tiago *et al.*, 2005b). Unless otherwise stated, all morphological examinations and biochemical and tolerance tests were performed on this medium after up to 6 days incubation as described previously (Tiago *et al.*, 2005b). The growth temperature range of the strain was examined in liquid medium in a reciprocal water bath between 20 and 45 °C. The NaCl range for growth of the organism was determined at pH 8.0 and 37 °C. The pH range for growth was determined at 37 °C in the same medium buffered at pH values between pH 6.0 and 9.5 as described previously (Tiago *et al.*, 2005b). Anaerobic growth was assessed at 37 °C in anaerobic chambers with an H₂/CO₂ atmosphere (bioMérieux). Single carbon source assimilation tests were performed in 20 ml screw-capped tubes as described previously (Tiago *et al.*, 2005a). Acid production from carbohydrates was also determined using API 50CH test

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B22^T is AM114102.

strips (Analytab Products; bioMérieux) containing 50 CHB/E medium, as described previously (Tiago *et al.*, 2006). Peptidoglycan analysis was performed according to Schleifer (1985) and Schleifer & Kandler (1972). Respiratory quinone analysis was performed according to Tindall (1989) and the fatty acid profile was determined as described by Tiago *et al.* (2005b) using the standard MIS library Generation Software (Microbial ID).

The DNA G+C content was determined by HPLC as described by Mesbah *et al.* (1989). The 16S rRNA gene was sequenced as described by Tiago *et al.* (2006). Phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004). Phylogenetic trees were constructed using maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) algorithms. Tree topologies were evaluated by performing bootstrap analysis (Felsenstein, 1985) of a dataset of 1000.

Strain B22^T formed small, beige-coloured, spherical colonies. Cells were Gram-positive, motile by two polar flagella and rod-shaped (0.5 µm in width by 3.0–7.0 µm in length), with oval terminal endospores in a non-swollen sporangium. The NaCl concentration for optimum growth was 1.0%, but growth occurred in the absence of NaCl and in medium containing up to 8.0% NaCl. Strains belonging to the phylogenetically most related genera had higher NaCl requirements for optimal growth and they could grow at much higher NaCl concentrations. Moreover, only species of the genus *Oceanobacillus* and strain B22^T were able to grow in media without NaCl (Table 1). The novel organism also had a narrower pH range (pH 7.0–9.0) for growth when compared with members of related genera. Strain B22^T did not reduce nitrate. Casein, starch, DNA, arbutin, aesculin, hippurate, elastin, gelatin and Tweens 20, 40, 60 and 80 were hydrolysed, but xylan and urea were not. Furthermore, B22^T utilized several sugars and proteinaceous substrates.

Strain B22^T had peptidoglycan type A4 α ; L-Lys was the diamino acid at position 3 of the peptidoglycan and the dicarboxylic amino acid present in the cross-linkage was D-Asp. The peptidoglycan composition of the novel strain is unique amongst members of phylogenetically related genera, which are characterized by direct cross-linkage between positions 3 and 4 and by the presence of *meso*-diaminopimelic acid as diamino acid (Table 1). Menaquinone-7 (MK-7) was the major respiratory quinone detected. The fatty acid composition of strain B22^T was characterized by the predominance of branched fatty acids, namely anteiso-15:0, anteiso-17:0 and iso-15:0, which made up 58.9, 18.3 and 15.4% of the total fatty acids, respectively (Table 2).

The DNA G+C content of strain B22^T was 37.9 mol%. Comparative analyses of 1547 nt positions of the 16S rRNA gene sequence of strain B22^T with those of other members of the *Bacillaceae* lineage showed that strain B22^T had a close relationship (98% similarity) with a clone sequence

recovered from an aerosol in an urban environment (GenBank accession no. DQ129343). The cultured and described taxa to which strain B22^T showed the closest 16S rRNA gene sequence similarity were species of the genera *Salinibacillus* (94.3–94.7%), *Virgibacillus* (92.8–95.1%), *Oceanobacillus* (93.2–94.7%) and *Lentibacillus* (92.3–93.1%). The novel isolate and these genera formed a coherent cluster supported by bootstrap analysis at a confidence level of 71%, thus showing the phylogenetic relatedness of this cluster (Fig. 1). Furthermore, this tree topology was found using the maximum-likelihood as well as the neighbour-joining algorithms.

The knowledge of bacteria of the family *Bacillaceae* that grow optimally in media containing NaCl has increased of late and, despite the fact that these organisms share similar characteristics and close phylogenetic relationships, a number of new genera have been described recently. Nevertheless, strain B22^T can be clearly distinguished from the type strains of species of closely related genera, namely by the NaCl requirement for growth, the peculiar peptidoglycan composition present in the cell wall and the relative amounts of the major fatty acid components, in addition to other phenotypic features.

On the basis of these findings, it is proposed that strain B22^T represents a novel species in a new genus, *Paucisalibacillus globulus* gen. nov., sp. nov.

Description of *Paucisalibacillus* gen. nov.

Paucisalibacillus (Pau'ci.sa.li.ba.cil'lus. L. adj. *paucus* few, little; L. n. *sal*, *salis* salt; L. masc. n. *bacillus* a small staff, a wand; N.L. masc. n. *Paucisalibacillus* a rod that needs only small amounts of salt).

Form rod-shaped cells, which stain Gram-positive, are motile by means of two polar flagella at one end and spore-forming. Strictly aerobic, oxidase-negative and catalase-positive. NaCl is not required for growth; small amounts of NaCl improve growth. Peptidoglycan is of the A4 α type with L-Lys as the diamino acid and D-Asp as the dicarboxylic amino acid present in the cross-linkage. Major respiratory quinone is MK-7. Fatty acids are predominantly saturated and branched. Belongs to the family *Bacillaceae*. The type species is *Paucisalibacillus globulus*.

Description of *Paucisalibacillus globulus* sp. nov.

Paucisalibacillus globulus [glo'bu.lus. L. n. *globulus* (nominative in apposition) a little ball, a globule, because the bacterium forms colonies that are similar to a little ball, a globule].

Exhibits the following characteristics in addition to those described for the genus. Cells are 0.5 µm in width by 3.0–7.0 µm in length. Oval endospores are formed in a terminal non-swollen sporangium. Heterotrophic. Colonies are small, smooth, spherical and beige-coloured. The

Table 1. Comparison of characteristics of strain B22^T (*Paucisolibacillus* gen. nov.) with those of phylogenetically related genera of the family *Bacillaceae*

Genera: 1, *Paucisolibacillus* gen. nov.; 2, *Salinibacillus* (data from Ren & Zhou, 2005); 3, *Virgibacillus* (Yoon *et al.*, 2005; Lee *et al.*, 2006); 4, *Oceanobacillus* (Lee *et al.*, 2006); 5, *Lentibacillus* (Jeon *et al.*, 2005). +, Positive result or growth; –, negative result or no growth; v, variable results between strains; ND, no data available. Members of all genera are catalase-positive.

Characteristic	1	2	3	4	5
Anaerobic growth	–	–	v	v	ND
NaCl requirement for growth	–	+	+	–	+
NaCl range (%)	0–8	1–30	0.5–25.0	0–22	1–23
NaCl optimum (%)	1	12–15	4–10	3	4–15
Growth temperature range (°C)	20–45	20–50	5–50	15–42	15–40
Optimum pH	8.0–8.5	7.0–8.0	7.0–7.4	7.0–9.5	6.0–8.0
pH range	7.0–9.0	6–10	5.5–10.0	6.5–10.0	ND
Oxidase	–	v	+	v	+
DNase	+	–	+	–	ND
Degradation/hydrolysis of:					
Starch	+	v	v	–	–
Aesculin	+	+	v	ND	v
Gelatin	+	+	v	+	ND
Casein	–	+	+	+	v
Tween 20	+	–	ND	ND	ND
Tween 40	+	+	ND	+	ND
Tween 60	+	–	ND	+	ND
Tween 80	+	–	v	ND	v
Major fatty acids*	ai-15:0, ai-17:0, i-15:0	ai-15:0, i-15:0, ai-17:0, i-16:0	ai-15:0, i-15:0, ai-17:0	ai-15:0, i-15:0, ai-17:0	ai-15:0, i-16:0, ai-17:0
Cell-wall type	A4 α	A1 γ	A1 γ	A1 γ	A1 γ

*Anteiso- and iso-branched fatty acids are respectively indicated by ai and i.

Table 2. Fatty acid composition of strain B22^T (*Paucisolibacillus* gen. nov.) and the type strains of species of the most closely related genera

Genera: 1, *Paucisolibacillus* (data for the type strain of the type species); 2, *Salinibacillus* (two species) (data from Ren & Zhou, 2005); 3, *Virgibacillus* (ten species) (Yoon *et al.*, 2005; Lee *et al.*, 2006); 4, *Oceanobacillus* (three species) (Lu *et al.*, 2001; Lee *et al.*, 2006); 5, *Lentibacillus* (four species) (Jeon *et al.*, 2005). Data are mean $\pm$ SD percentages of each fatty acid. Abbreviations: i, iso; ai, anteiso; tr, trace (<0.5%); –, not detected.

Fatty acid	1	2	3	4	5
i-13:0	tr	tr	1.2 $\pm$ 0.1	–	tr
ai-13:0	tr	tr	0.4 $\pm$ 0.1	–	–
i-14:0	1.4	2.6 $\pm$ 0.1	3.3 $\pm$ 1.6	5.7 $\pm$ 4.1	10.5 $\pm$ 4.4
14:0	0.5	0.5 $\pm$ 0.1	–	1.6 $\pm$ 2.8	tr
i-15:0	15.4	23.2 $\pm$ 6.8	26.1 $\pm$ 14.2	19.1 $\pm$ 14.7	7.6 $\pm$ 6.2
ai-15:0	58.9	31.5 $\pm$ 10.7	39.5 $\pm$ 9.3	42.6 $\pm$ 15.1	35.5 $\pm$ 6.8
15:0	–	–	0.5 $\pm$ 0.1	0.3 $\pm$ 0.5	tr
i-16:0	2.5	10.9 $\pm$ 1.5	6.4 $\pm$ 1.3	7.0 $\pm$ 3.6	25.5 $\pm$ 7.1
16:0	1.1	2.3 $\pm$ 1.1	0.9 $\pm$ 0.2	5.8 $\pm$ 7.4	1.7 $\pm$ 0.8
i-17:0	1.1	7.6 $\pm$ 5.4	5.0 $\pm$ 2.5	2.7 $\pm$ 2.3	2.4 $\pm$ 1.9
ai-17:0	18.3	21.1 $\pm$ 3.7	16.0 $\pm$ 10.6	12.3 $\pm$ 7.0	16.2 $\pm$ 6.9

Fig. 1. Phylogenetic dendrogram based on a comparison of 16S rRNA gene sequences of strain B22^T and its closest phylogenetic relatives. The tree was created using the neighbour-joining method. Numbers on the tree indicate the percentages of bootstrap sampling, derived from 1000 replications. Besides those indicated, 16S rRNA gene sequences from strains of the following species were used to produce the tree (GenBank accession nos are given in parentheses): *Salinibacillus aidingensis* (AY321436), *Salinibacillus kushneri* (AY321437), *Virgibacillus halodenitrificans* (AY543168), *Virgibacillus pantothenicus* (AB039331), *Virgibacillus marismortui* (AJ009793), *Virgibacillus proomii* (AJ012667), *Virgibacillus dokdonensis* (AY822043), *Virgibacillus salexigens* (Y11603), *Lentibacillus salarius* (AY667493), *Lentibacillus juripiscarius* (AB127980), *Lentibacillus salicampi* (AY057394), *Oceanobacillus iheyensis* (AB010863), *Oceanobacillus picturae* (AJ315060) and *Oceanobacillus oncorhynchi* (AB188089). Bar, 1 inferred nucleotide substitution per 100 nt.

optimum temperature for growth is about 37 °C and no growth occurs at 15 or 45 °C; the optimum pH is between 8.0 and 8.5 and no growth occurs at pH 6.0 or 9.5. Grows in 0–8 % NaCl; optimal growth occurs in media containing 1 % (w/v) NaCl. Predominant fatty acids are anteiso-15:0, anteiso-17:0 and iso-15:0, which comprise over 90 % of the total fatty acids. Hydrolyses casein, starch, DNA, arbutin, aesculin, hippurate, elastin, gelatin and Tweens 20, 40, 60 and 80. Xylan and urea are not hydrolysed. DNase is detected. Does not reduce nitrate. Assimilates glucose, mannose, galactose, fructose, L-sorbose, D-xylose, sucrose, maltose, lactose, trehalose, L-rhamnose, raffinose, L-fucose, ribitol, xylitol, L-erythritol, mannitol, 2-oxoglutarate, lactate, malate, pyruvate, acetate, L-glutamate, glycine, serine and threonine. Does not utilize arabinose, ribose, melibiose, cellobiose, melibiose, sorbitol, arabinol, *myo*-inositol, glycerol, succinate, citrate, aspartate, alanine, asparagine, cysteine, phenylalanine, histidine, isoleucine, lysine, methionine, proline, glutamine, arginine, valine or ornithine. Acid is produced from ribose, xylose, glucose, mannose, fructose, mannitol, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, sucrose, starch, glycogen, gentiobiose, turanose, tagatose and 5-ketogluconate, but not from the other substrates in the API 50CH test strips.

The type strain is B22^T (= CIP 108857^T = LMG 23148^T), isolated from potting soil. The DNA G + C content of strain B22^T is 37.9 mol%.

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References

- Edelstein, P. (1981). Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J Clin Microbiol* **14**, 298–303.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus pantothenicus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenicus*. *Int J Syst Bacteriol* **48**, 99–106.
- Jeon, C. O., Lim, J. M., Lee, J. C., Lee, G. S., Lee, J. M., Xu, L. H., Jiang, C. L. & Kim, C. J. (2005). *Lentibacillus salarius* sp. nov., isolated from saline sediment in China, and emended description of the genus *Lentibacillus*. *Int J Syst Evol Microbiol* **55**, 1339–1343.
- Lee, J.-S., Lim, J.-M., Lee, K. C., Lee, J.-C., Park, Y.-H. & Kim, C.-J. (2006). *Virgibacillus koreensis* sp. nov., a novel bacterium from a salt field, and transfer of *Virgibacillus picturae* to the genus *Oceanobacillus* as *Oceanobacillus picturae* comb. nov. with emended descriptions. *Int J Syst Evol Microbiol* **56**, 251–257.
- Lu, J., Nogi, Y. & Takami, H. (2001). *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge. *FEMS Microbiol Lett* **205**, 291–297.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- 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.
- Ren, P. G. & Zhou, P. J. (2005). *Salinibacillus aidingensis* gen. nov., sp. nov. and *Salinibacillus kushneri* sp. nov., moderately halophilic bacteria isolated from a neutral saline lake in Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 949–953.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

- 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.
- Tiago, I., Mendes, V., Pires, C., Morais, P. V. & Veríssimo, A. (2005a).** *Phenylobacterium falsum* sp. nov., an alphaproteobacterium isolated from a nonsaline alkaline groundwater, and emended description of the genus *Phenylobacterium*. *Syst Appl Microbiol* **28**, 295–302.
- Tiago, I., Pires, C., Mendes, V., Morais, P. V., da Costa, M. & Veríssimo, A. (2005b).** *Microcella putealis* gen. nov., sp. nov., a Gram-positive alkaliphilic bacterium isolated from a nonsaline alkaline groundwater. *Syst Appl Microbiol* **28**, 479–487.
- Tiago, I., Mendes, V., Pires, C., Morais, P. V. & Veríssimo, A. (2006).** *Chimaericella alkaliphila* gen. nov., sp. nov., a Gram-negative alkaliphilic bacterium isolated from a nonsaline alkaline groundwater. *Syst Appl Microbiol* **29**, 100–108.
- Tindall, B. J. (1989).** Fully saturated menaquinones in the archaeobacterium *Pyrobaculum islandicum*. *FEMS Microbiol Lett* **60**, 251–254.
- Yoon, J. H., Kang, K. H. & Park, Y. H. (2002).** *Lentibacillus salicampi* gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt field in Korea. *Int J Syst Evol Microbiol* **52**, 2043–2048.
- Yoon, J. H., Kang, S. J., Lee, S. Y., Lee, M. H. & Oh, T. K. (2005).** *Virgibacillus dokdonensis* sp. nov., isolated from a Korean island, Dokdo, located at the edge of the East Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1833–1837.