Paenibacillus phoenicis sp. nov., isolated from the Phoenix Lander assembly facility and a subsurface molybdenum mine

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A novel Gram-positive, motile, endospore-forming, aerobic bacterium was isolated from the NASA Phoenix Lander assembly clean room that exhibits 100 % 16S rRNA gene sequence similarity to two strains isolated from a deep subsurface environment. All strains are rod-shaped, endospore-forming bacteria, whose endospores are resistant to UV radiation up to 500 J m⁻². A polyphasic taxonomic study including traditional phenotypic tests, fatty acid analysis, 16S rRNA gene sequencing and DNA–DNA hybridization analysis was performed to characterize these novel strains. The 16S rRNA gene sequencing convincingly grouped these novel strains within the genus *Paenibacillus* as a separate cluster from previously described species. The similarity of 16S rRNA gene sequences among the novel strains was identical but only 98.1 to 98.5 % with their nearest neighbours *Paenibacillus barengoltzii* ATCC BAA-1209^T and *Paenibacillus timonensis* CIP 108005^T. The menaquinone MK-7 was dominant in these novel strains as shown in other species of the genus *Paenibacillus*. The DNA–DNA hybridization dissociation value was <45 % with the closest related species. The novel strains had DNA G + C contents of 51.9 to 52.8 mol%. Phenotypically, the novel strains can be readily differentiated from closely related species by the absence of urease and gelatinase and the production of acids from a variety of sugars including L-arabinose. The major fatty acid was anteiso-C_{15:0} as seen in *P. barengoltzii* and *P. timonensis* whereas the proportion of C_{16:0} was significantly different from the closely related species. Based on phylogenetic and phenotypic results, it was concluded that these strains represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus phoenicis* sp. nov. is proposed. The type strain is 3PO2SA^T (=NRRL B-59348^T =NBRC 106274^T).

Species of the endospore-forming genus *Bacillus* have been frequently isolated from spacecraft and associated surfaces (La Duc *et al.*, 2003, 2007; Puleo *et al.*, 1977). In addition, members of the genera *Paenibacillus*, *Filibacter*, *Sporosarcina* and *Streptomyces* have recently been isolated from these environments (Osman *et al.*, 2006; Venkateswaran *et al.*, 2001). Based on 16S rRNA gene sequencing analysis, group 3 members were separated from the genus *Bacillus* and assigned to the genus *Paenibacillus* (Ash *et al.*, 1991). At

the time of writing this manuscript, there were 110 species of this genus with validly published names (<http://www.bacterio.cict.fr/paenibacillus.html>) isolated from a variety of environmental niche, including *Paenibacillus barengoltzii* and *Paenibacillus pasadenensis* from spacecraft assembly clean rooms (Osman *et al.*, 2006). While characterizing strains isolated from the Payload Hazardous Servicing Facility (PHSF), where the Phoenix spacecraft was assembled (Ghosh *et al.*, 2010), it was observed that endospores of strain 3PO2SA^T exhibited greater tolerance to UV irradiation conditions than *Bacillus subtilis* 168 endospores (Venkateswaran *et al.*, 2003).

Two strains having phenotypic and 16S rRNA gene sequence similarity with 3PO2SA^T were collected in

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 3PO2SA^T, HM06-03 and HM06-05 are EU977789, EU004565 and EU004567, respectively.

Three supplementary figures are available with the online version of this paper.

March 2005 from the deep-seat fluids at 2141 m (7025 ft) below the surface of granite bore hole 1 of the Henderson molybdenum mine in Colorado, USA (Sahl *et al.*, 2008). Isolates from the iron oxide precipitates of the seat fluids were filtered and plated onto *Leptothrix* media (Mayhew *et al.*, 2008) and included in this study. A more detailed description of sample collection and analysis of these strains has been reported elsewhere (Mayhew *et al.*, 2008). Strain 3P02SA^T and the isolates retrieved from the deep subsurface mine shared 100 % 16S rRNA gene sequence similarity (all 3 strains), and yet were distantly related to previously described species of the genus *Paenibacillus* (~98 % similarity). Here we describe the phylogenetic affiliation of these novel strains using a polyphasic taxonomic approach. In addition to the three novel strains, *P. barengoltzii* ATCC BAA-1209^T, *Paenibacillus timonensis* CIP 108005^T and *Paenibacillus macerans* ATCC 8244^T were included in this study for all phenotypic, chemotaxonomic and genotypic characterization analyses.

The isolated strains described herein were routinely cultured on tryptone soy agar (TSA; BD) plates (72 h at 32 °C) for various analyses. The morphologies of all colonies were circular, raised, entire edged and beige in 72-hour-old cultures, with no pigmentation even after prolonged incubation. A modified Schaeffer's medium (MSM), which consisted of 0.1 % KCl, 0.012 % MgCl₂, 1.0 mM Ca(NO₃)₂, 0.01 mM MnCl₂, 0.001 mM FeSO₄ and 8 g nutrient broth in 1.0 litre distilled deionized water, was used to induce sporulation and then the spores were further purified as previously described (Kempf *et al.*, 2005). A single colony of each strain was inoculated in 10 ml MSM medium and incubated for 48 to 72 h at 32 °C, before being subjected to phase-contrast light microscopy (Olympus BX-90). All strains initially showed rod-shaped vegetative cells that occurred singly or in aggregates.

Surface details and endospore architecture were examined using scanning electron microscopy (NOVA nanoSEM600, EDAX; Cole & Popkin, 1981) and transmission electron microscopy (TEM; Technai 12, FEI) (Supplementary Figure S1 available in IJSEM Online). Samples were prepared for TEM as follows. Sporulating cells (500 µl; 10⁷) were gently concentrated at 3000 r.p.m. and fixed in 0.1 M sodium cacodylate buffer containing 2.5 % glutaraldehyde (pH 7.2) at 4 °C for 24 h. The suspension was mixed with 2 % agarose to bind sporulating cells, which were then fixed in 0.1 M sodium cacodylate buffer containing 2 % osmium tetroxide (pH 7.2) for 2 h. The reaction mixtures were further washed with increasing concentrations of alcohol and finally embedded in epoxy resin. Thin sections of these preparations (50 nm) were made using an ultramicrotome (Leica Microsystems), which were subsequently stained with aqueous 2 % uranyl acetate and Reynolds lead citrate to enhance contrast.

All strains were grown for 2 days at 32 °C, harvested and observed via phase-contrast microscopy (Olympus) to verify that 99 % of the images were endospores; these spore

suspensions were further studied for their resistance to UV₂₅₄ (Newcombe *et al.*, 2005). The cultures were washed with sterile PBS (pH 7.4) and diluted in sterile PBS to obtain an optical density at 600 nm of 0.01 (equivalent to ~10⁶ endospores ml⁻¹). Appropriate aliquots of the endospore suspension were placed in an uncovered sterile Petri dish and exposed to UV₂₅₄ radiation (0, 200, 500, 1000 and 1500 J m⁻²) using a hand-held UV instrument at a fixed distance (Newcombe *et al.*, 2005). At appropriate intervals, 100 µl of the reaction mixtures were removed, spread plated on TSA in duplicate and incubated at 32 °C for 2 to 5 days. All strains were resistant up to 500 J m⁻² and were ~2 to 3 times more resistant than well-studied *B. subtilis* endospores (Newcombe *et al.*, 2005).

The ability to grow with varying concentrations of NaCl (1–10 %, w/v) was determined in 1 % Bacto tryptone containing varying amounts of NaCl. Similarly, growth under various pH (5 to 11) and temperature (25 to 60 °C) conditions was measured as published elsewhere (Osman *et al.*, 2006). Biochemical tests to characterize phenotypes were carried out using API 20NE and API 50CH test strips with API 50CHB suspension medium (BioMérieux) according to the manufacturer's instructions. The results of carbon substrate utilization and acid production are given in Table 1. API 20NE tests were used to determine nitrate reduction, aesculin hydrolysis, β-galactosidase production, fermentation of glucose, ONPG, urease, gelatinase and indole production. API 50CH was used to determine all acid production from carbohydrates. Phenotypic characteristics that readily distinguish the novel strains from the closely related species *P. barengoltzii* and *P. timonensis* are the inability to produce urease, gelatinase and tryptophan deaminase. The deep subsurface isolates (HM06-03 and HM06-05) produced lysine- and ornithine decarboxylases whereas the novel clean room isolate produced indole. Unlike *P. barengoltzii* and *P. timonensis*, the novel strains produced acids from D-ribose, D-mannitol, xylitol and inulin. The other differential phenotypic characteristics are shown in Table 1.

Cellular fatty acid methyl esters (FAME) were analysed with the Microbial ID system (MIDI) according to manufacturer's instructions. The FAME profiles of the three strains of this study and other closely related species of the genus *Paenibacillus* are given in Table 2. No significant differences were observed when the cellular fatty acid profiles of three strains of *Paenibacillus phoenicis* sp. nov. were compared with one another, supporting their affiliation to a single species. C_{16:0} was found to be significantly higher in strain 3P02SA^T (29.3 %) compared with closely related *P. barengoltzii* ATCC BAA-1209^T (2.1 %). Significant differences in the proportions of iso-C_{15:0}, C_{16:0}, C_{12:0}, C_{14:0} and iso-C_{17:1}ω10c were also observed between the novel strains described herein and related species of the genus *Paenibacillus* (Table 2). The isoprenoid quinone (Akagawa-Matsushita *et al.*, 1992) and diaminopimelic acid in the whole cell (Staneck & Roberts, 1974) were analysed using established methods. All three

Table 1. Phenotypic characteristics of *Paenibacillus phoenicis* sp. nov. and related species of the genus *Paenibacillus*

Strains: 1, *Paenibacillus phoenicis* sp. nov. 3P02SA^T; 2, *P. phoenicis* sp. nov. HM06-03; 3, *P. phoenicis* sp. nov. HM06-05; 4, *P. barengoltzii* ATCC BAA-1209^T; 5, *P. timonensis* CIP 108005^T; 6, *P. macerans* ATCC 8244^T. Data for all six strains were determined in this study. All strains showed negative reactions for growth at pH 10, H₂S production, aesculin hydrolysis, nitrate and nitrite reduction, acid production from: glycerol, erythritol, L-xylose, D-adonitol, dulcitol, inositol, D-sorbitol, D-tagatose, D-fucose, L-fructose, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate; and positive reactions for growth at pH 7.0 and utilization of: citrate, glucose, mannitol, inositol, rhamnose, sorbitol, sucrose, melibiose, amygdalin, arabinose and aesculin ferric citrate. +, Positive; –, negative. ND, no data.

Characteristic	1	2	3	4	5	6
Growth at/with:						
37 °C	+	+	+	+	+	ND
50 °C	+	+	+	+	+	ND
5 % NaCl	+	+	+	–	–	ND
pH 5.0	–	–	–	+	ND	ND
pH 10.0	+	–	–	+	ND	ND
Urease	–	–	–	+	+	–
Hydrolysis of gelatin	–	–	–	+	+	–
Tryptophan deaminase	–	–	–	+	–	–
ONPG reaction	+	+	–	+	+	+
Lysine decarboxylase	–	+	+	+	+	+
Ornithine decarboxylase	–	+	+	+	+	+
Voges-Proskauer	+	–	–	+	–	+
Acid production from:						
Sucrose	+	+	+	–	–	–
L-Arabinose	+	+	+	–	+	–
D-Xylose	+	+	+	–	+	–
D-Galactose	+	+	+	–	+	–
D-Glucose	+	+	+	–	+	–
D-Fructose	+	+	+	–	+	–
D-Mannose	+	+	+	–	+	–
N-Acetylglucosamine	+	+	+	–	+	–
Amygdalin	+	+	+	–	+	–
Arbutin	+	+	+	–	+	–
Salicin	+	+	+	–	+	–
Lactose (bovine)	+	+	+	–	+	–
Starch	+	+	+	–	+	–
Glycogen	+	+	+	–	+	–
Gentiobiose	+	+	+	–	+	–
Trehalose	+	+	+	–	+	+
L-Sorbose	–	–	–	–	–	+
D-Arabinose	–	–	–	–	+	–
L-Rhamnose	–	–	–	–	+	–
D-Ribose	+	–	–	–	–	–
D-Mannitol	+	–	–	–	–	–
Xylitol	+	–	–	–	–	–
Methyl β-D-xylopyranoside	+	–	–	–	+	–
Melezitose	–	–	+	–	–	–
Turanose	–	–	+	–	–	–
D-Arabitol	–	–	+	–	–	–
Methyl α-D-mannopyranoside	–	–	+	–	+	–

Table 1. cont.

Characteristic	1	2	3	4	5	6
Methyl α-D-glucopyranoside	–	–	+	–	+	–
Inulin	+	–	+	–	–	–
Cellobiose	+	–	+	–	+	–
Maltose	+	–	+	–	+	–
D-Raffinose	+	–	+	–	+	–
Potassium gluconate	+	–	+	–	+	–
Melibiose	+	+	–	–	+	–

novel strains contained MK-7 menaquinone as the major isoprenoid quinone and *meso*-diaminopimelic acid that is normally observed in species of the genus *Paenibacillus*.

Genomic DNA was isolated from all three *Paenibacillus phoenicis* sp. nov. strains and the other type strains tested during this study using established procedures (La Duc *et al.*, 2009). The 16S rRNA gene was PCR-amplified using a universal primer set and sequenced bi-directionally as reported elsewhere (La Duc *et al.*, 2007). The 16S rRNA gene sequence (~1.4 kb) similarity was compared with existing sequences in the public database (<http://www.ncbi>).

Table 2. Cellular fatty acid profiles of *Paenibacillus phoenicis* sp. nov. and related species of the genus *Paenibacillus*

Strains: 1, *Paenibacillus phoenicis* sp. nov. 3P02SA^T; 2, *P. phoenicis* sp. nov. HM06-03; 3, *P. phoenicis* sp. nov. HM06-05; 4, *P. barengoltzii* ATCC BAA-1209^T; 5, *P. timonensis* CIP 108005^T (=2301032^T); 6, *P. macerans* ATCC 8244^T. Values represent percentages of the total fatty acid composition. Data for all six strains were determined in this study.

Fatty acid	1	2	3	4	5	6
Straight-chain saturated						
C _{12:0}	2.4	2.5	2.8			
C _{14:0}	8.9	14.8	11.0	0.3	5.0	0.3
C _{15:0}	0.6				0.4	
C _{16:0}	29.3	32.8	21.7	2.1	18.3	2.0
Branched saturated						
iso-C _{14:0}	1.2	2.6	1.8	0.8	3.4	0.9
iso-C _{15:0}	3.4		3.5	24.4	7.6	24.6
iso-C _{16:0}	8.1	10.8	10.9	2.6	9.7	2.7
iso-C _{17:0}	2.0		2.3	9.7	2.5	9.4
anteiso-C _{11:0}	0.7				0.3	
anteiso-C _{15:0}	30.9	30.9	35.1	40.7	46.3	40.9
anteiso-C _{13:0}	0.7		0.9		0.3	
anteiso-C _{17:0}	11.0	5.6	10.1	12.6	6.1	12.1
Monounsaturated						
C _{16:1} ω11c				1.3		1.4
C _{16:1} ω7c alcohol				0.8		0.8
iso-C _{17:1} ω10c				2.9		3.1
Unknown fatty acids						
iso-C _{17:1} I/anteiso-C _{17:1} B	0.8			1.93		1.98

nlm.nih.gov/) using the BLAST function. Neighbour-joining (Fig. 1) and maximum-parsimony (Supplementary Fig. S3) phylogenetic analysis was performed using the program MEGA4 (Tamura *et al.*, 2007). The three novel strains shared 100 % sequence similarity amongst each other and formed a well-defined cluster (Fig. 1). *P. barengoltzii* ATCC BAA-1209^T and *P. timonensis* CIP 108005^T shared high (98.5 % and 98.1 %, respectively) sequence similarity with type strain 3P02SA^T while all other previously reported type strains of species of the genus *Paenibacillus* exhibited less than 97.5 % sequence similarity.

In order to elucidate the genotypic variations among the three strains and related type strains of the genus *Paenibacillus*, the extracted DNA was amplified using the *Bacillus* DiversiLab DNA Fingerprinting kit (Spectral Genomics), according to the protocol established by Healy *et al.* (2005). The detailed report from this typing method and the resulting dendrogram are given in Supplementary Fig S2. The type strain, 3P02SA^T, clustered together with strains HM06-05 and HM06-03 with high similarity (99–93 %). Distinct DNA fingerprinting patterns were exhibited by *P. barengoltzii*, *P. macerans* and *P. timonensis* with very low similarity with type strain 3P02SA^T (<71 %). It was also observed that the DiversiLab DNA Fingerprinting kit used was not successful in differentiating some of the species of the genus *Paenibacillus* tested.

DNA from strains 3P02SA^T, HM06-03, HM06-05, *P. barengoltzii* ATCC BAA-1209^T, *P. macerans* ATCC 8244^T and *P. timonensis* CIP 108005^T was prepared by a standard method

(Sambrook *et al.*, 1989). The G + C content of the DNA was analysed by HPLC (Tamaoka & Komagata, 1984). The DNA G + C content of the type strain, 3P02SA^T, was 52.5 mol% and of the other two strains, HM06-05 and HM06-03, was 51.9 and 52.8 mol%, respectively. These values were similar to those of other related species of the genus *Paenibacillus* (*P. barengoltzii* ATCC BAA-1209^T 52.3 mol%, *P. macerans* ATCC 8244^T 53.0 mol% and *P. timonensis* CIP 108005^T 53.9 mol%). DNA–DNA relatedness of the three novel strains and the type strains of *P. barengoltzii*, *P. macerans* and *P. timonensis* was performed by the microplate hybridization method (Ezaki *et al.*, 1989). Means of the three most confident readings were taken for calculation purposes. Strain 3P02SA^T exhibited 70.3 % and 77.7 % similarity to the deep subsurface strains HM06-03 and HM06-05, respectively. On the other hand, strain 3P02SA^T showed less than 70 % similarity to *P. barengoltzii* ATCC BAA-1209^T (35.7 %), *P. timonensis* CIP 108005^T (43.7 %) and *P. macerans* ATCC 8244^T (18.3 %). The low (<70 %) DNA–DNA relatedness values between the novel strains and their close relatives preclude genomic relatedness and support designation of strain 3P02SA^T and the subsurface strains as representatives of a novel species within the genus *Paenibacillus* (Stackebrandt & Goebel, 1994; Wayne, 1988).

The results of the DNA hybridization combined with phenotypic comparisons indicate that all of the novel isolates belong to the genus *Paenibacillus* but are significantly distinct from any previously described species. Based on the data described herein, all three

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of *Paenibacillus phoenicis* sp. nov. with respect to closely related species of the genus *Paenibacillus*. Strain numbers and GenBank accession numbers (in parentheses) are shown. Bootstrap percentages based on 1000 replications (greater than 50 %) are shown at branch points of the tree. Bar, 0.005 substitutions per nucleotide position.

strains (type strain 3PO2SA^T) represent a novel species, for which the name *Paenibacillus phoenicis* sp. nov. is proposed.

Description of *Paenibacillus phoenicis* sp. nov.

Paenibacillus phoenicis (phoe'ni.cis. L. gen. n. *phoenicis* of phoenix, isolated from the surface of the clean room where the recently landed Mars Phoenix spacecraft was assembled).

Vegetative cells are rod-shaped with a diameter of 1 to 1.5 µm and a length of 4 to 6 µm, motile, Gram-positive, produce catalase and form endospores which are subterminal in position and have a diameter of 1 to 1.5 µm. Colonies form after 72 h of growth on TSA at 32 °C, are beige, smooth, glistening, have a diameter of 4 mm, show no pigmentation and possess entire edges. Grows at 32 °C on TSA (Difco), R2A (Difco) and TYG (5 g tryptone, 3 g yeast extract and 1 g glucose per litre). Produces oval endospores in MSM medium which are resistant to UV radiation up to 500 J m⁻². Tests for urease, tryptophan deaminase and hydrolysis of gelatin are negative. Assimilation of the following sole carbon sources is positive: glucose, mannitol, inositol, rhamnose, sorbitol, sucrose, melibiose, amygdalin, L-arabinose, D-arabinose, sucrose, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, arbutin, salicin, lactose (bovine), starch, aesculin ferric citrate, glycogen, gentiobiose and trehalose. Acid was not produced from the following substrates: glycerol, erythritol, L-xylose, D-adonitol, dulcitol, inositol, D-sorbitol, D-tagatose, D-fucose, L-fructose, L-arabitol, potassium 2-ketogluconate, potassium 5-ketogluconate, L-sorbose, D-arabinose and L-rhamnose. Positive for citrate utilization. Growth occurs between 21 and 50 °C and at pH 7.0–9.0 (pH 10.0 for strain 3PO2SA^T). Optimum growth occurs at 37 °C and at pH 7.0. Growth occurs in the presence of 5% NaCl. Growth is inhibited at 65 °C and in the presence of 10% NaCl. The major fatty acids are anteiso-C_{15:0} and C_{16:0}. Major isoprenoid quinone is MK-7 menaquinone and the cell wall contains *meso*-diaminopimelic acid. The DNA G+C content is 51.9 to 52.8 mol%.

The type strain, 3PO2SA^T (=NRRL B-59348^T =NBRC 106274^T), was isolated from surfaces of the Payload Hazardous Servicing Facility (PHSF) located at the Kennedy Space Center where the Phoenix Lander was assembled. The DNA G+C content of the type strain, 3PO2SA^T, is 52.5 mol%. The type strain, 3PO2SA^T, differs from the other two strains, HM06-03 and HM06-05, by producing β-galactosidase and being positive for the Voges–Proskauer reaction but lacks production of lysine and ornithine decarboxylases.

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References

- Akagawa-Matsushita, M., Itoh, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- Cole, R. M. & Popkin, T. J. (1981). Electron microscopy. In *Manual of Methods for General Bacteriology*, pp. 34–51. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilaw, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, D.C.: American Society for Microbiology.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Ghosh, S., Osman, S., Vaishampayan, P. & Venkateswaran, K. (2010). Recurrent isolation of extremotolerant bacteria from the clean room where Phoenix spacecraft components were assembled. *Astrobiology* **10**, 325–335.
- Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A. & other authors (2005). Microbial DNA typing by automated repetitive-sequence-based PCR. *J Clin Microbiol* **43**, 199–207.
- Kempf, M. J., Chen, F., Kern, R. & Venkateswaran, K. (2005). Recurrent isolation of hydrogen peroxide-resistant spores of *Bacillus pumilus* from a spacecraft assembly facility. *Astrobiology* **5**, 391–405.
- La Duc, M. T., Nicholson, W., Kern, R. & Venkateswaran, K. (2003). Microbial characterization of the Mars Odyssey spacecraft and its encapsulation facility. *Environ Microbiol* **5**, 977–985.
- La Duc, M. T., Dekas, A. E., Osman, S., Moissl, C., Newcombe, D. & Venkateswaran, K. (2007). Isolation and characterization of bacteria capable of tolerating the extreme conditions of clean room environments. *Appl Environ Microbiol* **73**, 2600–2611.
- La Duc, M. T., Osman, S. & Venkateswaran, K. (2009). Comparative analysis of methods for the purification of DNA from low-biomass samples based on total yield and conserved microbial diversity. *J Rapid Meth Auto Microbiol* **17**, 350–368.
- Mayhew, L. E., Swanner, E. D., Martin, A. P. & Templeton, A. S. (2008). Phylogenetic relationships and functional genes: distribution of a gene (mnxG) encoding a putative manganese-oxidizing enzyme in *Bacillus* species. *Appl Environ Microbiol* **74**, 7265–7271.
- Newcombe, D. A., Schuerger, A. C., Benardini, J. N., Dickinson, D., Tanner, R. & Venkateswaran, K. (2005). Survival of spacecraft-associated

microorganisms under simulated martian UV irradiation. *Appl Environ Microbiol* **71**, 8147–8156.

Osman, S., Satomi, M. & Venkateswaran, K. (2006). *Paenibacillus pasadenensis* sp. nov. and *Paenibacillus barengoltzii* sp. nov., isolated from a spacecraft assembly facility. *Int J Syst Evol Microbiol* **56**, 1509–1514.

Puleo, J. R., Fields, N. D., Bergstrom, S. L., Oxborrow, G. S., Stabekis, P. D. & Koukol, R. (1977). Microbiological profiles of the Viking spacecraft. *Appl Environ Microbiol* **33**, 379–384.

Sahl, J. W., Schmidt, R., Swanner, E. D., Mandernack, K. W., Templeton, A. S., Kieft, T. L., Smith, R. L., Sanford, W. E., Callaghan, R. L. & other authors (2008). Subsurface microbial diversity in deep-granitic-fracture water in Colorado. *Appl Environ Microbiol* **74**, 143–152.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* **44**, 846–849.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Venkateswaran, K., Satomi, M., Chung, S., Kern, R., Koukol, R., Basic, C. & White, D. C. (2001). Molecular microbial diversity of a spacecraft assembly facility. *Syst Appl Microbiol* **24**, 311–320.

Venkateswaran, K., Kempf, M., Chen, F., Satomi, M., Nicholson, W. & Kern, R. (2003). *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are gamma-radiation resistant. *Int J Syst Evol Microbiol* **53**, 165–172.

Wayne, L. G. (1988). International Committee on Systematic Bacteriology: announcement of the report of the ad hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Zentralbl Bakteriol Mikrobiol Hyg [A]* **268**, 433–434.