

Bacillus horneckiae sp. nov., isolated from a spacecraft-assembly clean room

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Five Gram-stain-positive, motile, aerobic strains were isolated from a clean room of the Kennedy Space Center where the Phoenix spacecraft was assembled. All strains are rod-shaped, spore-forming bacteria, whose spores were resistant to UV radiation up to 1000 J m⁻². The spores were subterminally positioned and produced an external layer. A polyphasic taxonomic study including traditional biochemical tests, fatty acid analysis, cell-wall typing, lipid analyses, 16S rRNA gene sequencing and DNA–DNA hybridization studies was performed to characterize these novel strains. 16S rRNA gene sequencing and lipid analyses convincingly grouped these novel strains within the genus *Bacillus* as a cluster separate from already described species. The similarity of 16S rRNA gene sequences among the novel strains was >99%, but the similarity was only about 97% with their nearest neighbours *Bacillus pocheonensis*, *Bacillus firmus* and *Bacillus bataviensis*. DNA–DNA hybridization dissociation values were <24% to the closest related type strains. The novel strains had a G + C content 35.6 ± 0.5 mol% and could liquefy gelatin but did not utilize or produce acids from any of the carbon substrates tested. The major fatty acids were iso-C_{15:0} and anteiso-C_{15:0} and the cell-wall diamino acid was meso-diaminopimelic acid. Based on phylogenetic and phenotypic results, it is concluded that these strains represent a novel species of the genus *Bacillus*, for which the name *Bacillus horneckiae* sp. nov. is proposed. The type strain is 1P01SC^T (=NRRL B-59162^T =MTCC 9535^T).

During microbial diversity surveys performed since 2000, several hundred aerobic microbial strains have been isolated from spacecraft-associated surfaces (NASA, 1980) and characterized for their taxonomic affiliation (Kempf *et al.*, 2005; La Duc *et al.*, 2004b, c; Venkateswaran *et al.*,

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Abbreviations: DAP, diaminopimelic acid; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Bacillus horneckiae* strains 1P01SC^T, 1P02SA, 1P08SA, 1P10SC and 1P10SEA are EU861362–EU861366, respectively.

Micrographs of the novel strains, 2D TLC of polar lipids and details of rep-PCR-based fingerprinting are available as supplementary material with the online version of this paper.

2003a). The majority of these strains (85%) were Gram-positive bacteria that almost all belonged to the genus *Bacillus*. In addition to prevalent species such as *Bacillus licheniformis* (25%) and *Bacillus pumilus* (16%), several novel species have been described (La Duc *et al.*, 2003; Osman *et al.*, 2006, 2007; Satomi *et al.*, 2006). Although spacecraft-assembly environments are clean, desiccated and controlled for relative humidity (La Duc *et al.*, 2007), the micro-organisms that persist within these facilities frequently demonstrate tolerance to desiccation, chemical oxidizing agents and UV irradiation (La Duc *et al.*, 2003, 2004a, 2007; Venkateswaran *et al.*, 2001). Microbes isolated from these environments are problematic, as they have the potential to adhere to robotic mission flight hardware (Crawford, 2005; La Duc *et al.*, 2004c; Osman *et al.*, 2006; Venkateswaran *et al.*, 2003b) and subsequently to be transferred to other planetary bodies when spacecraft land.

While characterizing strains isolated from the Payload Hazardous and Servicing Facility (PHSF), in which the Phoenix spacecraft was assembled (Ghosh *et al.*, 2010), five strains were found that possessed external layer-like structures, similar to those noticed in *Bacillus nealsonii*, that are presumed to enhance the survival of spores under UV irradiation conditions (Venkateswaran *et al.*, 2003b). The isolated strains clustered together based on 16S rRNA gene sequence analysis (>99% similarity), yet were distantly related to any previously described *Bacillus* species (<97.5% similarity). Here, we describe the phylogenetic affiliation of these novel strains using a polyphasic taxonomic approach.

The PHSF is a class 100K, HEPA-filtered clean-room facility, 32.6 m wide, 18.4 m long and 28.9 m high, with a consistently maintained temperature of 21.7 ± 3.3 °C and a relative humidity between 39 and 42%. Samples were collected from different locations of the clean-room floor using biological sampling kits (BiSKits; QuickSilver Analytics) as described elsewhere (La Duc *et al.*, 2007). The samples were heat-shocked for 15 min at 80 °C, as called for by the NASA standard assay, to ensure inactivation of all vegetative cells and enhance detection of spores (NASA, 1980). Samples were then plated on tryptic soy agar (TSA; Difco) and incubated for 2–7 days at 32 °C. The isolated strains described herein were routinely cultured on TSA plates (24 h at 32 °C) for various analyses. Colonies of all strains were circular, convex, irregularly edged and yellowish in 24 h 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 per litre distilled deionized water, was used to induce sporulation, as described previously (Kempf *et al.*, 2005). A single colony of each strain was inoculated in 10 ml MSM medium and incubated for 24–48 h at 28 °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. Spores purified after 48 h growth and vegetative cells with subterminal endospores or sporangia (Supplementary Fig. S1a, available in IJSEM Online) exhibited either single or aggregated forms. Scanning electron microscopy (NOVA nano-SEM600; EDAX) was used to determine the morphology of the cells and spores in detail (Cole & Popkin, 1981). As shown in Supplementary Fig. S1(b, d), spores exhibited an external layer that appeared to attract other spores and result in the formation of a biofilm or aggregates. Transmission electron microscopy (Supplementary Fig. S1c) confirmed that these structures are not exosporia, as observed in *Bacillus odyseeyi* (La Duc *et al.*, 2004c), but are loosely attached external layers around the spore coat, similar to those seen in *B. nealsonii* (Venkateswaran *et al.*, 2003b). Unlike *B. nealsonii*, however, spores of the novel strains exhibited a tendency to attach to one another, although spore aggregates could be separated by incubating

spore solutions in 50% ethanol for 10–15 min (not shown). Further chemical characterization of the external layer is not reported here as it is beyond the scope of this communication.

All strains showed about 99% sporulation when grown for 2 days at 32 °C and were harvested to study 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 OD₆₀₀ of 0.1 (equivalent to about 10⁶ spores ml⁻¹). Appropriate aliquots of the spore suspension were placed in uncovered sterile Petri dishes 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 aliquots of the reaction mixtures were removed, inoculated into 5 ml tryptic soy broth (TSB; Difco) and incubated at 32 °C for 2–5 days. All strains were resistant up to 1000 J m⁻² and were about five times more resistant than the well-studied spores of *Bacillus subtilis* (Newcombe *et al.*, 2005).

A number of metabolic assays were performed to evaluate the novel strains. 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 tests are given in Table 1. API 20NE tests were used to determine nitrate reduction, aesculin hydrolysis, β-galactosidase production, fermentation of glucose, the ONPG test (β-galactosidase), urease, gelatinase and indole production. API 50CHB was used to determine acid production from and assimilation of carbohydrates. The ability of the strains to grow in varying concentrations of NaCl (0–20% w/v) was determined in 1% (w/v) Bacto tryptone prepared in deionized water as basal medium, with increasing concentrations of NaCl (Venkateswaran *et al.*, 1999). A single colony was suspended in sterile tryptone water and used for inoculation, and growth was assessed after 5 days of incubation at 32 °C. Compared with their nearest neighbour *Bacillus pocheonensis*, which is able to grow at up to 12% (w/v) NaCl, all five novel strains were only able to grow in 0–10% (w/v) NaCl, and were considered to be halotolerant. Growth at varying pH was determined as described previously (La Duc *et al.*, 2007). Sterile TSA was adjusted to pH 3, 9, 10.8 and 13 after autoclaving using 0.5 M sterile citric acid or 10% (w/v) Na₂CO₃ as appropriate. A single colony of each strain was streaked onto appropriate plates and incubated for 5 days at 32 °C. All strains showed growth at pH 7, 9 and 10.8 but not at pH 3 or 13. Growth at different temperatures was assessed in TSB; inoculated cultures were incubated at 4, 10, 32 and 50 °C. All five novel strains showed growth at 4–32 °C after incubation for 7 days. Phenotypic characteristics that distinguish the novel strains readily from the closely related *B. pocheonensis* type strain are motility, growth in 0.5% NaCl (TSA), tolerance of pH 9.0, liquefaction of gelatin and the inability to hydrolyse aesculin and to produce acids from a variety of sugars. Other differential phenotypic characteristics are shown in Table 1.

Table 1. Differential characteristics of the five novel strains and type strains of related *Bacillus* species

Strains: 1, 1P01SC^T, 1P08SA, 1P10SC, 1P10SE and 1P02SA (*B. horneckiae* sp. nov.); 2, *B. pocheonensis* DSM 18135^T; 3, *B. drentensis* DSM 15600^T; 4, *B. novalis* DSM 15603^T; 5, *B. firmus* IAM 12464^T; 6, *B. niacini* DSM 2923^T; 7, *B. bataviensis* DSM 15601^T. All data were obtained in this study. All strains showed negative reactions for indole production, urease, assimilation of capric acid and phenylacetic acid and activities of lipase, valine arylamidase, cystine arylamidase, trypsin, α -mannosidase and α -fucosidase and positive reactions for catalase and nitrate reduction. +, Positive; -, negative; ND, not determined; v, variable; w, weak.

Characteristic	1	2	3	4	5	6	7
Gram staining	+	+	+/v	+	+	v	+/v
Growth at 50 °C	-	-	+	+	-	-	+
Growth on TSA	+	-	+	+	+	+	+
Maximum pH	10.8	8.5	10	10	10.5	8	10
Maximum NaCl concentration (% w/v)	10	12	ND	ND	10	ND	ND
Growth in the absence of NaCl	+	+	ND	ND	-	ND	ND
DNA G + C content (mol%)	35.6–36.1	44.9	39.4	40.5	46.1–47.4	37	40.1
Oxidase	-	+	-	+	-	+	+
API 20NE tests							
Glucose fermentation	-	-	-	w	+	-	-
Arginine dihydrolase	-	-	+	-	-	-	-
β -Galactosidase production	-	w	+	+	-	+	+
Hydrolysis of:							
Aesculin	-	+	-	+	-	+	-
Gelatin	+/w	-	-	+	+	-	-
Assimilation of:							
Adipic acid	+	w	-	-	-	-	-
L-Arabinose	-	+	-	+	-	+	-
D-Glucose	-	+	+	+	+	+	+
Malic acid	+	+	-	+	-	+	-
Maltose	+	+	+	+	+	+	+
D-Mannitol	-	-	-	+	+	-	+
D-Mannose	-	+	-	+	-	+	+
N-Acetyl-D-glucosamine	-	+	+	+	+	+	+
Potassium gluconate	+	+	-	+	-	+	+
Trisodium citrate	+	w	-	+	-	-	-
Production of:							
Alkaline phosphatase	w	+	w	+	-	w	+
Esterase	w	+	-	+	w	+	+
Esterase lipase	w	+	-	+	+	+	+
Leucine arylamidase	-	+	+	+	-	-	+
α -Chymotrypsin	-	-	-	-	-	+	-
Acid phosphatase	-	+	-	+	-	-	+
Naphthol-AS-BI-phosphohydrolase	w	+	+	+	+	w	+
α -Galactosidase	-	+	-	+	-	-	-
β -Galactosidase	-	+	-	-	-	w	+
β -Glucuronidase	-	-	+	-	-	-	-
α -Glucosidase	-	+	-	+	+	-	-
β -Glucosidase	-	w	-	+	-	-	-
N-Acetyl- β -glucosaminidase	-	-	w	-	-	-	-

Cellular fatty acid methyl esters (FAMES) were analysed using the Microbial ID system (MIDI, Inc.) according to the manufacturer's instructions, which did not identify the novel strains as members of any known *Bacillus* species. The FAME profiles of all five strains of this study and strains of other closely related *Bacillus* species are given in Table 2. The fatty acid profile was not significantly different among the five tested strains and was somewhat similar to

that of *Bacillus firmus* but significantly different from that of any other closely related *Bacillus* species. iso-C_{15:0} was predominant in all five strains, with a relative concentration as high as 61 %, and the second most dominant fatty acid was anteiso-C_{15:0} (20 %). The FAME profile of TSA-grown cells of strain 1P01SC^T exhibited negligible change in the content of most fatty acids when compared with the FAME profile of R2A-grown cells (not shown).

Table 2. Fatty acid analysis of strains of *B. horneckiae* and related species of the genus *Bacillus*

Values are percentages of total fatty acids. Data for the five novel strains (*B. horneckiae*) and *B. niacini* DSM 2923^T are from this study; data for *B. firmus* (Kämpfer, 1994), for *B. novalis*, *B. bataviensis* and *B. drentensis* (Heyrman *et al.*, 2004) and for *B. subtilis* (Kämpfer, 2002) were reproduced from the publications indicated.

Fatty acid	1P01SC ^T	1P10SC	1P10SE	1P02SA	1P08SA	<i>B. firmus</i>	<i>B. niacini</i>	<i>B. novalis</i>	<i>B. bataviensis</i>	<i>B. drentensis</i>	<i>B. subtilis</i>
Straight-chain saturated											
C _{10:0}			0.1				3.6				
C _{12:0}		0.2					3.6				
C _{13:0}											
C _{14:0}	1.1	0.4	0.4	0.5	0.4	1.1	4.8	3.0	1.5	1.4	
C _{15:0}		1.3	1.4	1.3	1.4	0.3					
C _{16:0}	0.5	0.4	1.0	1.0	1.1	1.5	11.7	4.8	7.7	3.4	1.0
C _{18:0}						0.1		<1.0	2.7	1.3	
Branched saturated											
iso-C _{13:0}	0.2		0.2								
iso-C _{14:0}	4.0	2.6	2.6	2.5	2.2	2.4	3.5	5.3	6.9	8.7	1.0
iso-C _{15:0}	54.3	56.8	60.7	51.1	53.3	45.8	41.2	43.9	36.9	32.2	27.0
iso-C _{16:0}	2.5	1.5	2.5	3.0	2.9	2.9	7.3	2.7	2.4	2.1	1.0
iso-C _{17:0}	1.3	1.9	1.8	1.9	1.5	2.9	4.7	<1.0	1.4	2.6	8.0
anteiso-C _{15:0}	19.9	18.4	16.9	17.6	19.0	19.8	14.2	31.0	20.5	21.8	39.0
anteiso-C _{17:0}	2.3	2.6	3.4	4.2	3.5	3.7	5.0	1.9	1.1	1.1	1.0
Monounsaturated											
C _{16:1} ω11 <i>c</i>	2.0	2.8	2.8	3.7	3.9	3.2		3.2	11.3	13.2	
C _{16:1} ω7 <i>c</i> alcohol	6.8	5.6	6.0	6.7	5.2	4.5		1.7	2.3	3.1	
iso-C _{17:1}											3.0
iso-C _{17:1} ω10 <i>c</i>	2.3	2.6	2.7	3.3	3.9	0.1		<1.0	1.7	4.5	
anteiso-C _{17:1}											10.0
Unknown fatty acids	2.9	3.9	3.0	3.9	3.6	8.4		<1.0	<1.0	<1.0	

The diastereoisomer of diaminopimelic acid (DAP) in cell-wall hydrolysates of strains 1P01SC^T and 1P08SA was determined (Staneck & Roberts, 1974). Briefly, 20 mg freeze-dried cells was hydrolysed with 200 µl 6 M HCl in a 100 °C water bath for 18 h. Following hydrolysis, the cell mixture was filtered through an activated charcoal column and dried completely in a concentrator (Turbo-Vap; Zymarck) and then dissolved in 200 µl sterile double-distilled water. A total of 5 µl hydrolysate was applied on a TLC plate (Merck 5577) along with standards (LL-DAP and *meso*-DAP) and developed in a two-dimensional TLC tank containing methanol/water/10 M HCl/pyridine (80:17.5:2.5:10, by vol.) for 3 h. The TLC plate was dried under a fume hood and sprayed with 0.2% ninhydrin solution. The plates were finally heated to 100–105 °C. After about 5 min, DAP was visualized as a grey–green colour when compared against the standard DAP. Analysis of cell-wall hydrolysates of strains 1P01SC^T and 1P08SA revealed the presence of *meso*-DAP as the diagnostic cell-wall diamino acid, which was also present in other *Bacillus* type strains analysed in this study (not shown).

Extraction (Bligh & Dyer, 1959) and identification (Komagata & Suzuki, 1987) of polar lipids were carried out according to established procedures. Lipid spots were detected using the following spray reagents: molybdato-

phosphoric acid (5%, w/v, in absolute ethanol), molybdenum blue spray reagent (Sigma), ninhydrin (0.2%, w/v, in acetone) and anisaldehyde reagent (Sigma) for detection of total lipids, phospholipids, aminolipids and glycolipids, respectively. Strains 1P01SC^T and 1P08SA displayed a complex polar lipid profile, which consisted of seven unknown phospholipids, two unknown aminolipids and one unknown aminophospholipid (Supplementary Fig. S2). The lipid profiles of strains 1P01SC^T and 1P08SA are quite similar to each other (10 out of a total of 14 spots detected in both). One of the unknown aminolipids and the seven phospholipids are seen in both strains but were not reported for *B. subtilis*. However, the major lipids of these two strains are somewhat similar to the profile of *B. subtilis* (Table 3). Respiratory quinones were extracted according to established methods (Nakagawa & Yamasato, 1993), and MK-7 was dominant in strains 1P01SC^T and 1P08SA. Based on the cell-wall composition, menaquinone analyses and major polar lipids, the newly described strains belong to *Bacillus* (Table 3).

Genomic DNA was isolated from strain 1P01SC^T and other type strains (detailed below) using established techniques (Johnson, 1981). The G+C content of the novel strains was determined spectrophotometrically (Lambda 35;

Table 3. Characteristics that distinguish *Bacillus horneckiae* sp. nov. from related genera

Data for *B. horneckiae* are from this study; data for *B. subtilis* (Kämpfer, 1994), *Viridibacillus* (Albert *et al.*, 2007), *Kurthia* (Shaw & Keddie, 1983), *Lysinibacillus* (Ahmed *et al.*, 2007) and *Rummeliibacillus* (Vaishampayan *et al.*, 2009) were taken from the publications indicated.

Characteristic	<i>B. horneckiae</i>	<i>B. subtilis</i>	<i>Viridibacillus</i>	<i>Kurthia</i>	<i>Lysinibacillus</i>	<i>Rummeliibacillus</i>
Spore formation	Present	Present	Present	Absent	Present	Present
Cell-wall composition	<i>meso</i> -DAP	<i>meso</i> -DAP	L-Lys-D-Glu or L-Lys-D-Asp	L-Lys- D-Asp	Lys-Asp	L-Lys-D-Glu or Lys-Asp
Green pigment formation	Absent	Absent	Present	Absent	Absent	Absent
Quinone system	MK-7	MK-7	MK-8, MK-7	MK-7	MK-7	MK-7
Polar lipids*	DPG, PE, PG, APL, 2AL, 7PL	DPG, PE, PG, GBG, APL	DPG, PE, PG, APL, 2PL	DPG, PE, PG	DPG, PG, APGL	DPG, PE, PG, APL, 2PL, AL
Major fatty acids†	i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , ai-C _{15:0} , ai-C _{17:1}	i-C _{15:0} , (ai-C _{15:0})	i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , (ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0} , C _{16:1} ω7c alcohol)	i-C _{15:0} , ai-C _{15:0}

*AL, Unknown aminolipid; APGL, unknown aminophosphoglycerol; APL, unknown aminophospholipid; DPG, diphosphatidylglycerol; GBG, gentiobiosyldiacylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid.

†ai, Anteiso-branched; i, iso-branched. Compounds making up more than 20% of the total fatty acids; compounds present at 10–20% are indicated in parentheses.

Perkin Elmer) as 35.6 ± 0.5 mol% using a thermal denaturation method (Mandel & Marmur, 1968). The 16S rRNA gene was amplified by PCR using a universal primer set and sequenced bidirectionally as reported elsewhere (La Duc *et al.*, 2007). The 16S rRNA gene sequence (about 1.4 kb) was compared to publicly available sequences using BLAST. Neighbour-joining phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004). Sequence similarities between the five novel strains were 99.5–100%, and the strains formed a well-defined and separate cluster supported by high bootstrap values (Fig. 1). The sequences of the novel strains formed a separate cluster with a dissimilarity of more than 2.5% from several other *Bacillus* species. *B. pocheonensis* Gsoil 420^T exhibited the highest 16S rRNA gene sequence similarity (97.5%) to strain 1P01SC^T. Strains of all other *Bacillus* species with validly published names demonstrated sequence similar-

ities of 97.3% or less; notably, the similarity was only 93.8% with the type strain of *B. subtilis*, the type species of the genus *Bacillus*. Close relatives included the type strains of *Bacillus niacini* (97.3%), *B. firmus* (97.3%), *Bacillus bataviensis* (97.2%) and *Bacillus drentensis* (97.1%).

DNA–DNA hybridization of 1P01SC^T and type strains of various closely related *Bacillus* species was performed with freshly isolated genomic DNA and was carried out in quadruplicate using the membrane filter method (Tourova & Antonov, 1987). Means of the three most confident readings were taken for calculation purposes. Strain 1P01SC^T exhibited 87.8% DNA–DNA relatedness to strain 1P08SA, 24.3% to *B. niacini* MTCC 9480^T, 7.6% to *B. pocheonensis* DSM 18135^T, 5.2% to *B. firmus* MTCC 488^T, 3.3% to *B. drentensis* MTCC 9481^T, 2.5% to *Bacillus soli* MTCC 9482^T and 1.5% to *B. bataviensis* MTCC 7309^T. The low (<70%) DNA–DNA relatedness between the novel strains and their close relatives precludes genomic

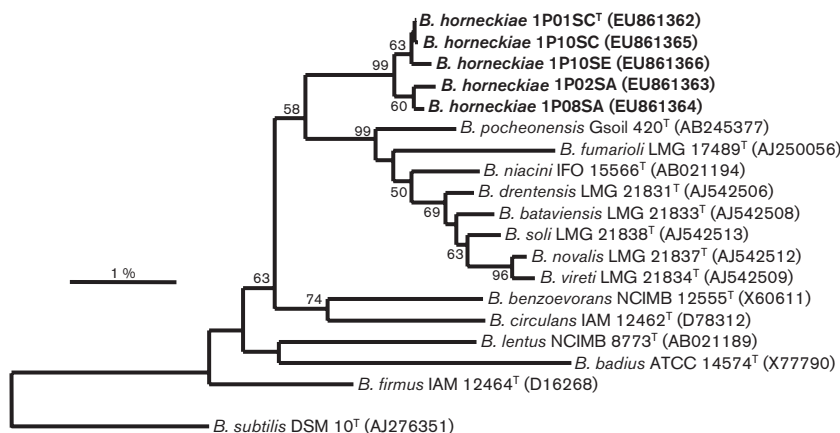


Fig. 1. Phylogenetic tree for strains of *Bacillus horneckiae* sp. nov. and other closely related *Bacillus* species based on neighbour-joining analysis of the 16S rRNA gene nucleotide sequence. Strain numbers and GenBank accession numbers (in parentheses) are shown. Bootstrap percentages based on 1000 replications (≥50%) are shown at branch points. Bar, 1% sequence divergence.

relatedness at the species level and supports the designation of strains 1P01SC^T and 1P08SA as representatives of a novel species within the genus *Bacillus* (Stackebrandt & Goebel, 1994; Wayne, 1988).

In order to elucidate the genotypic variation among the five strains, extracted DNA was amplified using the *Bacillus* DiversiLab DNA fingerprinting kit (Spectral Genomics, Inc.) according to the protocol established by Healy *et al.* (2005). The detailed report and resulting dendrogram are given in Supplementary Fig. S3. Strain 1P01SC^T clustered together with strain 1P10SC and formed the first genospecies, a second cluster consisted of strains 1P02SA and 1P08SA and strain 1P10SE was the sole member of the third genospecies. A similar clustering pattern of the five strains was also noticed in the 16S rRNA gene sequence analysis (Fig. 1).

The results of DNA–DNA hybridization combined with phenotypic comparisons indicate that all five isolates belong to the genus *Bacillus* but are significantly distinct from any previously described species. Based on the data described herein, the five strains represent a novel species, for which the name *Bacillus horneckiae* sp. nov. is proposed.

Description of *Bacillus horneckiae* sp. nov.

Bacillus horneckiae (hor.nec'ki.ae. N.L. gen. fem. n. *horneckiae* of Horneck, named after Dr Gerda Horneck, a German space microbiologist who carried out pioneering work in exposing spores to several space and radiation conditions).

Vegetative cells are rod-shaped, 1–1.5 µm in diameter and 4–6 µm long, motile and Gram-stain-positive and form endospores that are subterminal in position. Grows under aerobic conditions only. Catalase is produced. Colonies formed after 24 h of growth on TSA at 32 °C are yellowish, plain, 3 mm in diameter, show no pigmentation and possess irregular edges. Optimum growth temperature is 30 °C; known strains do not grow below 4 °C or at 50 °C. Cells grow in the absence of NaCl and with up to 10% (w/v) NaCl and up to pH 10.8 with optimum growth at pH 7.0. Produces oval spores in MSM which are resistant to UV radiation up to 1000 J m⁻². Reduces nitrates to nitrites and hydrolyses gelatin. Tests for oxidase, indole production, fermentation of glucose, arginine dihydrolase, urease, hydrolysis of aesculin and β-galactosidase (ONPG) are negative. Assimilates the following as sole carbon sources: maltose, potassium gluconate, adipic acid, malic acid and trisodium citrate. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, decanoic acid and 2-phenylacetic acid are not assimilated. Acid is not produced from the following substrates: glycerol, erythritol, D- and L-arabinose, D-ribose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amyg-

dalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. Gelatin liquefaction is positive for strains 1P01SC^T and 1P02SA and weakly positive for three other strains. The major fatty acid of strains 1P01SC^T and 1P08SA is iso-C_{15:0}. The diagnostic diamino acid in the peptidoglycan is meso-DAP. The DNA G + C content of the type strain is 35.6 ± 0.5 mol%.

The type strain, 1P01SC^T (=NRRL B-59162^T =MTCC 9535^T), was isolated from surfaces of the PHSF located at the Kennedy Space Center, where the Phoenix spacecraft was assembled. Strains 1P08SA (=MTCC 9536), 1P10SC, 1P10SE and 1P02SA are additional strains of the species, isolated from the same source.

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