Description of *Rummeliibacillus stabekisii* gen. nov., sp. nov. and reclassification of *Bacillus pycnus* Nakamura *et al.* 2002 as *Rummeliibacillus pycnus* comb. nov.

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Strains of aerobic, Gram-positive, rod-shaped, round-spore-forming bacteria were isolated from different geographical locations and a subsequent polyphasic study was undertaken to clarify the taxonomic position of the round-spore-forming isolates strain KSC-SF6g^T, strain M32 and strain NBRC 12622. 16S rRNA gene sequence similarities demonstrated that these strains were most closely affiliated with *Bacillus pycnus* NRRL NRS-1691^T (98 %), with species of *Kurthia* (96 %) and *Viridibacillus* (94–96 %) as the next nearest relatives. However, while DNA–DNA hybridization studies showed approx. 70 % reassociation among strains KSC-SF6g^T, M32 and NBRC 12622, DNA–DNA hybridization values between these strains and *B. pycnus* NRRL NRS-1691^T never exceeded 13 %. Differences in the molecular structure of the cell-wall peptidoglycan could not differentiate these strains sufficiently from other closely related genera (*Viridibacillus* and *Kurthia*). However, Lys–Asp was present in strains KSC-SF6g^T, M32 and NBRC 12622, whereas L–Lys–D–Glu was reported in *B. pycnus* NRRL NRS-1691^T. The menaquinone MK-7 was dominant in strains KSC-SF6g^T, M32 and NBRC 12622 and members of the genus *Kurthia*, whereas MK-8 was abundant in *Viridibacillus* species. Strains KSC-SF6g^T, M32 and NBRC 12622 exhibited fatty acid profiles consisting of major amounts of anteiso-C_{15:0} (~50 %) and iso-C_{15:0} (~25 %) and moderate amounts of anteiso-C_{17:0} (~7 %), which discriminated them from closely related *B. pycnus* NRRL NRS-1691^T and species of *Viridibacillus* (iso-C_{15:0}; 46–74 %). The authors propose that strains KSC-SF6g^T, M32 and NBRC 12622 and *B. pycnus* NRRL NRS-1691^T be reclassified into a separate genus based on clear-cut differences in discriminative taxonomic markers and the distant placement of *B. pycnus* and the novel strains described herein from other species of this clade according to current 16S rRNA gene sequence-based relatedness (~4 % difference in sequence). We propose the placement of these isolates into the novel genus *Rummeliibacillus* gen. nov. For the new taxon comprising strains KSC-SF6g^T, M32 and NBRC 12622, we propose the name *Rummeliibacillus stabekisii* gen. nov., sp. nov. (the type species of *Rummeliibacillus*), represented by the type strain KSC-SF6g^T (=NRRL B-51320^T =NBRC 104870^T). In addition, *Bacillus pycnus*, which bears traits distinct from other round-spore-forming species [i.e. absence of growth at high NaCl (7 %), positive reaction for gelatin liquefaction], is reclassified as *Rummeliibacillus pycnus* comb. nov. (type strain JCM 11075^T =NRRL NRS-1691^T) based on phylogenetic affiliations and phenotypic characterization.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KSC-SF6g^T, M32 and NBRC 12622 are DQ870754, AB116126 and AB271737, respectively.

A comparison of the fatty acid profiles of the novel strains and related strains is available as a supplementary table with the online version of this paper.

The round-spore-forming, mesophilic bacterial species *Bacillus sphaericus* was first described in 1904 (Neide, 1904). Inadequate taxonomic classification tools and the lack of modern-day molecular approaches restricted further phylogenetic subdivision of this heterogeneous species. The introduction of DNA-based comparative methodologies has recently triggered the reclassification of the species *B. sphaericus*, leading to the discovery of novel mesophilic species such as *Bacillus fusiformis* (Priest *et al.*, 1988), *Bacillus silvestris* (Rheims *et al.*, 1999) and *Bacillus odysseyi* (La Duc *et al.*, 2004). Likewise, *B. sphaericus*-like species were segregated into seven groups (Nakamura, 2000) based on a molecular approach, and groups 6 and 7 were further characterized as *Bacillus pycnus* and *Bacillus neidei*, respectively (Nakamura *et al.*, 2002). As members of this *B. sphaericus*-like group shared 95.1–96.6 % 16S rRNA gene sequence similarity with non-spore-forming species of *Kurthia*, Ash *et al.* (1991) deemed *Kurthia* species to be members of *Bacillus* rRNA group 2. The species *Bacillus arvi*, *Bacillus arenosi* and *B. neidei* exhibited high 16S rRNA gene sequence similarity (97.1–99.8 %) with one another, whereas *B. pycnus* appeared to be distantly related to these three species (95.2–96.7 %). Recently, *B. neidei* was reclassified along with *B. arvi* and *B. arenosi* into the novel genus *Viridibacillus* (Albert *et al.*, 2007). The same study suggested that *B. pycnus* be examined and considered as a representative of a possible novel genus.

To understand better the physiological breadth enveloped within the microbial populations encountered while surveying spacecraft-associated microbial diversity, polyphasic taxonomic studies are routinely undertaken to clarify the taxonomic positions of isolates. Several novel physiologically recalcitrant, phylogenetically distinct micro-organisms have been encountered while examining spacecraft surfaces (La Duc *et al.*, 2007; Osman *et al.*, 2006; Satomi *et al.*, 2006). Some of these micro-organisms form round spores surrounded by an exosporium (La Duc *et al.*, 2004; Venkateswaran *et al.*, 2003), which probably renders such entities much more tolerant of various clean-room conditions and enhances the adhesion of spores to spacecraft surfaces. A novel spore-forming bacterial species comprising three strains was isolated from various geographical locations: the floor of a class 100K spacecraft-assembly clean room at the Kennedy Space Center, Florida, USA (strain KSC-SF6g^T; La Duc *et al.*, 2007), a field-scale composter in Japan (strain M32) and an unknown source (strain NBRC 12622, deposited in the culture collection of the Institute of Fermentation, Osaka, Japan). This novel species, along with previously described strains of *B. pycnus*, are here reclassified into a novel genus based on polyphasic taxonomic analyses. The type strain of *B. pycnus*, NRRL NRS-1691^T, was included for comparative analyses.

Strains KSC-SF6g^T, M32 and NBRC 12622 showed growth on tryptic soy agar (TSA; Difco) after 24 h at 32 °C. Colonies were regular-edged, non-pigmented, translucent,

smooth and circular, with a mean diameter of about 1 mm after 24 h of incubation on TSA at 32 °C. Upon extended incubation, colonies did not yield any pigmentation. A modified Schaeffer's medium (MSM) was used to induce sporulation (Kempf *et al.*, 2005). MSM consists 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 l distilled deionized water. A single purified colony of strains KSC-SF6g^T, M32 and NBRC 12622 was inoculated individually in MSM broth. After 24 h of incubation at 32 °C, cultures were examined by wet-mount light microscopy to determine the level of sporulation. Once the number of free spores was greater than 99 %, they were harvested by centrifugation and purified by treatment with lysozyme and washing with salts and detergents (Nicholson & Setlow, 1990). Purified spores were resuspended in sterile deionized water, heat-treated (80 °C for 15 min) to ensure inactivation of the vegetative population and stored at 4 °C in glass tubes.

The morphologies of vegetative cells (24 h incubation at 32 °C on TSA) and purified spores were observed by phase-contrast microscopy using an Olympus microscope (BX-60). Scanning electron microscopy (NOVA nanoSEM600; EDAX) (Cole & Popkin, 1981) and transmission electron microscopy (FEI Inc.) were performed to examine surface details and spore architecture. Samples were prepared for transmission electron microscopy as follows. Sporulating cells (500 µl; 10⁷ cells) 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 cell suspension was 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) and subsequently stained with aqueous 2 % uranyl acetate and Reynolds' lead citrate to enhance contrast.

All strains yielded positive Gram reactions, according to established Gram-stain procedures (Smibert & Krieg, 1994). Fig. 1(a) depicts endospore-forming cells terminally swollen with phase-bright spore bodies, as viewed under a phase-contrast light microscope. The spherical nature (~1 µm in diameter) of the spore bodies was apparent when viewed with a scanning electron microscope, as shown in Fig. 1(b). Transmission electron micrographs (Fig. 1c, d) showed the presence of an exosporium, a unique layer that is an integral part of certain *Bacillus* spores, such as those of *B. odysseyi* (La Duc *et al.*, 2004).

Bacterial 16S rRNA genes were amplified by PCR using the eubacteria-biased B27f and universal 1492r primer set, as described previously (Satomi *et al.*, 2006). The phylogenetic relationships of isolates included in this study were determined by comparison of individual 16S rRNA gene

Fig. 1. (a, b) Microscope images of *Rummeliibacillus stabekisii* gen. nov., sp. nov. KSC-SF6g^T showing sporulating (bright structures) and vegetative cells using light microscopy (a) and round-spore structures using scanning electron microscopy (b). (c, d) Transmission electron micrographs of spore architecture of strain KSC-SF6g^T with cross section of a single spore (c) and a mother cell engulfing a spore (d). EX, Exosporium; SC, spore coat. Bars, 2.5 µm (a), 4 µm (b), 0.2 µm (c) and 0.5 µm (d).

sequences to existing sequences in public databases (<http://www.ncbi.nlm.nih.gov/>). The similarities observed in 16S rRNA gene sequences demonstrated that strain KSC-SF6g^T is phylogenetically affiliated most closely with strains M32 and NBRC 12622 (99.86%) and *B. pycnus* NRRL NRS-1691^T (97.96%). According to 16S rRNA gene sequence similarity, members of *Viridibacillus* (maximum similarity of 95.85%) and several species of *Kurthia* (95.35–96.29%) were the next nearest relatives to the novel strains. *Bacillus subtilis* IAM 12118^T exhibited very low sequence similarity (90.17%). When neighbour-joining phylogenetic analyses were performed with the program MEGA4 (Tamura *et al.*, 2007), strains KSC-SF6g^T, M32 and NBRC 12622 formed a

distinct cluster with *B. pycnus* NRRL NRS-1691^T (Fig. 2). It is clear from the resulting phylogenetic tree that these strains are distant relatives of members of the genera *Kurthia*, *Viridibacillus*, *Caryophanon* and *Lysinibacillus* (Fig. 2).

The ability to grow in varying concentrations of NaCl (1–10%, w/v) was determined in 1% Bacto tryptone containing varying amounts of NaCl. Isolates were also grown in 1% tryptone to examine their ability to grow in the absence of NaCl (Satomi *et al.*, 2006). The novel strains described herein were halotolerant and capable of growth in the presence of up to 7% NaCl, in contrast to *B. pycnus*

Fig. 2. Phylogenetic analysis based on 16S rRNA gene sequences using a neighbour-joining algorithm showing the position of strains of *Rummeliibacillus stabekisii* gen. nov., sp. nov. with their closest relatives. Bar, 0.005 substitutions per nucleotide position. Bootstrap values at nodes are percentages based on estimation of 1000 trees by the neighbour-joining method.

NRRL NRS-1691^T, which did not exhibit any growth at 7 % NaCl. Commercially available API 20 NE and API 50 CH test strips (bioMérieux) were used, according to the manufacturer's specifications, to characterize each of the strains biochemically. Table 1 summarizes the physiological and biochemical traits of each of the strains examined in this study. Notable discriminative factors not shared between the novel strains and *B. pycnus* include the novel strains' ability to liquefy gelatin, their inability to break down tryptophan to indole and their inability to produce acid from glucose (Table 1).

Cellular fatty acid methyl esters were analysed with the Microbial ID system (MIDI, Inc.) according to the manufacturer's instructions and the results are provided in Supplementary Table S1 (available in IJSEM Online). No significant differences were observed when the cellular fatty acid profiles of strains KSC-SF6g^T, M32 and NBRC 12622 were compared with one another, supporting their affiliation to a single species. The profile of *B. pycnus* NRRL NRS-1691^T differed from the profiles of strains KSC-SF6g^T, M32 and NBRC 12622 in the content of iso-C_{15:0}, C_{14:0} and anteiso-C_{15:0} and the lack of C_{12:0}, C_{15:0} and C_{16:0}. When the fatty acid methyl ester profiles of members of the genus *Viridibacillus* (Albert *et al.*, 2007) were compared with those of *B. pycnus* NRRL NRS-1691^T and the novel strains (Supplementary Table S1), significant differences were observed in the content of iso-C_{14:0}, iso-C_{15:0}, iso-C_{17:1}ω10c, iso-C_{17:0} and iso-C_{17:1} I/anteiso-C_{17:1} B and the lack of C_{15:0}. The fatty acid profile comparison clearly segregates isolates KSC-SF6g^T, M32 and NBRC 12622 from *B. pycnus* NRRL NRS-1691^T and species of *Viridibacillus*, which, coupled with its deep-branching molecular phylogeny, advocates novel genus status for this group. Characteristics used to differentiate the novel genus described herein from related genera are shown in Table 2.

Respiratory quinones were extracted according to the method of Nakagawa & Yamasato (1993) and determined by using LC-MS (8000x; Shimadzu). The quinone system

of *B. pycnus* NRRL NRS-1691^T was dominated (>99 %) by the menaquinone MK-7, with trace amounts of MK-8 (Table 2). However, for *Viridibacillus arvi* DSM 16317^T, *V. arenosi* DSM 16319^T and *V. neidei* NRRL BD-87^T, the primary isoprenoid quinone was MK-8 (69–81 %), with MK-7 present at 19–30 % and MK-6 present in trace amounts (Albert *et al.*, 2007). Cell walls were prepared by mechanical disruption with an ultrasonic oscillator and were purified as described elsewhere (Schleifer & Kandler, 1972). The amino acid compositions of complete cell-wall hydrolysates were determined by HPLC with a model LC-20A (Shimadzu). The novel strains contained peptidoglycan with alanine, glutamic acid, lysine and aspartic acid, indicating that the peptidoglycan type is A4x (Lys-Asp) according to Schleifer & Kandler (1972), while *B. pycnus* NRRL NRS-1691^T was reported to contain L-Lys-D-Glu (Nakamura *et al.*, 2002).

DNA was extracted from all strains according to established protocols (Johnson, 1981). The genomic DNA of strain NBRC 12622 was found to have a G+C content of 37.3 mol%, as determined by adhering to previously published protocols (Saha *et al.*, 2005). DNA-DNA hybridization was performed using previously reported microplate hybridization methods (Ezaki *et al.*, 1989). Isolates KSC-SF6g^T, M32 and NBRC 12622 were closely related to each other (approx. 70 % DNA relatedness), indicating that these novel strains were indeed members of the same species. These strains exhibited relatively low levels of reassociation with *B. pycnus* NRRL NRS-1691^T (13 %) and strains of *Kurthia* (15–17 %). Nakamura *et al.* (2002) have previously reported high levels (100 %) of DNA relatedness between *B. pycnus* NRRL NRS-1691^T and other strains (NRRL NRS-1694, NRRL NRS-1693 and NRRL NRS-1695). Thus, according to the results of DNA-DNA hybridizations, strains KSC-SF6g^T, M32 and NBRC 12622 are members of a species which is distinct from *B. pycnus* NRRL NRS-1691^T.

Phylogenetic analyses based on the 16S rRNA gene sequences of strains KSC-SF6g^T, M32 and NBRC 12622

Table 1. Differentiating characteristics between the novel strains and *B. pycnus* NRRL NRS-1691^T

Strains: 1, *B. pycnus* NRRL NRS-1691^T; 2, KSC-SF6g^T; 3, NBRC 12622; 4, M32. All data were obtained in this study. All strains are motile and positive for growth in 5 % NaCl and negative for oxidase, growth in 10 % NaCl, starch hydrolysis, citrate utilization and acid production from D-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-lactose, D-mannose, D-mannitol and cellobiose.

Characteristic	1	2	3	4
Temperature range (°C)	10–45	28–55	15–45	28–55
Growth in 7 % NaCl	–	+	+	+
Gelatin hydrolysis	–	+	+	+
Cell-wall type	L-Lys-D-Glu	Lys-Asp	Lys-Asp	Lys-Asp
Indole production	+	–	–	–
Acid production from D-glucose	+	–	–	–
Major fatty acid	iso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}	anteiso-C _{15:0}

Table 2. Characteristics that distinguish members of the genus *Rummeliibacillus* gen. nov. from related genera and *B. subtilis*

Data for reference taxa were taken from Nakamura *et al.* (2002) (*B. pycnus*), Albert *et al.* (2007) (*Viridibacillus*), Shaw & Keddie (1983) (*Kurthia*), Ahmed *et al.* (2007) (*Lysinibacillus*) and Kämpfer (1994) (*B. subtilis*). Dap, Diaminopimelic acid.

Characteristic	<i>Rummeliibacillus</i>	<i>Viridibacillus</i>	<i>Kurthia</i>	<i>Lysinibacillus</i>	<i>B. subtilis</i>
Spore formation	Present	Present	Absent	Present	Present
Cell-wall composition	L-Lys-D-Glu or Lys-Asp	L-Lys-D-Glu or L-Lys-D-Asp	L-Lys-D-Asp	Lys-Asp	meso-Dap direct
Green pigment formation	Absent	Present	Absent	Absent	Absent
Quinone system	MK-7	MK-8, MK-7	MK-7	MK-7	MK-7
Polar lipids*	DPG, PE, PG, APL, 2PL, AL	DPG, PE, PG, APL, 2PL	DPG, PE, PG	DPG, PG, APGL	PG, DPG, PE, GBG, APL
Major fatty acids (>10%)†	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , (anteiso-C _{15:0})	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , (anteiso- C _{15:0} , iso-C _{16:0} , anteiso-C _{17:0} , C _{16:1} ω7c alcohol)	anteiso-C _{15:0} , iso-C _{15:0} , anteiso-C _{17:1}

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

†Fatty acids given in parentheses are present at >10% in some members of the genus.

and *B. pycnus* NRRL NRS-1691^T portray this assemblage as a distinct sublineage, having sequence divergence of >4% from members of the closely related genera *Kurthia* and *Viridibacillus*. This discriminatory phylogenetic inference was supported by phenotypic differences (Table 1) and DNA–DNA hybridization. On the basis of distinguishing chemotaxonomic and genotypic attributes, we propose the classification of strains KSC-SF6g^T, M32 and NBRC 12622 and all previously described *B. pycnus* strains into a novel genus designated *Rummeliibacillus* gen. nov. The name *Rummeliibacillus stabekisii* sp. nov. is proposed for strains KSC-SF6g^T, M32 and NBRC 12622. We further propose the reclassification of *Bacillus pycnus* as *Rummeliibacillus pycnus* comb. nov.

Description of *Rummeliibacillus* gen. nov.

Rummeliibacillus (Rum.me'li.i.ba.cil'lus. N.L. n. *Rummelius* Rummel; L. masc. n. *bacillus* a rod, and also a bacterial genus name; N.L. masc. n. *Rummeliibacillus* a bacterium close to the genus *Bacillus* and named in honour of former NASA Planetary Protection Officer Dr John Rummel, an astrobiologist responsible for bringing planetary protection into the public domain).

Gram-positive, spore-forming, motile rods. Endospores are round and are located terminally with an exosporium. Growth occurs at 28–55 °C and in the presence of up to 5% NaCl (w/v). Sporulation is observed in MSM broth after 72 h at 32 °C. The quinone system consists of MK-7 as the most prevalent compound. The polar lipid profile consists of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, moderate amounts of an unknown aminophospholipid (APL1), minor amounts of two unknown phospholipids (PL1, PL2)

and an unknown aminolipid (AL). Fatty acid profiles consist largely of anteiso-C_{15:0} (approx. 50%) and iso-C_{15:0} (approx. 25%) acids along with moderate amounts of anteiso-C_{17:0} (approx. 7%). Cell-wall peptidoglycan is L-Lys-D-Glu or Lys-Asp. The G + C content of the genomic DNA of the type strain of the type species of the genus is 34.3 mol%. The type species is *Rummeliibacillus stabekisii*.

Description of *Rummeliibacillus stabekisii* sp. nov.

Rummeliibacillus stabekisii (sta.be.ki'si.i. N.L. gen. masc. n. *stabekisii* of Stabekis, in honour of Perry Stabekis, a great source of advice and wisdom to the NASA Planetary Protection Program and its officers).

Displays the following characteristics in addition to those given in the genus description. Rods are 1.07–1.14 × 2.64–3.32 µm (from scanning electron micrographs). Agar colonies are non-pigmented, translucent, smooth, circular and entire with a mean diameter of about 1 mm after 24 h of incubation at 32 °C on TSA. Optimum growth temperature is 28–32 °C (no growth at 5–10 or 65 °C). Strictly aerobic, oxidase-negative, does not reduce nitrate to nitrite, liquefies gelatin, grows in up to 7% NaCl, hydrolyses starch, utilizes citrate and produces acid from D-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-lactose, D-mannose, D-mannitol and cellobiose. Cell-wall peptidoglycan is of type Lys-Asp.

The type strain, KSC-SF6g^T (=NRRL B-51320^T =NBRC 104870^T), was isolated from the Payload Hazardous Servicing Facility at the Kennedy Space Center, FL, USA. Strains M32 and NBRC 12622 are additional strains of the species.

Description of *Rummeliibacillus pycnus* (Nakamura *et al.* 2002) comb. nov.

Rummeliibacillus pycnus (pyc'nus. N.L. masc. adj. *pycnus* from Gr. adj. *puknos* thick, referring to the thick cells).

Basonym: *Bacillus pycnus* Nakamura *et al.* 2002.

In addition to the characteristics of *B. pycnus* summarized by Nakamura *et al.* (2002), the species exhibits the traits listed in the genus description above. The type strain is NRRL NRS-1691^T (=JCM 11075^T).

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References

- Ahmed, I., Yokota, A., Yamazoe, A. & Fujiwara, T. (2007). Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *Int J Syst Evol Microbiol* 57, 1117–1125.
- Albert, R. A., Archambault, J., Lempa, M., Hurst, B., Richardson, C., Gruenloh, S., Duran, M., Worliczek, H. L., Huber, B. E. & other authors (2007). Proposal of *Viridibacillus* gen. nov. and reclassification of *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* as *Viridibacillus arvi* gen. nov., comb. nov., *Viridibacillus arenosi* comb. nov. and *Viridibacillus neidei* comb. nov. *Int J Syst Evol Microbiol* 57, 2729–2737.
- 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. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determined genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Johnson, J. L. (1981). Genetic characterization. In *Manual of Methods for General Bacteriology*, pp. 450–472. 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.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* 17, 86–98.
- 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., Satomi, M. & Venkateswaran, K. (2004). *Bacillus odysseyi* sp. nov., a round-spore-forming bacillus isolated from the Mars Odyssey spacecraft. *Int J Syst Evol Microbiol* 54, 195–201.
- La Duc, M. T., Dekas, A. E., Osman, S., Moissi, 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.
- Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* 139, 1155–1161.
- Nakamura, L. K. (2000). Phylogeny of *Bacillus sphaericus*-like organisms. *Int J Syst Evol Microbiol* 50, 1715–1722.
- Nakamura, L. K., Shida, O., Takagi, H. & Komagata, K. (2002). *Bacillus pycnus* sp. nov. and *Bacillus neidei* sp. nov., round-spored bacteria from soil. *Int J Syst Evol Microbiol* 52, 501–505.
- Neide, E. (1904). Botanische Beschreibung einiger sporenbildenden Bakterien. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg* 12, 337–352 (in German).
- Nicholson, W. L. & Setlow, P. (1990). Sporulation, germination and outgrowth. In *Molecular Biological Methods for Bacillus*, pp. 391–450. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.
- 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.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* 134, 1847–1882.
- Rheims, H., Frühling, A., Schumann, P., Rohde, M. & Stackebrandt, E. (1999). *Bacillus silvestris* sp. nov., a new member of the genus *Bacillus* that contains lysine in its cell wall. *Int J Syst Bacteriol* 49, 795–802.
- Saha, P., Krishnamurthi, S., Mayilraj, S., Prasad, G. S., Bora, T. C. & Chakrabarti, T. (2005). *Aquimonas voraii* gen. nov., sp. nov., a novel gammaproteobacterium isolated from a warm spring of Assam, India. *Int J Syst Evol Microbiol* 55, 1491–1495.
- Satomi, M., La Duc, M. T. & Venkateswaran, K. (2006). *Bacillus safensis* sp. nov., isolated from spacecraft and assembly-facility surfaces. *Int J Syst Evol Microbiol* 56, 1735–1740.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 36, 407–477.
- Shaw, S. & Keddle, R. M. (1983). A numerical taxonomic study of the genus *Kurthia* with a revised description of *Kurthia zopfii* and a description of *Kurthia gibsonii* sp. nov. *Syst Appl Microbiol* 4, 253–276.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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., 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.