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.

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A polyphasic study was undertaken to clarify the taxonomic position of endospore-forming strains 433-D9, 433-E17 and 121-X1. BOX-PCR-generated fingerprints indicated that they may be members of a single species. 16S rRNA gene sequence similarity demonstrated that a representative of this group, 433-D9, is affiliated closely with *Bacillus arvi* DSM 16317^T (100 %), *Bacillus arenosi* DSM 16319^T (99.8 %) and *Bacillus neidei* NRRL BD-87^T (97.1 %). Sequence similarities revealed *Bacillus pycnus* NRRL NRS-1691^T and several *Kurthia* species as the next nearest relatives. DNA–DNA hybridization results showed that strain 433-D9 is a member of *B. arvi*. Detection of L-Lys–D-Asp-based peptidoglycan in strain 433-D9, *B. arvi* DSM 16317^T and *B. arenosi* DSM 16319^T was in agreement with their close relationship, but differentiated these strains from *B. neidei* NRRL BD-87^T and *B. pycnus* NRRL NRS-1691^T, for which L-Lys–D-Glu was reported. A similar quinone system was detected in strains 433-D9, 433-E17, 121-X1, *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T and *B. neidei* NRRL BD-87^T. This system, unusual for bacilli, consisted of the major compound menaquinone MK-8 (69–80 %) and moderate amounts of MK-7 (19–30 %). This observation was in contrast to the predominance of MK-7 of the closest relative *B. pycnus* NRRL NRS-1691^T, as also reported for representatives of the closely related non-endospore-forming genus *Kurthia*. Strains 433-D9, *B. arvi* DSM 16317^T and *B. arenosi* DSM 16319^T exhibited homogeneous and discriminative polar lipid profiles and fatty acid profiles consisting of major acids i-C_{15:0} and ai-C_{15:0} and moderate amounts of i-C_{17:1}ω10c and i-C_{17:1}/ai-C_{17:1} B that discriminated them from closely related strains such as *B. neidei* NRRL BD-87^T. On the basis of clear-cut discriminative chemotaxonomic markers, we

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BOX-PCR profiles, phase-contrast micrographs of cells of strain 433-D9 and photographs of growth of strain 433-D9 are available as supplementary material with the online version of this paper.

propose strains 433-D9, 433-E17 and 121-X1, *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T and *B. neidei* NRRL BD-87^T to be reclassified within a separate genus. For this new taxon, we propose the name *Viridibacillus* gen. nov., and we propose the reclassification of *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* as *Viridibacillus arvi* gen. nov., comb. nov. (the type species of *Viridibacillus*, with the type strain DSM 16317^T = LMG 22165^T), *Viridibacillus arenosi* comb. nov. (type strain DSM 16319^T = LMG 22166^T) and *Viridibacillus neidei* comb. nov. (type strain NRRL BD-87^T = DSM 15031^T = JCM 11077^T).

Since the publication of Ash *et al.* (1991), in which the subdivision of the genus *Bacillus* into at least five groups was demonstrated, it has been split into numerous newly described genera (Ash *et al.*, 1993; Fortina *et al.*, 2001; Heyndrickx *et al.*, 1998; Ishikawa *et al.*, 2002; Lu *et al.*, 2001; Nakamura *et al.*, 2004; Nazina *et al.*, 2001; Schlesner *et al.*, 2001; Shida *et al.*, 1996; Spring *et al.*, 1996; Wainø *et al.*, 1999; Wisotzkey *et al.*, 1992; Yoon *et al.*, 2001c). Many of these reclassifications are well supported by relatively conserved phenotypic traits such as quinone system, peptidoglycan composition and polar lipid and/or cellular fatty acid profiles. In conclusion, the genus *Bacillus sensu stricto* should be restricted to species that share high 16S rRNA gene sequence similarities with the type species of the genus, *Bacillus subtilis*, and its chemotaxonomic traits. The latter would include the presence of menaquinone MK-7, a peptidoglycan with the characteristic diamino acid *meso*-diaminopimelic acid, a polar lipid profile with the major to moderate compounds diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown aminophospholipid and gentiobiosyldiacylglycerol and a cellular fatty acid profile in which branched fatty acids predominate (Kämpfer *et al.*, 2006).

The recently described species *Bacillus pycnus*, *Bacillus neidei*, *Bacillus arvi* and *Bacillus arenosi* (Nakamura *et al.*, 2002; Heyrman *et al.*, 2005) exhibit a high degree of 16S rRNA gene sequence similarity to species of the genus *Kurthia* (a genus lacking endospore formation), ranging from 95.1 to 96.6 %. In conclusion, they can be considered as members of *Bacillus* rRNA group 2 (Ash *et al.*, 1991; Farrow *et al.*, 1994). Furthermore, *B. pycnus* and *B. neidei* exhibit L-Lys-D-Glu in the cell-wall peptidoglycan (Nakamura *et al.*, 2002), which corresponds to peptidoglycan types A4 α (Schleifer & Kandler, 1972) and A11.33 (<http://www.dsmz.de/species/murein.htm>). This trait is in agreement with the characteristics of *Bacillus* rRNA group 2. The species *B. arvi*, *B. arenosi* and *B. neidei* share among each other sequence similarities in the range 97.1–99.8 %, whereas *B. pycnus* appears to be more distantly related, exhibiting 95.2–96.7 % similarity to the other three species. These values indicate almost the same degree of relatedness for *B. pycnus* and for representatives of the genus *Kurthia* to the group consisting of *B. arvi*, *B. arenosi* and *B. neidei* and hence the latter group might be considered members of a new genus. Based upon sequence similarities, *B. pycnus* can be considered as a representative of another genus.

The present study was undertaken to classify strains 433-D9, 121-X1 and 433-E17 which, in the course of this study, were affiliated to *B. arvi*. The three strains were isolated from organic-matter-rich samples from a sphagnum bog, river sediment and forest soil, respectively, in south-eastern Wisconsin using methods described previously (Albert *et al.*, 2005). After heat shocking, samples were subcultured in plate count broth (PCB; Difco) and incubated aerobically at 10 °C. Periodically, samples were streaked onto plate count agar (PCA; Difco) and incubated at 10 °C.

In order to provide evidence that strains 433-D9, 121-X1 and 433-E17 may belong to a single species, they were subjected to BOX-PCR (Wieser & Busse, 2000). All three strains exhibited a product at approximately 800 bp (see Supplementary Fig. S1 in IJSEM Online), suggesting that they are strains of the same species. Strains 121-X1 and 433-E17 showed identical genomic fingerprints, indicating that they might be clonally related. A representative of this group, strain 433-D9, was subjected to 16S rRNA gene sequence analysis as reported previously (Albert *et al.*, 2005).

Sequence comparisons (Pearson & Lipman, 1988) of its 16S rRNA-encoding gene showed that strain 433-D9 shared highest sequence identities with *B. arvi* DSM 16317^T (100 %), *B. arenosi* DSM 16319^T (99.8 %) and *B. neidei* NRRL BD-87^T (97.1 %) and 96.7 % similarity with *B. pycnus* NRRL NRS-1691^T. *Kurthia* species (95.7–96.5 % similarity), *Sporosarcina macmurdoensis* CMS 21w^T (95.7 %) and *Bacillus sphaericus* DSM 28^T (95.0 %) were identified as moderately related. Phylogenetic analyses (Fig. 1) were performed by the use of the program ARB (Ludwig *et al.*, 2004) as indicated in the figure legend. The results supported the observed relationships. DNA–DNA hybridizations were carried out with strain 433-D9, *B. arvi* DSM 16317^T and *B. arenosi* DSM 16319^T, following the method described by Ziemke *et al.* (1998). DNA–DNA relatedness of 88 % (reverse 80 %) with *B. arvi* DSM 16317^T and 55 % with *B. arenosi* DSM 16319^T identified isolate 433-D9 as a strain of the species *B. arvi*. Strains 121-X1 and 433-E17 can be also considered to be strains of *B. arvi*, because they share with strain 433-D9 similar BOX fingerprints.

In order to compare strains 433-D9, 121-X1 and 433-E17 with *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T, *B. neidei* NRRL BD-87^T and *B. pycnus* NRRL NRS-1691^T, this set of

Fig. 1. Phylogenetic reconstruction based on a neighbour-joining algorithm showing the affiliation of strain 433-D9, *Viridibacillus* (*Bacillus*) *arvi* DSM 16317^T, *Viridibacillus* (*Bacillus*) *arenosi* LMG 22166^T and *Viridibacillus* (*Bacillus*) *neidei* NRRL BD-87^T with their closest relatives. The tree is a result of a consensus topology obtained after the evaluation of multiple trees by using neighbour-joining, maximum-parsimony and maximum-likelihood algorithms with different datasets and masks as implemented in the ARB program. Multifurcations show those branching orders that could not be resolved unambiguously. Bootstrap values are based on evaluation of 1000 trees by the neighbour-joining method with the same dataset as shown in the tree and indicate the percentage of reconstructions where a given branch appeared consistently. Bar, 2% sequence divergence.

strains was subjected to physiological and biochemical characterization and analyses of their cellular fatty acids, polar lipids, cell-wall composition and respiratory quinone systems. Respiratory quinones, cell-wall composition and polar lipids had not been reported previously for *B. arvi* or *B. arenosi* (Heyrman *et al.*, 2005), while Nakamura *et al.* (2002) did not report the polar lipid profiles or quinone system of *B. neidei* and *B. pycnus*.

The physiological and biochemical characterizations reported in Table 1 as part of this investigation were performed as follows. All tests were performed at 25 °C, except growth temperature range, using 24 h-old cultures grown on PCA. Indole production, lysine decarboxylase, casein and starch hydrolysis, motility testing, catalase activity, oxidase test and Gram staining were assessed using standard procedures (Smibert & Krieg, 1994).

Gelatin hydrolysis was determined by inoculating tubes of 12% nutrient gelatin (Difco) and incubating with shaking aeration for 7 days. Temperature tolerance was determined by visual examination for growth on PCA plates. Prior to inoculation, the plates were incubated at the test temperature for 24 h. After inoculation, plates were incubated for 2–7 days. Anaerobic growth was determined by monitoring for growth visually in thioglycolate broth supplemented with glucose (Difco). After inoculation, the tubes were placed in the anaerobe jars and anaerobic conditions were generated using H₂ and CO₂ GasPaks (BBL). Ten days later, the tubes were removed from the anaerobic conditions and examined visually for growth. Turbidity was used to indicate growth. All tubes were streaked onto PCA to determine the identity of the bacterium present. Growth at NaCl concentrations of 2, 5, 7 and 10% (w/w) was determined by inoculating tubes

Table 1. Differentiating characteristics between *B. neidei*, *B. arvi*, *B. arenosi* and *B. pycnus*

Strains: 1, *B. arvi* DSM 16317^T, 433-D9, 121-X1 and 433-E17; 2, *B. neidei* NRRL BD-87^T; 3, *B. arenosi* DSM 16319^T; 4, *B. pycnus* NRRL NRS-1691^T. All data were obtained in this study. All strains are motile and positive for catalase and growth in 2% NaCl and negative for oxidase, growth in 7% NaCl, starch and casein hydrolysis, indole production, the Voges–Proskauer test, citrate utilization and acid production from D-glucose, D-mannitol, D-xylose, D-lactose, glycerol, sucrose, D-ribose, D-mannose, D-galactose, L-arabinose and D-cellobiose.

Character	1	2	3	4
Temperature range (°C)	5–40	5–45	5–37	10–45
Growth in 5% NaCl	–	+	–	–
Gelatin hydrolysis	+	+	+	–
Acid production from D-fructose	+	+	–	–
Cell-wall type	L-Lys–D-Asp	L-Lys–D-Glu	L-Lys–D-Asp	L-Lys–D-Glu

containing 10 ml PCB at the defined NaCl concentrations. After inoculation, the tubes were incubated with shaking aeration for 2–7 days. Appearance of turbidity was used to indicate growth. All tubes were streaked on PCA to determine the identity of the bacterium present. Citrate utilization, the Voges–Proskauer test and acid production from D-glucose, D-mannitol, D-xylose, D-lactose, glycerol, sucrose, D-ribose, D-mannose, D-galactose, fructose, L-arabinose and D-cellobiose were assessed using the methods described by Gordon *et al.* (1973). Tubes were incubated for up to 7 days.

Results for the physiological and biochemical characterizations performed as part of this study are similar to those reported previously (Nakamura *et al.*, 2002; Heyrman *et al.*, 2005) except for the following. This study found that all strains including the type strains of *B. arvi*, *B. neidei* and *B. arenosi* were able to liquefy gelatin. *B. neidei* NRRL BD-87^T was found to produce acid from fructose and grow weakly anaerobically. Differences are probably due to differences in test methods.

Colonies of isolates 433-D9, 433-E17 and 121-X1 developed irregular to lobate edges with a slight greenish tint when grown on PCA at 25 °C for 48 h. With longer incubation, the greenish colour became more obvious. Employing the same conditions, *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T, *B. neidei* NRRL BD-87^T and *B. pycnus* NRRL NRS-1691^T did not show the green colour. The relationship between the appearance of the green pigment and sporulation was further examined by using different growth media. The media used were PCA, TSA and R2A (all from Difco), which represent a range of nutrient contents. All strains were grown aerobically at 25 °C. Colour development was determined by examining the colony colour visually and using a sterile cotton swab to remove cells from the heavy growth area of the plate and examining the colour on the swab visually. The sporulation process was monitored by using microscopic observations of wet mounts from the heavy streak areas.

On PCA after 48–72 h of growth, endospores were observed for all strains, although only strains 433-D9, 433-E17 and 121-X1 displayed the development of the green pigment (Supplementary Fig. S2). After 48 h of growth on PCA, strains 433-D9, 433-E17 and 121-X1 displayed the development of the green pigment, when approximately 60–70 % of the cells contained endospores, with a few free spores present. By 72 h, the green pigment was obvious, with an increase in the number of free spores. Growth of all strains on TSA was excellent. However, sporulation for all strains was poor. Under these conditions, all strains, including strains 433-D9, 433-E17 and 121-X1, did not develop the green colour. After 24 h of growth on R2A, all strains grew readily and developed endospores (>95 %) but did not display the green colour. However, after 48 h of growth, strains 433-D9, 433-E17, 121-X1, *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T and *B. neidei* NRRL BD-87^T developed the green pigment

(Supplementary Fig. S3), while strain *B. pycnus* NRRL NRS-1691^T did not. The green colour was similar for all strains that developed the green pigment, although the pigment for *B. arenosi* DSM 16319^T was a lighter green. For all strains, after 48 h of growth on R2A, over 95 % of the endospores had been released from the mother cells as free spores.

The cellular fatty acid profile was determined by growing the strains on TSBA (Difco) at 23 °C for 21 and 30 h. Analysis was performed at two time points in order to minimize the effect of sporulation on the fatty acid profile, while the incubation temperature reflects the psychrotolerant nature of the strains. After growth, bacteria were saponified and the liberated fatty acids were methylated and analysed by capillary GLC (Hewlett Packard 6890) by the Sherlock system (MIDI 4.5), according to the manufacturer's instructions. No significant differences in the fatty acid profiles were observed in relation to the different incubation times. When compared to *B. arvi* DSM 16317^T and to each other, strains 433-D9, 121-X1 and 433-E17 showed similar fatty acid profiles, supporting their affiliation to a single species. *B. neidei* NRRL BD-87^T exhibited a profile that did not allow differentiation from the former group, whereas *B. arenosi* DSM 16319^T differed from this group only in a slightly lower content of anteiso-C_{15:0} and higher content of C_{16:1}ω11c. *B. pycnus* NRRL NRS-1691^T could be distinguished from the other strains based on significant differences in the content of iso-C_{14:0}, iso-C_{15:0}, iso-C_{17:1}ω10c and iso-C_{17:1} I/anteiso-C_{17:1} B and the lack of C_{15:0}, which is in agreement with its more distant relatedness to the other species. Once more, our results confirmed the common knowledge that fatty acid data should be only compared when highly standardized conditions for growth of biomass are applied. In comparison with data reported by Nakamura (2000) and Nakamura *et al.* (2002), examination of our results (Table 2) indicated some significant differences for *B. neidei* and *B. pycnus* in respect to the relative amounts of iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0} and C_{16:0}ω11c and the presence/absence of other fatty acids (*B. neidei*) and relative amounts of C_{16:1}ω7c alcohol and the presence/absence of other acids (*B. pycnus*). Our results from fatty acid analyses of *B. arvi* DSM 16317^T and *B. arenosi* DSM 16319^T also differed from corresponding strains examined by Heyrman *et al.* (2005). Compared with reported results, *B. arvi* DSM 16317^T contained significantly lower contents of iso-C_{14:0}, C_{16:1}ω7c alcohol and iso-C_{16:0} and higher contents of iso-C_{15:0}, iso-C_{17:1}ω10c and iso-C_{17:1} I/anteiso-C_{17:1} B and *B. arenosi* DSM 16319^T contained smaller amounts of iso-C_{14:0} and iso-C_{16:0} and larger amounts of iso-C_{15:0}. The differences might be caused by different cultivation conditions. This study used TSBA, an incubation temperature of 23 °C and incubation times of 21 and 30 h, while the previously published studies used TSA, an incubation temperature of 30 °C and incubation times of 24–48 h (Nakamura *et al.*, 2002; Heyrman *et al.*, 2005).

Respiratory lipoquinones and polar lipids were extracted and analysed as reported previously (Tindall, 1990a, b;

Table 2. Cellular fatty acid profiles of strains 433-D9, 433-E17 and 121-X1 and the type strains of *B. neidei*, *B. arvi*, *B. arenosi* and *B. pycnus*

Strains: 1, 121-X1; 2, 433-E17; 3, 433-D9; 4, *B. neidei* NRRL BD-87^T; 5, *B. arvi* DSM 16317^T; 6, *B. arenosi* DSM 16319^T; 7, *B. pycnus* NRRL NRS-1691^T. Strains were grown on TSBA at 23 °C for 21 or 30 h as indicated. —, Not detected.

Fatty acid	1		2		3		4		5		6		7	
	21 h	30 h	21 h	30 h	21 h	30 h	21 h	30 h	21 h	30 h	21 h	30 h	21 h	30 h
i-C _{14:0}	1.1	1.1	0.9	0.8	1.5	—	1.5	1.4	1.4	1.4	1.0	1.0	4.7	4.7
C _{14:0}	—	0.7	0.9	0.6	0.6	—	1.0	1.0	0.8	0.8	1.6	1.0	—	0.4
i-C _{15:0}	47.0	46.8	53.7	53.6	54.9	57.4	52.4	54.7	44.7	46.2	55.2	55.5	74.3	74.2
ai-C _{15:0}	21.9	21.4	17.1	16.3	15.5	15.2	15.4	15.3	21.6	21.7	11.3	11.2	10.0	9.9
C _{15:0}	2.2	2.2	2.7	1.6	3.3	2.8	2.1	1.9	3.0	2.7	3.4	2.8	—	—
C _{16:1} ω7c alcohol	3.3	3.2	2.4	2.5	3.2	3.3	4.8	4.2	3.9	3.7	3.1	3.7	1.8	1.8
i-C _{16:0}	—	0.6	—	0.6	0.6	—	0.8	0.7	0.9	0.8	—	0.7	2.1	2.1
C _{16:1} ω11c	3.6	3.5	4.0	3.3	2.9	2.9	4.1	3.8	3.8	3.9	8.2	7.0	0.7	0.6
i-C _{17:1} ω10c	6.3	5.7	6.6	7.6	6.2	7.0	7.0	6.8	5.4	5.2	7.0	7.1	0.8	0.8
i-C _{17:1} I/ai-C _{17:1} B	7.7	7.8	6.2	7.2	5.3	5.6	6.3	5.8	7.2	6.8	5.1	5.1	0.7	0.6
i-C _{17:0}	2.1	2.0	1.9	2.2	2.0	2.5	1.6	1.7	2.0	2.0	2.3	2.5	2.3	2.1
ai-C _{17:0}	4.8	4.9	3.7	3.3	3.6	3.2	2.8	2.4	5.3	4.8	1.8	1.8	1.2	1.2

Altenburger *et al.*, 1996). The primary isoprenoid quinones present in strains *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T, *B. neidei* NRRL BD-87^T, 433-D9, 433-E17 and 121-X1 were menaquinone MK-8 (69–81%), menaquinone MK-7 (19–30%) and traces of menaquinone MK-6. In contrast, *B. pycnus* NRRL NRS-1691^T exhibited a quinone system with the predominant compound menaquinone MK-7 (>99%) and only traces of MK-8. Quinone systems with the predominant compound MK-8 have been reported only rarely among bacilli and representatives of *Bacillus* group 2, and the type species of the genus *Bacillus*, *B. subtilis*, contains a quinone system with menaquinone MK-7 predominant (Collins & Jones, 1981). Polar lipid profiles of strains 433-D9, 433-E17, 121-X1, *B. arvi* DSM 16317^T (Fig. 2), *B. arenosi* DSM 16319^T and *B. neidei* NRRL BD-87^T were nearly identical, exhibiting the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, major to moderate amounts of an unknown aminophospholipid (APL1) and moderate to minor amounts of two phospholipids (PL1, PL2) and four unknown polar lipids (L1–L4). *B. pycnus* NRRL NRS-1691^T differed from this group by the presence of moderate amounts of an unknown phospholipid (PL3) and minor amounts of another unknown phospholipid (PL4), traces of another aminophospholipid and the lack of unknown phospholipid PL1 and aminophospholipid APL1 (results not shown). The type strains of *Kurthia gibsonii* and *Kurthia zopfii*, which were also analysed, also exhibited these major characteristics, but the relative proportion of phosphatidylethanolamine was significantly higher than in the other strains, whereas no additional aminophospholipids could be detected. This polar lipid profile lacking any glycolipid also distinguishes the group of strains analysed here from *B. subtilis* DSM 36^T. Another distinguishing trait is the presence of another aminophospholipid in *B. subtilis* DSM 36^T (Kämpfer *et al.*, 2006).

Analyses of peptidoglycan structures were carried out as described by Groth *et al.* (1996). Cell-wall peptidoglycan of strains 433-D9, *B. arvi* DSM 16317^T and *B. arenosi* DSM 16319^T was determined to be of the A4α type with L-Lys–D-Asp as described by Schleifer & Kandler (1972), which corresponds to type A11.31 (<http://www.dsmz.de/species/murein.htm>), which is also characteristic of species of the closely related genus *Kurthia* (Shaw & Keddle, 1983). This

Fig. 2. Two-dimensional TLC of polar lipids of *B. arvi* DSM 16317^T. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL1–2, unknown phospholipids; APL1, unknown aminophospholipid; L1–4, unknown polar lipids.

trait distinguishes the three strains from *B. neidei* NRRL BD-87^T and *B. pycnus* NRRL NRS-1691^T, which were reported to contain a peptidoglycan based on L-Lys-D-Glu (type A11.33).

The G + C content was determined as described previously (Albert *et al.*, 2005). Results of the analysis found that the G + C content for strain 433-D9 was 40.4 mol%, while that for strain 121-X1 was 39.2 mol%. The G + C contents for *B. arvi* DSM 16317^T, *B. arenosi* DSM 16319^T, *B. neidei* NRRL BD-87^T and *B. pycnus* NRRL NRS-1691^T were reported to be 35 mol% (Nakamura *et al.*, 2002; Heyrman *et al.*, 2005).

It is evident from the highly similar phenotype (biochemical and physiological traits, quinone system, polar lipids, peptidoglycan type, fatty acid profile), similar genomic fingerprints and DNA-DNA hybridizations that our isolates 433-D9, 433-E17 and 121-X1 are members of the species *B. arvi*. The species *B. arvi*, *B. arenosi* and *B. neidei* exhibit an extraordinarily high degree of similarity in respect to their fatty acid profiles, quinone systems and polar lipid profiles and also share the peptidoglycan type A4 α , based on the characteristic diamino acid L-lysine in their peptidoglycan, and the combination of these traits distinguishes them from related taxa of *Bacillus* group 2 (Ash *et al.*, 1991) as well as from the type species of the genus *Bacillus*, *B. subtilis* (Table 3). Although the presence of D-glutamic acid in the interpeptide bridge of the peptidoglycan of *B. neidei* distinguishes it from *B. arvi* and *B. arenosi*, which were shown to contain aspartic acid

in the interpeptide bridge, it appears to be justified to assign the three species to a single genus. Variations in the peptidoglycan interpeptide bridge have also been reported among certain species of the neighbouring genera *Sporosarcina* and *Planomicrobium* (Yoon *et al.*, 2001a, b; Reddy *et al.*, 2003), indicating that this trait can be less conserved than other chemotaxonomic traits. We refrain from assignment of *B. pycnus* to the same novel genus because it is distinguished by its phylogenetic position, quinone system, polar lipid composition and significantly smaller amounts of i-C_{17:1} I/ai-C_{17:1} B and i-C_{17:0} in its fatty acid profile. These data suggest that *B. pycnus* is a representative of another novel genus. However, we do not propose a novel genus for this species here because we think this should await substantiation of the characteristic traits by examination of additional closely related species. It is therefore proposed that the species *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* are assigned to the novel genus *Viridibacillus* gen. nov. as *Viridibacillus arvi* comb. nov. (the type species), *Viridibacillus arenosi* comb. nov. and *Viridibacillus neidei* comb. nov.

Description of *Viridibacillus* gen. nov.

Viridibacillus (Vi.ri.di.ba.cil'lus. L. adj. *viridis* green; L. masc. n. *bacillus* rod; N.L. masc. n. *Viridibacillus* the green bacillus/rod).

Gram-positive, spore-forming, motile rods. Endospores are round and are located terminally in a swollen or slightly swollen sporangium. Growth occurs below 10 °C and in

Table 3. Characteristics that distinguish the genus *Viridibacillus* gen. nov. from related taxa and the type species of genus *Bacillus*, *B. subtilis*

Data were obtained as follows: *Viridibacillus* gen. nov., this study; *B. pycnus*, this study and Nakamura *et al.* (2002); *Kurthia*, Shaw & Keddie (1983); *Lysinibacillus*, Ahmed *et al.* (2007); *Bacillus sylvestris*, Rheims *et al.* (1999); *Sporosarcina*, Ranft & Kandler (1970), Hess *et al.* (1979), Fahmy *et al.* (1985), Stackebrandt *et al.* (1987), Claus & Fritze (1989), Rheims *et al.* (1999), Yoon *et al.* (2001a) and Reddy *et al.* (2003); *B. subtilis*, Kämpfer (1994), Kämpfer *et al.* (2006) and Claus & Berkeley (1986). Dap, Diaminopimelic acid.

Characteristic	<i>Viridibacillus</i>	<i>B. pycnus</i>	<i>Kurthia</i>	<i>Lysinibacillus</i>	<i>B. sylvestris</i>	<i>Sporosarcina</i>	<i>B. subtilis</i>
Spore formation	Present	Present	Absent	Present	Present	Present	Present
Peptidoglycan	L-Lys-D-Glu or L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Asp	Lys-Asp	L-Lys-D-Glu	L-Lys-L-Ala-D-Asp or L-Lys-Gly-D-Glu or L-Lys-D-Glu	meso-Dap direct
Quinone system	MK-8, MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7
Polar lipids*	DPG, PE, PG, APL, 2PL	DPG, PE, PG, APL, 2PL, AL	DPG, PE, PG	DPG, PG, APGL	PG, DPG, PE, PS, PL	Unknown	PG, DPG, PE, GBG, APL
Major fatty acids (>10 %)†	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_{15:0} , (ai-C _{15:0}), i-C_{16:0} , ai-C _{17:0} , C _{16:1} ω7c alcohol)	iso-C_{15:1} , i-C_{16:1}	ai-C_{15:0} , (i-C_{16:1}), (ai-C _{17:0} , i-C _{14:0} , i-C _{17:1} I/ai-C _{17:1} B)	ai-C_{15:0} , i-C_{15:0} , ai-C _{17:1}

*Major compounds are in bold. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; APL, unknown aminophospholipid; PL, unknown phospholipid; AL, unknown aminolipid; GBG, gentiobiosyldiacylglycerol; APGL, unknown aminophosphoglycolipid.

†Components making up >20 % of the total fatty acids are in bold. Components listed in parentheses are present at the indicated level (>10 or >20 %) in some members of the taxon.

the presence of 2 % NaCl but not at 7 % NaCl (w/w). Sporulation and release of endospores (free spores) are abundant on R2A agar after 24 and 48 h, respectively, at 25 °C. On R2A, all members of the genus develop a green pigment. The quinone system consists of the major compound MK-8 (69–81 %) and moderate amounts of MK-7 (19–30.5 %). In the fatty acid profile, the major fatty acids are iso-C_{15:0} (approx. 40–60 %), anteiso-C_{15:0} (approx. 10–25 %), iso-C_{17:1}ω10c (approx. 5–8 %) and iso-C_{17:1}/anteiso-C_{17:1}. B. The polar lipid profile consists of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine and major to moderate amounts of an unknown aminophospholipid (APL1) and moderate to minor amounts of two unknown phospholipids (PL1, PL2) and three unknown polar lipids. Cell wall peptidoglycan is A4z, either L-Lys–D-Glu or L-Lys–D-Asp type. The G+C content of genomic DNA of species of the genus ranges from 35 to 40.4 mol%. The type species is *Viridibacillus arvi*.

Description of *Viridibacillus arvi* (Heyrman *et al.* 2005) comb. nov.

Viridibacillus arvi (ar'vi. L. gen. n. *arvi* of a field).

Basonym: *Bacillus arvi* Heyrman *et al.* 2005.

In addition to the characteristics of *B. arvi* summarized by Heyrman *et al.* (2005), the species exhibits the traits listed in the genus description. The previous description (Heyrman *et al.*, 2005) is emended with data for strains 433-D9, 121-X1 and 433-E17 and the data generated as a result of this study. All strains, including the type strain, share the following attributes. Cells are straight, round-ended and Gram-positive to Gram-variable, occurring singly and in pairs. On PCA, colonies are shiny, opaque, irregular and flat with a lobate edge and range from 3 × 5 to 6 × 6 mm in size when grown at 25 °C for 48 h. On PCA, strains 433-D9, 121-X1 and 433-E17 develop a green pigment in 48–72 h of growth at 25 °C, while the type strain does not. Growth occurs at 5 and 40 °C. Gelatin is liquefied, while starch is not hydrolysed. Strains grow at 2 % (w/w) NaCl but not at 5 %. Catalase-positive and oxidase-negative. Acid is not produced from D-glucose, D-mannitol, D-xylose, D-lactose, glycerol, sucrose, D-ribose, D-mannose, D-galactose, L-arabinose or D-cellobiose. Acid is produced from fructose. Cell wall peptidoglycan is of the L-Lys–D-Asp type.

The type strain, DSM 16317^T (=LMG 22165^T), was isolated from soil. Three other strains, 433-D9 (=DSM 16704 =NRRL B-23969), 121-X1 and 433-E17, were isolated from organic-matter-rich samples in Wisconsin, USA.

Description of *Viridibacillus arenosi* (Heyrman *et al.* 2005) comb. nov.

Viridibacillus arenosi (a.re.no'si. L. gen. n. *arenosi* of a sandy place).

Basonym: *Bacillus arenosi* Heyrman *et al.* 2005.

In addition to the characteristics of *B. arenosi* summarized by Heyrman *et al.* (2005), the species exhibits the traits listed in the genus description. Cell wall peptidoglycan is of the L-Lys–D-Asp type. The type strain is DSM 16319^T =LMG 22166^T.

Description of *Viridibacillus neidei* (Nakamura *et al.* 2002) comb. nov.

Viridibacillus neidei (nei'de.i. N.L. gen. n. *neidei* of Neide, in recognition of the early microbiologist E. Neide).

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

In addition to the characteristics of *B. neidei* summarized by Nakamura *et al.* (2002), the species exhibits the traits listed in the genus description. The type strain is DSM 15031^T =NRRL BD-87^T =JCM 11077^T.

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