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.

Richard A. Albert,1 † Julieta Archambault,1 † Melissa Lempa,1 Beth Hurst,1 Christine Richardson,1 † Stephanie Gruenloh,1,2 Metin Duran,3 Hanna Lucia Worliczek,4 Birgit E. Huber,4 Ramon Rosselló-Mora,5 Peter Schumann6 and Hans-Jürgen Busse4

1 Semco BioScience, 630 East Keefe, Milwaukee, WI 53212, USA
2 Medical College, University of Wisconsin, Milwaukee, WI, USA
3 Department of Civil and Environmental Engineering, Villanova University, Villanova, PA, USA
4 Institut für Bakteriologie, Mykrologie und Hygiene, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Vienna, Austria
5 Grup de Microbiologia Marina, Institut Mediterrani d’Estudis Avançats, E-07190 Esporles, Mallorca, Spain
6 DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany

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 16317T (100 %), Bacillus arenosi DSM 16319T (99.8 %) and Bacillus neidei NRRL BD-87T (97.1 %). Sequence similarities revealed Bacillus pycnus NRRL NRS-1691T 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 16317T and B. arenosi DSM 16319T was in agreement with their close relationship, but differentiated these strains from B. neidei NRRL BD-87T and B. pycnus NRRL NRS-1691T, 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 16317T, B. arenosi DSM 16319T and B. neidei NRRL BD-87T. 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-1691T, as also reported for representatives of the closely related non-endospore-forming genus Kurthia. Strains 433-D9, B. arvi DSM 16317T and B. arenosi DSM 16319T exhibited homogeneous and discriminative polar lipid profiles and fatty acid profiles consisting of major acids i-C15:0 and ai-C15:0 and moderate amounts of i-C17:1ω9c and i-C17:1ω0c that discriminated them from closely related strains such as B. neidei NRRL BD-87T. On the basis of clear-cut discriminative chemotaxonomic markers, we
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; Heyman 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 A4z (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 subject 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 16317T (100 %), B. arenosi DSM 16319T (99.8 %) and B. neidei NRRL BD-87T (97.1 %) and 96.7 % similarity with B. pycnus NRRL NRS-1691T. Kurthia species (95.7–96.5 % similarity), Sporosarcina macmurodensis CMS 21wT (95.7 %) and Bacillus sphaericus DSM 28T (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 16317T and B. arenosi DSM 16319T, following the method described by Ziemke et al. (1998). DNA–DNA relatedness of 88 % (reverse 80 %) with B. arvi DSM 16317T and 55 % with B. arenosi DSM 16319T 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 16317T, B. arenosi DSM 16319T, B. neidei NRRL BD-87T and B. pycnus NRRL NRS-1691T, this set of
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 anaerobic 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

![Fig. 1. Phylogenetic reconstruction based on a neighbour-joining algorithm showing the affiliation of strain 433-D9, Viridibacillus (Bacillus) arvi DSM 16317T, Viridibacillus (Bacillus) arenosi LMG 22165T and Viridibacillus (Bacillus) neidei NRRL BD-87T 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.](http://ijs.sgmjournals.org)

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

Strains: 1, B. arvi DSM 16317T, 433-D9, 121-X1 and 433-E17; 2, B. neidei NRRL BD-87T; 3, B. arenosi DSM 16319T; 4, B. pycnus NRRL NRS-1691T. 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, lysine decarboxylase, casein and starch hydrolysis, motility testing, catalase activity, oxidase test and Gram staining were assessed using standard procedures (Smibert & Krieg, 1994).
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 characterization 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-87T 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 16317T, B. arenosi DSM 16319T, B. neidei NRRL BD-87T and B. pycnus NRRL NRS-1691T 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 16317T, B. arenosi DSM 16319T and B. neidei NRRL BD-87T developed the green pigment (Supplementary Fig. S3), while strain B. pycnus NRRL NRS-1691T did not. The green colour was similar for all strains that developed the green pigment, although the pigment for B. arenosi DSM 16319T 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 16317T 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-87T exhibited a profile that did not allow differentiation from the former group, whereas B. arenosi DSM 16319T differed from this group only in a slightly lower content of anteiso-C15:0 and higher content of C16:1ω9t1c. B. pycnus NRRL NRS-1691T could be distinguished from the other strains based on significant differences in the content of iso-C14:0, iso-C15:0, iso-C17:1ω0t1c and iso-C17:1 I/anteiso-C17:1ω0t1c B and the lack of C15:0ω7c 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-C14:0, iso-C15:0, iso-C16:0 and C16:0ω9t1c and the presence/absence of other fatty acids (B. neidei) and relative amounts of C16:1ω0t7c alcohol and the presence/absence of other acids (B. pycnus). Our results from fatty acid analyses of B. arvi DSM 16317T and B. arenosi DSM 16319T also differed from corresponding strains examined by Heyrman et al. (2005). Compared with reported results, B. arvi DSM 16317T contained significantly lower contents of iso-C14:0, C16:1ω0t7c alcohol and iso-C16:0 and higher contents of iso-C15:0, iso-C17:1ω0t1c and iso-C17:1 I/anteiso-C17:1ω0t1c B and B. arenosi DSM 16319T contained smaller amounts of iso-C14:0 and iso-C16:0 and larger amounts of iso-C15:0. 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).
Altenburger et al., 1996). The primary isoprenoid quinones present in strains B. arvi DSM 16317\textsuperscript{T}, B. arenosi DSM 16319\textsuperscript{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\textsuperscript{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\textsuperscript{T}, B. arenosi DSM 16319\textsuperscript{T}, B. neidei NRRL BD-87\textsuperscript{T}, 433-D9, 433-E17 and 121-X1, B. arvi DSM 16317\textsuperscript{T}, B. arenosi DSM 16319\textsuperscript{T}, B. neidei NRRL BD-87\textsuperscript{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\textsuperscript{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\textsuperscript{T}. Another distinguishing trait is the presence of another aminophospholipid in B. subtilis DSM 36\textsuperscript{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\textsuperscript{T} and B. arenosi DSM 16319\textsuperscript{T} was determined to be of the A4a 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 & Keddie, 1983).

### 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

<table>
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<tr>
<th>Fatty acid</th>
<th>Strain</th>
<th>21 h</th>
<th>30 h</th>
<th>21 h</th>
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<td>0.6</td>
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**Fig. 2.** Two-dimensional TLC of polar lipids of B. arvi DSM 16317\textsuperscript{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-87T and *B. pycnus* NRRL NRS-1691T, 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 16317T, *B. arenosi* DSM 16319T, *B. neidei* NRRL BD-87T and *B. pycnus* NRRL NRS-1691T 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 peptidoglycan type, fatty acid profile), similar genomic 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 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-C17:1/ai-C17:1 B and i-C17:0 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.

**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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Viridibacillus</th>
<th>B. pycnus</th>
<th>Kurthia</th>
<th>Lysinibacillus</th>
<th>B. sylvestris</th>
<th>Sporosarcina</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore formation</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Quinone system</td>
<td>MK-8, MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PE, PG, APL, 2PL</td>
<td>DPG, PE, PG, APL, 2PL</td>
<td>DPG, PE, PG</td>
<td>DPG, PG, APGL</td>
<td>PG, DPG, PE, PS, PL</td>
<td>iso-C15:0, (ai-C15:0, ai-C15:0, C16:1ω7c alcohol)</td>
<td>PK, DPG, PE, GBG, APL</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>i-C15:0, ai-C15:0</td>
<td>i-C15:0, ai-C15:0</td>
<td>i-C15:0, ai-C15:0</td>
<td>i-C15:0, ai-C15:0, i-C16:0, ai-C17:0, C16:1ω7c alcohol</td>
<td>iso-C15:1, i-C16:1, ai-C15:0, (ai-C15:0, ai-C16:0, i-C17:1, B)</td>
<td>ai-C15:0, ai-C15:0, ai-C17:1</td>
<td></td>
</tr>
<tr>
<td>(&gt;10%)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Major compounds are in bold. DPG, Diphosphatidylglycerol; PG, Phosphatidylglycerol; PE, Phosphatidylethanolamine; APL, unknown aminophospholipid; PL, unknown phospholipid; AL, unknown aminolipid; GBG, Gentiobiosydilicacylglycerol; 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_{17:0}3-OH (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).


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-xylene, D-lactose, glycerol, sucrose, D-ribose, D-mannose, D-galactose, L-arabinobiose or D-cellobiose. Acid is produced from fructose. Cell wall peptidoglycan is of the L-Lys–D-Asp type.

The type strain, DSM 16317T (=LMG 22165T), 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).


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 16319T =LMG 22166T.

**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).


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 15031T =NRRL BD-87T =JCM 11077T.

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We would like to acknowledge the excellent technical assistance of Jerri Woodards. 16S rRNA gene sequencing of strain 433-D9 was performed by Accugenix (Newark, DE). Photomicrographs were taken with the assistance of the Electron Microscopic Laboratory at UW-Milwaukee Department of Biological Sciences.

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