Re-examination of the taxonomic position of Bacillus silvestris Rheims et al. 1999 and proposal to transfer it to Solibacillus gen. nov. as Solibacillus silvestris comb. nov.

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Following the transfer of three of the six species enclosed in the original definition of rRNA group 2 of Bacillus to the genus Sporosarcina and two to Lysinibacillus, other species of this group, some of which were added later, still await taxonomic revision. In a recent publication, a set of ‘core’ characteristics was proposed for species to be included in the genus Bacillus (Kämpfer et al., 2006). Except for Bacillus silvestris, however, several or none of these properties are available for members of rRNA group 2. According to our analysis of data including the ‘core’ characteristics, Bacillus silvestris should not be a member of the genus Bacillus. We therefore propose the establishment of a new genus, Solibacillus gen. nov., and transfer Bacillus silvestris to this genus as Solibacillus silvestris comb. nov., with the type strain HR3-23T (=DSM 12223T = ATCC BAA-269T = CIP 106059T).

In a major systematic study based on the 16S rRNA gene sequence analyses of the members of the genus Bacillus, Ash et al. (1991) recognized five distinct groups and proposed that absence of phenotypically definable criteria ‘should not preclude the recognition of these groups as distinct genera’. Subsequently, species belonging to rRNA group 3 were placed in a new genus, Paenibacillus (Ash et al., 1993; Shida et al., 1997). Bacillus rRNA group 2 comprises six Bacillus species: Bacillus fusiformis, Bacillus globisporus, Bacillus insolitus, Bacillus pasteurii, Bacillus psychrophilus and Bacillus sphaericus, and also the non-Bacillus species Sporosarcina ureae. An increasing number of species descriptions indicated the need to properly define the properties of the genus Bacillus (Farrow et al., 1994; Rheims et al., 1999; Stackebrandt & Swiderski, 2002; Kämpfer et al., 2006; Zhang et al., 2007) and according to Farrow et al. (1994) the ‘simplest and most phylogenetically consistent policy would be to exclude the group 2 bacilli from the genus Bacillus’. The same authors also suggested the transfer of B. globisporus, B. pasteurii and B. psychrophilus to the genus Sporosarcina. These three species were subsequently described as members of Sporosarcina by Yoon et al. (2001). Recently Ahmed et al. (2007) proposed the transfer of two additional rRNA group 2 Bacillus species, i.e. B. fusiformis and B. sphaericus, into a newly created genus Lysinibacillus and Albert et al. (2007) proposed a new genus Viridibacillus to include the species Bacillus arvi, Bacillus arenosi and Bacillus neidei. Five of the six species originally placed in Bacillus rRNA group 2 were excluded from this genus and reclassified as members of existing or new genera. In parallel to the exclusion of certain species, several new species were affiliated to this group [Bacillus silvestris (Rheims et al., 1999); Bacillus pycnus and B. neidei (Nakamura et al., 2002); Bacillus psychrodurans and Bacillus psychrotolerans (Abd El-Rahman et al., 2002); Bacillus odysseyi (La Duc et al., 2004); B. arvi and B. arenosi (Heyrman et al., 2005); Bacillus massiliensis (Glazunova et al., 2006); Bacillus decisifrondis (Zhang et al., 2007)]. In order to clarify the taxonomic situation within the genera Bacillus and Paenibacillus, Kämpfer et al. (2006) strongly recommended the inclusion of polar lipid pattern, menaquinone type and fatty acid profiles as important criteria for making taxonomic conclusions. According to the authors, the presence of diphasphatidylglycerol, phosphatidylglycerol, diphosphatidylethanolamine, an aminoacylphosphatidylglycerol and a glycolipid (β-gentiobiosyldiacylglycerol) as polar lipids, MK-7 as a major isoprenoid quinone and

Abbreviations: AL, aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PGL, phosphoglycolipid; PL, phospholipid.

Supplementary tables showing 16S rRNA gene sequence similarity between Bacillus silvestris and other closely related members of Bacillus rRNA group 2; and comparative fatty acid profiles of B. silvestris, Caryophanon latum and Bacillus subtilis are available with the online version of this paper.
predominance of iso- and anteiso-C_{15:0} as the major fatty acids ‘should in future, constitute the ‘core characteristics’ of the genus Bacillus’. Unfortunately, except for B. silvestris, information of this dataset is not available for most of the species of Bacillus rRNA group 2.

In order to redetermine the topology of the phylogenetic dendrogram of Bacillus rRNA group 2 the 16S rRNA gene sequences of Bacillus silvestris, related members of Bacillus and Lysinibacillus, and type species of genera of the families Planococcaceae (Planococcus, Planomicrobium, Sporosarcina, Filibacter) and Caryophanaceae (Caryophanon) were retrieved from GenBank. The sequences were aligned using the CLUSTAL_X software (Thompson et al., 1997); the gaps at the end of the sequences were removed manually. A phylogenetic tree based on the generation of the neighbour-joining method (Saitou & Nei, 1987) was constructed using the TREECON software package (Van de Peer & De Wachter, 1997; the gaps in the branching point of the lineage embracing Filibacter limicola and Sporosarcina ureae when the maximum-likelihood method was used. In all trees B. silvestris appeared as a sister clade of the Caryophanon lineage, a situation already depicted in the original description of B. silvestris (Rheims et al., 1999). Pairwise sequence similarities were calculated using the BioEdit software package (Hall, 1999). Supplementary Table S1 (available in IJSEM Online) presents the sequence similarity matrix constructed on the basis of 1477 unambiguous nucleotides of B. silvestris and those members of Bacillus rRNA group 2 bacilli which are neighbours in the phylogenetic tree. B. silvestris shows 96.6 % sequence similarity to Caryophanon latum followed by C. tenue (95.5 %), B. odysseyi (95.1 %) and Lysinibacillus fusiformis (95.1 %). Sequence similarities with other members of Bacillus rRNA group 2 were less than 95.0 %.

Although C. latum and C. tenue show closest similarity in the 16S rRNA gene sequence with B. silvestris and share the same clade in the phylogenetic tree, they are distinguishable on the basis of morphological and chemotaxonomic characteristics. B. silvestris differs from Caryophanon in the type of menaquinones present, i.e. presence of MK-7 in B. silvestris as against MK-6 in Caryophanon. Secondly, Caryophanon species are known to occur in trichomes (multicellular forms) and endospores have not been reported in this genus, whereas B. silvestris is endospore-forming and occurs only in rod shape. Thirdly, the G+C content of Caryophanon varies from 41.0 to 46.0 mol%, compared with 39.3 mol% for B. silvestris. Differences in fatty acid profiles between Caryophanon latum DSM 24151^T and Bacillus silvestris DSM 12223^T grown on the same medium and at the same temperature support their affiliation into different genera. Though both strains share the main component iso-C_{15:0}, quantitative and qualitative differences do occur (Supplementary Table S2, available in IJSEM Online). Moreover, the habitat of Caryophanon seems to be very restricted (mostly cattle dung), and is quite different from the habitats of the taxa under consideration here. B. silvestris can also be differentiated from species of Kurthia, Planococcus, Planomicrobium and Filibacter on the basis of sporulation and other phenotypic characteristics (Table 1). Except for B. silvestris, data for peptidoglycan composition, polar lipids and often fatty acids are lacking for those Bacillus species that together with B. silvestris belong to the same clade (B. odysseyi, B. massiliensis, B. pycnus). Many investigators have suggested that species placed within the genus Bacillus in Bacillus rRNA group 2 do not belong to it and should be reclassified either into novel genera or into pre-existing genera within the group (Farrow et al., 1994; Yoon et al., 2001; Ahmed et al., 2007; Albert et al., 2007). As for the taxonomic position of B. odysseyi, B. massiliensis and B. pycnus, all three species seem to be phylogenetically distantly related to B. silvestris (Supplementary Table S1 and Fig. 1). All three species are also present in a different clade separated from B. silvestris. B. odysseyi and B. massiliensis are present in a single clade sharing common ancestry with the genus Lysinibacillus, whereas B. pycnus forms a clade along with Kurthia with both nodes supported by a high bootstrap value (Fig. 1). It has to be pointed out that B. odysseyi is more closely related to Lysinibacillus boronitolerans strain 10a^T which with it shares a sequence similarity of 96.1 %, as described by Ahmed et al. (2007). As for B. pycnus and B. massiliensis, they are more distantly related at the 16S rRNA gene sequence level to B. silvestris (<94.0 % similarity, Supplementary Table S1). Polar lipid profile of B. pycnus as described by Albert et al. (2007) consists of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), one aminophospholipid (APL), two phospholipids (PL) and one aminolipid (AL). This is considerably different from that of B. silvestris that consists of DPG, PG, PE, phosphatidylserine (PS) and one phospholipid (PL). Because of these differences, Albert et al. (2007) have even suggested that B. pycnus should be considered as representative of a novel genus.

Kämpfer et al. (2006) recommended that, for inclusion of any species in the genus Bacillus, it should share certain phenotypic and chemotaxonomic characteristics present in the type species of the genus, B. subtilis. B. silvestris differs from B. subtilis in cell-wall type, pattern of polar lipids (Table 1) and fatty acid composition (Supplementary Table S2, available in IJSEM Online). It differs from the type species and other members of the genus Bacillus, exhibiting A1\_ type peptidoglycan, in containing A4\_ type peptidoglycan (Schleifer & Kandler, 1972). The fatty acid profile of the species shows major qualitative and quantitative differences when compared with that of B. subtilis (Supplementary Table S2). Whereas iso fatty acids (iso-C_{16:1} and iso-C_{15:0}) are predominant fatty acids in
Table 1. Characteristics that differentiate *B. silvestris* from members of closely related genera

+ Present; −, absent; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; UPL, unidentified phospholipid; GL, glycolipid (β-gentiobiosyldiacylglycerol); PGL, phosphoglycolipid. V, Variable reaction; NA, data not available; ID, identical sequence.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>B. silvestris</em></th>
<th>Lysinibacillus†</th>
<th>Viridibacillus‡</th>
<th>Caryophanon§</th>
<th>Kurthia†</th>
<th>Sporosarcina†</th>
<th>Bacillus∥</th>
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<tr>
<td>Rod-shaped cells</td>
<td>+</td>
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<td>−/−</td>
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<td>Round</td>
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<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>NA</td>
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<tr>
<td>Cell wall type</td>
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<td>Lys-Asp</td>
<td>Lys-Glu or Lys-Asp</td>
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<td>Lys-Asp</td>
<td>Lys-Glu-tGlu</td>
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<td>Menaquinone system</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-8, MK-7</td>
<td>MK-6</td>
<td>MK-7</td>
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<td>Polar lipids</td>
<td>PG, DPG, PE, PS, UPL</td>
<td>DPG, PG, PGL</td>
<td>DPG, PG, PE, APL, 2PL</td>
<td>NA</td>
<td>DPG, PG, PE</td>
<td>NA</td>
<td>PG, DPG, PE, aminoacylphosphatidylglycerol, GL</td>
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<td>92.6</td>
<td>96.6</td>
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<td>92.3</td>
<td>91.5</td>
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</table>

*Data from Rheims *et al.* (1999).
†Data from Ahmed *et al.* (2007).
‡Data from Albert *et al.* (2007).
§Fatty acid data for Caryophanon are from this study.
∥Data from Kämpfer *et al.* (2006).
B. silvestris, members of the genus Bacillus, including B. subtilis, contain iso- and anteiso-C₁₅ : 0 as their major fatty acids. The polar lipid profile of B. silvestris shows the presence of PS as a minor component, but no glycolipid which is present in B. subtilis (Kämpfer et al., 2006). To our knowledge the presence of PS has not been reported so far in any species of Bacillus. In addition, B. silvestris can be differentiated from other closely related genera Lysinibacillus and Viridibacillus by the oxidase test, menaquinone types, major fatty acids and polar lipids (Table 1). Phylogenetically also B. silvestris was distantly related to these two genera and was always retrieved in a separate clade in different tree-drawing methods (Fig. 1). Therefore, on the basis of phylogenetic analyses (Fig. 1), differences in cell-wall type, polar lipids and fatty acid profiles (Table 1 and Supplementary Tables S1 and S2), we propose to reclassify B. silvestris in a new genus, Solibacillus gen. nov., as Solibacillus silvestris comb. nov.

Description of Solibacillus gen. nov.

Solibacillus (So.li.ba.cil’lus. L. n. solum soil; L. n. Bacillus a bacterial genus; N.L. n. Solibacillus a Bacillus-like organism isolated from soil).

Gram-positive, rod-shaped cells; round endospores are formed terminally in swollen sporangia; catalase-positive; oxidase, Voges–Proskauer, nitrate reduction and indole formation tests are negative; cell-wall type A4 (lysine, glutamic acid, alanine); major isoprenoid quinone is MK-7; iso-C₁₅ : 0 and iso-C₁₆ : 1 are the predominant fatty acids; polar lipids are PG, DPG, PE, PS and one unknown PL.

Description of Solibacillus silvestris comb. nov.

Description of the species is the same as given by Rheims et al. (1999). Type strain is HR3-23T (DSM 12223T = ATCC BAA-269T = CIP 106059T).

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References


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