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.

Srinivasan Krishnamurthi,1 Tapan Chakrabarti1 and Erko Stackebrandt2

1Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Sector 39A, Chandigarh 160036, India
2DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7b, D-38124 Braunschweig, Germany

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); *B. odyssey* (La Duc et al., 2004); *B. arvi* and *B. arenosi* (Heyrman et al., 2005); *Bacillus massiliensis* (Glazunova et al., 2006); *Bacillus decisfrondis* (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 diphosphatidylglycerol, phosphatidylglycerol, diphosphatidylethanolamine, an aminoacylphosphatidylglycerol and a glycolipid (β-gentiobiosyldictyoglycerol) as polar lipids, MK-7 as a major isoprenoid quinone and

Abbreviations: AL, aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylsereine; 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 re-determine 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 *Planococaceae* (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 generated on the basis of the neighbour-joining method (Saitou & Nei, 1987) using the neighbour-joining method (Saitou & Nei, 1987) was constructed using the TREECON software package (Van de Peer & De Wachter, 1997) while trees based on maximum-parsimony and maximum-likelihood methods were constructed using the PHYLIP software package, version 3.67 (Felsenstein, 1993). The topology of the neighbour joining tree agreed with that generated by maximum-parsimony method, but differed slightly 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 24151T and *Bacillus silvestris* DSM 12223T 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* with which 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 A1γ: type peptidoglycan, in containing A4γ 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

| Characteristic                      | *B. silvestris* | Lysinibacillus† | Viridibacillus‡ | Caryophanon§ | Kurthia† | Sporosarcina† | Bacillus|| |
|-----------------------------------|----------------|-----------------|-----------------|--------------|---------|--------------|----------|
| Rod-shaped cells                  | +              | +               | +               | Trichome rods | +       | –/+          | +        |
| Endospore formation               | +              | +               | –/+             | –/++         | –/++   | +            | +        |
| Endospore shape                   | Round          | Round           | Round           | NA           | NA      | Round        | Oval/ellipsoidal |
| Oxidase                           | –              | +               | –               | V            | –       | +            | +        |
| Cell wall type                    | Lys-Glu        | Lys-Asp         | Lys-Glu or Lys-Asp | Lys-Glu     | Lys-Glu | Lys-Asp      | Lys-Gly-Glu |
| Menaquinone system                | MK-7           | MK-7            | MK-8, MK-7     | MK-6         | MK-7    | MK-7         | MK-7     |
| Major fatty acids                 | Iso-C_{15:0}, anteiso-C_{15:0} | Iso-C_{15:0} | Iso-C_{15:0} | Iso-C_{15:0} | Anteiso-C_{15:0} | Anteiso-C_{15:0} | Anteiso-C_{15:0} |
| Polar lipids                      | PG, DPG, PE, PS, UPL | DPG, PG, PGL | DPG, PG, PE, APPL, 2PL | NA | DPG, PG, PE | NA | PG, DPG, PE, amino-acylphosphatidylglycerol, GL |
| DNA G+C content (mol%)            | 39.3           | 35–38           | 35–40.4         | 41–46        | 36–38   | 40–42        | 32–69    |
| 16S rRNA gene sequence similarity | ID             | 92.9            | 92.6            | 96.6         | 91.4    | 92.3         | 91.5     |

*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-C\textsubscript{15}: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\(\alpha\) (lysine, glutamic acid, alanine); major isoprenoid quinone is MK-7; iso-C\textsubscript{15}:0 and iso-C\textsubscript{16}: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 HR3-23\(^T\) (=DSM 12223\(^T\) = ATCC BAA-269\(^T\) = CIP 106059\(^T\)).

**Acknowledgements**

Financial assistance from DBT, Government of India and CSIR is duly acknowledged. S. K. is a recipient of CSIR fellowship. This is IMTECH communication number 042/2007. We are extremely thankful to Dr Rüdiger Pukall (DMSZ, Braunschweig, Germany) for his help in fatty acid analysis. We thank the anonymous reviewers for their constructive suggestions on the manuscript. We also thank Dr Jean Euzeby (Ecole Nationale Vétérinaire, Toulouse, France) for his advice on nomenclature.

**References**


Felsenstein, J. (1993). PHYLIP (Phylogeny inference package), version 3.5c. distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.


