Transfer of *Bacillus schlegelii* to a novel genus and proposal of *Hydrogenibacillus schlegelii* gen. nov., comb. nov.

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Analysis of the 16S rRNA gene sequences of species currently assigned to the genus *Bacillus* has shown an extensive intrageneric phylogenetic heterogeneity. The 16S rRNA gene sequence of *Bacillus schlegelii* ATCC 43741T shows only 82.2–85.9% sequence similarity to type strains of other members of the genus *Bacillus* and ≈88.5% sequence similarity to recognised species of the most closely related genera, *Calditerricola* (88.4–88.5%), *Planifilum* (87.3–87.8%) and *Caldalkalibacillus* (87.2–87.9%). Furthermore, *B. schlegelii* ATCC 43741T could not be assigned to an existing family by phylogenetic analysis. The predominant menaquinone was MK-7. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and two unidentified glycolipids. The major fatty acids were iso-C16:0, C16:0, iso-C17:0 and anteiso-C17:0. Both the polar lipid profile and the fatty acid composition clearly distinguished *B. schlegelii* DSM 2000T from the type species of the genus *Bacillus, Bacillus subtilis*. Hence, there is no evidence for a clear phenotypic grouping of this organism into the genus *Bacillus* nor to the genera *Calditerricola, Caldalkalibacillus* or *Planifilum*. A proposal is made to transfer *Bacillus schlegelii* to a novel genus and species, *Hydrogenibacillus schlegelii* gen. nov., comb. nov., and to emend the species description. The type strain of the type species is DSM 2000T (=ATCC 43741T=CCUG 26017T=CIP 106933T).

*Bacillus schlegelii* was proposed by Schenk and Aragno (1979) as a novel species of thermophilic, facultatively anaerobic, chemolithotrophic bacteria that are able to oxidize hydrogen. This study was a consequence of the initial study of Aragno (1978), who isolated several strains of spore-forming thermophilic hydrogen bacteria from the superficial layer of the sediment of a little eutrophic lake. The name was validated in Validation List 6 (Euzeby, 1981) and only one further detailed physiological study on the hydrogenase of this organism has been published (Pinkwart et al., 1983). Here we present the results of a re-examination of this species and propose formally that it is reassigned to a novel genus, *Hydrogenibacillus* gen. nov.

*B. schlegelii* DSM 2000T was obtained from the DSMZ and cultured under the conditions as described by Schenk and Aragno (1979). Cell morphology was checked under a Zeiss light microscope at magnification 1000 using cells that had been grown for 24 h at 65 °C Medium on 260 (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium260.pdf). Oxidase activity was tested using oxidase reagent (bioMérieux), according to the instructions of the manufacturer. Cells were Gram-positive, spore-forming, oxidase-positive, long rods. The results on the cell morphology and other details are given in the species description and by Schenk and Aragno (1979).

Before phylogenetic analysis, the 16S rRNA gene sequence of DSM 2000T was confirmed by resequencing and was identical to the sequence from ATCC 43741T deposited under accession number AB042060. All phylogenetic calculations were performed with this previously published sequence, which was a continuous stretch of 1515 nt spanning *Escherichia coli* positions 16–1543 (Brosius et al., 1978). Detailed phylogenetic analysis was performed in ARB release 5.2 (Ludwig et al., 2004) using the All-Species Living Tree Project (LTP; Yarza et al., 2008) ARB database release LTPs106 (August 2011). Sequences not included in the LTP database were aligned with SINA (version 1.2.9) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse et al. (2007) and implemented in the ARB
Phylogenetic analysis based on 16S rRNA gene sequences (E. coli numbering 101–1451) showing the relationships of strain ATCC 43741\textsuperscript{T} with type strains of selected species of the most closely related genera of the families Bacillaceae, Paenibacillaceae, Alicyclobacillaceae, Sporolactobacillaceae, Thermoactinomycetaceae, Thermoanaerobacteraceae, and Thermolithobacteraceae. The phylogenetic tree was generated in ARB using RAxML (GTR-GAMMA, Rapid Bootstrap analysis, 100 bootstraps). Bootstrap values (>70\%) based on 100 replicates are shown at branch nodes. *Jiangella gansuensis* YIM 002\textsuperscript{T} was used as an outgroup. Bar, 0.10 substitutions per site.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences (E. coli numbering 101–1451) showing the relationships of strain ATCC 43741\textsuperscript{T} with type strains of selected species of the most closely related genera of the families Bacillaceae, Paenibacillaceae, Alicyclobacillaceae, Sporolactobacillaceae, Thermoactinomycetaceae, Thermoanaerobacteraceae, and Thermolithobacteraceae. The phylogenetic tree was generated in ARB using RAxML (GTR-GAMMA, Rapid Bootstrap analysis, 100 bootstraps). Bootstrap values (>70\%) based on 100 replicates are shown at branch nodes. *Jiangella gansuensis* YIM 002\textsuperscript{T} was used as an outgroup. Bar, 0.10 substitutions per site.

16S rRNA gene sequence similarity between ATCC 43741\textsuperscript{T} and type strains of members of the genus *Bacillus* ranged from 82.2 to 85.9\%, with 83.6\% sequence similarity to *Bacillus subtilis* subsp. *subtilis* DSM 10\textsuperscript{T} (type species). The most closely related genera were *Calditerricola* (88.4–88.5\% 16S rRNA gene sequence similarity), *Planifilum* (87.3–87.8\%) and *Caldalkalibacillus* (87.2–87.9\%). All phylogenetic trees showed the same topology between ATCC 43741\textsuperscript{T} and the most closely related genera of several families, including the *Bacillaceae*, *Paenibacillaceae*, *Alicyclobacillaceae*, *Sporolactobacillaceae*, *Thermoactinomycetaceae*, *Thermoanaerobacteraceae* and *Thermolithobacteraceae* (Fig. 1).

For chemotaxonomic analyses, respiratory quinones and polar lipids (Tindall, 1990a, b; Altenburger *et al.*, 1996; Stolz *et al.*, 2007) and the fatty acids (Kämpfer &
Kroppenstedt, 1996) were determined. The predominant menaquinone was MK-7 (97 %) and only trace amounts of MK-6 (1.7 %) and MK-8 (0.4 %) were detected. The predominance of MK-7 has also been determined for the most closely related genera Caldalkalibacillus (Xue et al., 2006), Geobacillus (Nazina et al., 2001), and Planifilum (Hatayama et al., 2005) of the families Bacillaceae and Thermoactinomycetaceae.

The polar lipid profile of B. schlegelii DSM 2000T (Fig. 2) contained major amounts of diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid (PL5) and two unidentified glycolipids (GL5, GL7) and minor amounts of additional unidentified phospholipids (PL1–PL4, PL6–PL9), glycolipids (GL1–GL4, GL6, GL8–GL12) and one unidentified phosphoglycolipid. This polar lipid profile is substantially different from that of Bacillus subtilis, the type species of the genus Bacillus (Kämpfer et al., 2006), in the absence of phosphatidylethanolamine and an unidentified aminophospholipid and the presence of numerous unidentified phospho- and glycolipids and the unidentified phosphoglycolipid. The polar lipid profile also clearly distinguishes B. schlegelii DSM 2000T from members of the genera Geobacillus and Aeribacillus, which have similar temperature preferences for growth. In addition to diphosphatidylglycerol and phosphatidylglycerol, members of the genus Geobacillus are characterized by the presence of major amounts of phosphatidylethanolamine, aminolipids and an aminophospholipid (Coorevits et al., 2012), and the single species of Aeribacillus, Aeribacillus pallidus, contains four unidentified glycolipids and an unidentified phosphoglycolipid but only one unidentified phospholipid (Miñana-Galbis et al., 2010).

The fatty acid composition, which was obtained after growth of B. schlegelii DSM 2000T for 72 h on medium 260 at 65 °C, revealed major amounts of iso-C_{16:0} (36.7 %), C_{16:0} (18.9 %), iso-C_{17:0} (17.6 %), anteiso-C_{17:0} (12.9 %), iso-C_{18:0} (7.5 %) and C_{18:0} (6.4 %). This composition is clearly distinct from that of B. subtilis, which shows predominantly iso-C_{15:0} and anteiso-C_{15:0} and that of Geobacillus and Aeribacillus, which both contain predominantly iso-C_{15:0} (Kämpfer et al., 2006; Miñana-Galbis et al., 2010; Coorevits et al., 2012).

The results of the physiological characterization of DSM 2000T are given in the novel combination description, using methods that were described previously (Kämpfer et al., 1991). Strain DSM 2000T was able to utilize some organic acids, but not sugars or sugar-related compounds. A clear differentiation from the genera Calditerricola, Caldalkalibacillus and Planifilum is possible.

B. schlegelii occupies a separate phylogenetic position among the endospore-forming taxa and their relatives, shows low 16S rRNA gene sequence similarity with the type species of the genus Bacillus and other taxa (<89 %) and exhibits substantial differences in the polar lipid profile and fatty acid composition. These data suggest that B. schlegelii is misclassified in the genus Bacillus, so it is proposed to reassign to a novel genus and species, Hydrogenibacillus schlegelii gen. nov., comb. nov.

**Description of Hydrogenibacillus gen. nov.**

Hydrogenibacillus (Hy.dro.ge.ni.ba’cil.lus. N.L. n. hydro-genum hydrogen; L. masc. n. bacillus a small staff, a wand, a rod, and also a generic name; N.L. masc. n. Hydrogenibacillus hydrogen bacillus, referring to the ability of the organisms to oxidize hydrogen).

Cells are Gram-variable, long, straight, rods. Thermoacidophilic; growth occurs above 42 °C and below 75 °C, with good growth at 55 °C. Growth occurs at pH 4.2–7.5. The predominant menaquinone is MK-7. The polar lipid profile is composed of major amounts of diphosphatidylglycerol, phosphatidylglycerol, two unidentified glycolipids, and one unidentified phospholipid. Minor amounts of other unidentified phospholipids and glycolipids and a phosphoglycolipid may also be present. The fatty acid profile contains predominantly branched fatty acids of iso- and anteiso-types but significant amounts of straight-chain fatty acids (~25 %) are also present. The major fatty acids are iso-C_{16:0}, C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}. The type species is Hydrogenibacillus schlegelii. The G+C content of the type species is 67–68 mol%.

**Description of Hydrogenibacillus schlegelii comb. nov.**

Hydrogenibacillus schlegelii (schle.ge1.i.i. N.L. masc. gen. n. schlegelii of Schlegel; named after H. G. Schlegel, a German microbiologist).
Basonym: Bacillus schlegelii Schenk and Aragno 1981.

The description is identical to that given by Schenk and Aragno (1979): long, straight, rod-shaped cells, 0.6 μm × 2.5–5 μm, with peritrichous flagellation; spherical, terminal endospores formed, 0.8–1 μm in diameter, distinctly distending the sporangium. Gram-variable. Colonies are cream, round or spreading over the agar surface. Neither a carotenoid pigment nor poly-β-hydroxybutyrate are produced (confirmed in this study). No growth factors required. Strictly respiratory metabolism, with oxygen as the terminal electron acceptor. Cannot grow anaerobically with nitrate. Nitrate is reduced to nitrite. Either chemo-organoheterotrophic, using H2 as the electron donor and CO2 as the carbon source, or chemoorganoheterotrophic. Phenol, 1-propanol and a small number of amino acids can serve as sole carbon sources, with ammonium ions as the nitrogen source. Sugars are not metabolized (confirmed with the method of Kämpfer et al. 1991). Ammonium ions, urea and asparagine can be utilized as sole nitrogen sources. Hydrogenase constitutive, membrane-bound, and not NAD(P) specific; hydrogenase activity is strongly thermophilic, with a temperature optimum between 70 and 75 °C. Good growth is, however, also observed at 55 °C. No growth at 37 or 80 °C. The pH for optimum growth is pH 6 to 7; more details have been reported by Pinkwart et al. (1983). Catalase- and oxidase-positive. Urease is not produced. Weak hydrolysis of casein, but no hydrolysis of starch or gelatin. H2S is not produced from cysteine. Indol is not produced. Growth occurs in the presence of 3 % NaCl, but not in 5 % NaCl. No growth in the presence of 1 % glucose. Minimum inhibitory penicillin G concentration: 0.005 U ml⁻¹. The quinone system is composed of a major amount of MK-7 and minor amounts of MK-6 and MK-8. In addition to the polar lipids listed in the genus description, one unidentified phosphoglycolipid, ten unidentified glycolipids and eight phospholipids are present.

The type strain is DSM 2000¹ (=ATCC 43741¹=CCUG 26017¹=CIP 106933¹), isolated from the surface layer of the sediment of a small eutrophic lake near Neuchatel, Switzerland.

References


