Bacillus herbersteinensis sp. nov.

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Two bacterial strains, designated D-1,5aT and D-1,5b, were isolated from a medieval wall painting in the chapel of Castle Herberstein, Styria (Austria). The Gram-positive, heterotrophic, aerobic, spore-forming rods showed nearly identical whole-cell protein patterns, identical genomic fingerprints and identical physiological profiles, demonstrating their relationship at the species level. Both strains contained meso-diaminopimelic acid in their peptidoglycan, possessed a quinone system comprising menaquinone MK-7 and had fatty acid profiles in which C15:0 iso and C15:0 anteiso were predominant. The 16S rRNA gene sequence of D-1,5aT showed the highest similarity (99.5%) to the sequence of Bacillus sp. LMG 20243, and Bacillus flexus IFO 15715T was the next most closely related established species (96.5%). Other type strains, such as Bacillus fastidiosus DSM 91T, Bacillus indicus SD/3T, Bacillus cibi JG-30T, Bacillus megaterium IAM 13418T, Bacillus cohnii DSM 6308T, Bacillus bataviensis LMG 21833T and Bacillus soli LMG 21838T, shared 96.0–96.1% 16S rRNA gene sequence similarity with D-1,5aT. The combination of physiological and chemotaxonomic traits distinguishes the two strains from those species sharing the highest sequence similarities (96.0–96.5%). On the basis of these characteristics and the phylogenetic position of strain D-1,5aT (=DSM 16534T =CCM 7228T), this strain is assigned as the type strain of a novel species of the genus Bacillus, for which the name Bacillus herbersteinensis sp. nov. is proposed.

From microbiological examinations of a medieval wall painting in the chapel of Castle Herberstein in Styria (Austria), several bacterial strains have been isolated and classified as novel species, including Agrococcus citreus, Micrococcus luteus (Wieser et al., 1999, 2002), Brachybacterium fresconis, Brachybacterium sacelli, Halomonas muralis, Bacillus decolorationis, Virgibacterium picturae and Brevibacterium picturae (Heyrman et al., 2002a, b, 2003a, b, 2004). During our studies of the microbiological diversity of the wall painting, we isolated, in addition to A. citreus and M. luteus, other isolates, which were preliminarily identified as members of the Bacillaceae (four isolates), the Microbacteriaceae (one isolate) and the Moraxellaceae (one isolate) (M. Wieser, unpublished results). Here we report the detailed characterization of two strains from this collection, which, in preliminary examinations, differed by only a single band in the protein patterns after SDS-PAGE (results not shown) performed as described by Altenburger et al. (1996).

Strains were isolated and investigated morphologically as described by Wieser et al. (1999). NaCl tolerance was tested on PYES agar (0.3% peptone, 0.3% yeast extract, 0.23% disodium succinate, 1.5% agar, pH 7.2) supplemented with 1, 3, 5, 7 and 10% NaCl, respectively. Tolerance towards different pH values was examined on PYES agar adjusted with HCl (37%) or 4 M NaOH to pH 6, 7, 8, 9, 10, 11 and 12, respectively, or on buffered medium as described by Nielsen et al. (1995). Hydrolysis of starch was examined on PYES medium as described by Sneath (1986). Other tests were done as described by Kämpfer et al. (1991). Optimal growth was obtained on PYES agar or broth, pH 8.0, supplemented with 1% NaCl (w/v). The characteristics are listed in the species description below and in Table 1.

Identical genomic fingerprints (see Supplementary Fig. S1 available in IJSEM Online) of strains D-1,5aT and D1,5b obtained after enterobacterial repetitive intergenic consensus (ERIC) sequence PCR (Wieser & Busse, 2000) confirmed indications from protein patterns (results not shown) that these strains were members of a single species.
The 16S rRNA gene sequence (1464 bases) of D-1,5aT was analysed and sequenced according to Wieser et al. (1999), using primers 27f, 342f, 519r and 1992r (Lane, 1991). It should be mentioned here that sequencing of the 16S rRNA-encoding gene of D-1,5aT caused unexpected problems. When primer 27f was used, the sequence could be determined only up to position 192. Ambiguous results were obtained for subsequent bases. A similar observation was made at the same position in the sequence when primer 519r was employed, and the base at position 193 (A or G) could not be determined in either of the two sequencing directions. This observation might be explained by the presence of two rRNA-encoding genes in D-1,5aT, one of which contains a gap or insertion at this position. Sequence comparisons (Pearson & Lipman, 1988) revealed the highest scores with the type strains of established species Bacillus flexus IFO 15715T (96.5%), Bacillus fastidiosus DSM 91T (96.1%), Bacillus indicus SD/3T (96.1%), Bacillus cibi JG-30T (96.0%), Bacillus megaterium IAM 13418T (96.0%), Bacillus cohnii DSM 6308T (96.0%), Bacillus bataviensis LMG 21833T (96.0%), Bacillus soli LMG 21838T (96.0%), Bacillus asahii MA001T (95.8%), Bacillus muralis LMG 0114T (95.4%), and Bacillus herbersteinensis sp. nov. (data from this study); 2, Bacillus bataviensis (Heyrman et al., 2004); 3, B. cibi (Yoon et al., 2005); 4, B. cohnii (Priest et al., 1988; Täubel et al., 2003; Suresh et al., 2004); 5, B. fastidiosus (Claus & Berkeley, 1986); 6, B. flexus (Priest et al., 1988; Täubel et al., 2003; Suresh et al., 2004); 7, B. indicus (Suresh et al., 2004); 8, B. megaterium (Priest et al., 1988; Täubel et al., 2003; Suresh et al., 2004); 9, B. soli (Heyrman et al., 2004). +, Positive; −, negative; w, weakly positive; v, variable; Ng, no growth; na, not analysed.

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<td>Cell-wall diaminoc acid†</td>
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<td>Na</td>
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<td>mDpm</td>
<td>L-Orn</td>
<td>mDpm</td>
<td>Na</td>
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</table>

*Values in parentheses indicate conditions under which no growth was observed. †mDpm, meso-Diaminopimelic acid; Orn, ornithine.
1,5αT and any of them at the species level. Interestingly, the
indicate that strain D-1,5αT may be considered as a species
moderate bootstrap value (81 %). However, these results
rRNA gene sequence similarity, and (ii)
http://ijs.sgmjournals.org 2121
ation of strain D-1,5αT among other
Fig. 1.
Maximum-likelihood phylogenetic tree showing the posi-
tion of strain D-1,5αT among other Bacillus species on the
basis of 16S rRNA gene sequences. Bootstrap values
(expressed as percentages of 100 replications) greater than
70 % are shown at branching points. Bar, 10 % sequence
divergence. The tree was rooted using Paenibacillus polymyxa
DSM 36 % as an outgroup.

Bacillus herbersteinensis sp. nov.

\begin{align*}
20238^\mathrm{T} & (95-8\%), Bacillus novalis \text{ LMG } 21837^\mathrm{T} (95-7\%), \\
Bacillus niacini \text{ IFO } 15366^\mathrm{T} (95-7\%) & \text{ and } Bacillus firmus \\
\text{IAM } 12464^\mathrm{T} (95-6\%), & \text{ indicating no relatedness between D-} \\
1,5αT \text{ and any of them at the species level. Interestingly, the} \\
16S \text{ rRNA gene sequence of } D-1,5αT \text{ shared the highest} \\
similarity (99-5 \%) & \text{ with the sequence of Bacillus sp. LMG} \\
20243, \text{ which was recently isolated from a mural painting in} \\
St. Martins church in Greene–Kreiensen (Lower Saxony, \\
Germany; Gorbushina et al, 2004) \text{ and shared } 97-1 \% \\
similarity with Bacillus sp. LMG 19415, \text{ which, like } D-1,5αT, \\
had been isolated from the wall painting in the chapel of \\
Castle Herberstein (Gurtner et al., 1997) \text{ and shared } 98-7 \% \\
\text{ divergence. The tree was rooted using } \\
Paenibacillus polymyxa \\
\text{type species of the genus \\
Bacillus sensu stricto. For G+C content}
\end{align*}
determination, DNA was extracted and purified as described
by Auling et al. (1985). The DNA G+C contents of strains
D-1,5αT \text{ and } D1,5β \text{ were determined by HPLC (Kaneko et al.,} \\
1986) \text{ as } 36-2 \text{ and } 36-9 \text{ mol\%}, \text{ respectively. These values}
\text{ differ only slightly from those reported for } Bacillus fasti-
diosus (34-3–35-1 \text{ mol\%}; Claus & Berkeley, 1986), Bacillus \\
flexus (37-0–37-9 \text{ mol\%}; Priest et al., 1988), Bacillus mega-
therium (37-3 \text{ mol\%}; Claus & Berkeley, 1986) \text{ and Bacillus } \\

Isoprenoid quinones and polar lipids were extracted and
analysed as described previously (Tindall, 1990; Ventosa \\
et al., 1993; Altenburger et al., 1996). Fatty acid methyl esters
were extracted from biomass grown on nutrient agar (1–1;
5 g peptone from casein, 3 g yeast extract, 15 g agar) \text{ and was analysed as described by} \\
Kämpfer (1994). The diagnostic cell-wall diamino acid was
determined as described by Schleifer (1985).

The major respiratory menaquinone in the two strains
was found to be MK-7 and the diagnostic diamino acid in their
cell walls was meso-diaminopimelic acid. These character-
istics are in agreement with those of numerous species of the

genus Bacillus, including the type species, Bacillus subtilis
(Claus & Berkeley, 1986). The polar lipid profiles of D-1,5αT
(see Supplementary Fig. S2 in IJSEM Online) and D1,5b
(results not shown) were identical. Major to moderate
amounts of diphosphatidylglycerol, phosphatidyglycerol,
two unknown glycolipids, which might correspond to
monoglcuosyl diacylglycerol and diglucosyldiacyl glycerol
(as reported to be present in a strain of Geobacillus steacro-
thermophilus; Minnikin et al., 1974), and moderate to minor
amounts of two unknown phospholipids and two unknown
polar lipids were detected. The presence of two glycolipids
and the absence of phosphatidylethanolamine clearly distin-
tinguish the two strains from Bacillus flexus DSM 1320T,
Bacillus cohnii LMG 16678T \text{ and Bacillus megaterium DSM} \\
32T (Täubel et al., 2003). On the other hand, the presence of
the two glycolipids might confirm the closer phylogenetic
relatedness to Bacillus subtilis (Fig. 1), which has been
reported to contain glycolipids (Brundish et al., 1965; 
Bishop et al., 1967). The fatty acid profiles of strains D-1,5αT
and D1,5b contained the major compounds C15:0 iso, C15:0
anteiso, C14:0 iso, C16:0 iso, C16:0 and C17:0 iso, which are
characteristic of numerous taxa within the bacilli (Kämpfer, 
1994). The relative fatty acid concentrations are listed below
in the species description.

All of the characteristics determined for strain D-1,5αT \text{ are in}
accordance with those of the genus Bacillus. On the basis of
phylogenetic distance from established Bacillus species, also
indicated by relatively low 16S RNA gene sequence simi-
larities (<97 %) and the combination of unique pheno-
typic characteristics, it is demonstrable that D-1,5αT is not
affiliated with any species of this genus. In conclusion,
we describe D-1,5αT as the type strain of a novel species,
for which we propose the name Bacillus herbersteinensis
sp. nov.
Description of Bacillus herbersteinensis sp. nov.

Bacillus herbersteinensis (her.ber.stein en.sis. N.L. masc. adj. herbersteinensis pertaining to Castle Herberstein in Styria, in which the chapel with the medieval wall painting is located from which the type strain was isolated).

Cells are motile, rod-shaped, Gram-positive in the KOH and aminopeptidase tests, rod-shaped and produce oval spores in terminal, unswnollen sporangia. Colonies on PYES agar are 2–3 mm in diameter, slightly raised, irregular and cream-coloured to beige. Older colonies are more translucent. Catalase- and oxidase-positive. Growth occurs at 4 and 28 °C, in the presence of 0, 1, 3 and 5 % NaCl (w/v) but not at 7 or 10 % NaCl (w/v). On buffered medium, growth is observed at pH 7 (weakly), 8, 9, 10, 11 and 12 but not at pH 6. Negative for nitrate reduction and haemolysis. Starch, p-nitrophenyl (pNP) β-D-galactopyranoside, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside and bis-pNP phosphate are hydrolysed. pNP β-D-xylene, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxyxymidine-5′-pNP phosphate, L-alanine pNA (pNA, p-nitroanilide), L-glutamate γ-3-carboxy-pNA, L-proline pNA and pNP β-D-glucoside are not hydrolysed. Acid is not produced from adonitol, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl D-glucoside, raffinose, rhamnose, sorbitol, sucrose, trehalose or D-xylene. N-Acetyl-D-glucosamine, L-arabinose (weakly), p-arbutin (weakly), D-cellobiose, D-fructose, D-glucalose, glucic acid, D-glucose, D-maltose, D-mannitol, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, L-rhamnose, D-sorbitol, D-sucrose, D-trehalose, D-xylene, acetate (weakly), cis-aconitate (weakly), L-aspartate, citrate, fumarate, DL-3-hydroxybutyrate, DL-lactate, D-malate, L-ornithine (weakly), 2-oxoglutarate (weakly), pyruvate and L-proline are assimilated. Adonitol, D-inositol, maltitol, putrescine, azelate, glutarate, itaconate, trans-aconitate, adipate, propionate, 4-aminoobutyric acid, 4-hydroxybenzoic acid, mesaconate, succinate, L-alanine, β-alanine, L-serine, L-histidine, L-leucine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoic acid and phenylacetate are not assimilated. The fatty acid profile consists of C_{14:0} iso (8:7–14:2 %), C_{14:0} (0–0·5 %), C_{15:0} iso (17:7–27:4 %), C_{15:0} anteiso (17:0–23:4 %), C_{16:0} (1:9–3:2 %), C_{16:1ω7c} (2:9–5:1 %), C_{16:1ω11c} (1:9–2:4 %), C_{16:0} iso (8:4–15:8 %), C_{16:0} (4:8–5:9 %), C_{17:0}ω10c iso (2:4–2:5 %), C_{17:0}ω10c iso (1·0 %), C_{17:0}ω10c iso (3:7–5:1 %), C_{17:0} anteiso (1·1–1·2 %) and C_{17:0} (1·1–1·6 %). The diamino acid in the cell wall is meso-diaminopimelic acid. The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid. Additionally, moderate to minor amounts of a second unknown glycolipid, two phospholipids and four polar lipids are present. The major respiratory quinone is MK-7. The DNA G+C content is 36·2–36·9 mol% (HPLC).

The type strain is strain D-1,5αT ( = DSM 16534T = CCM 7228T). Strains D-1,5αT and D-1,5b were both isolated from a damaged wall painting in the chapel of Castle Herberstein, Styria (Austria).

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References


