**Paenibacillus lactis** sp. nov., isolated from raw and heat-treated milk

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Endospore-forming bacteria were recovered from individual packages from different processing lines in a dairy plant during a tenacious periodical contamination of their UHT-milk production.

Two colony types were seen, one of which was identified as *Bacillus sporothermodurans*. Analysis of the 16S rRNA gene of the second colony type placed these isolates within the genus *Paenibacillus*, with *Paenibacillus lautus* as the closest known relative. Moreover, over 99% similarity was observed to the 16S rDNA sequence of MB 2035, a strain isolated previously from raw milk during a survey at dairy farms for very heat-resistant spore-forming bacteria. Nine other potentially closely related strains among the dairy farm isolates were found using rep-PCR typing. The taxonomic positions of these 19 isolates were further investigated using 16S rRNA gene sequencing and DNA–DNA hybridizations of representative strains. All 19 isolates shared a high degree of phenotypic similarity and were easily distinguished from closely related members of the genus. Anteiso-C15:0, C16:0 and iso-C15:0 were among the major fatty acids and the genomic DNA G+C content was 51.6–51.7 mol%. Therefore, based on their phenotypic, phylogenetic and genomic distinctiveness, these 19 strains, isolated from both raw and heat-treated milk, are placed in the genus *Paenibacillus* as *Paenibacillus lactis* sp. nov. The type strain is MB 1871T (=LMG 21940T =DSM 15596T).

*Paenibacillus* strains have been isolated from a variety of sources including soil, the rhizosphere, water, diseased insect larvae and foods (Daane et al., 2002) and display physiologically diverse characteristics. One distinctive characteristic of members of the genus is their ability to degrade a variety of macromolecules, amongst others proteins (Ash et al., 1993), polysaccharides (Kanzawa et al., 1995; Claus & Berkeley, 1986) and polyaromatic hydrocarbons (Daane et al., 2002). Another is the ability of paenibacilli associated with plants to fix nitrogen. These include *Paenibacillus azotofixans* (Seldin et al., 1984), *Paenibacillus polymyxa* (Grau & Wilson, 1962) and *Paenibacillus macerans* (Witz et al., 1967). More recently, four more nitrogen-fixing species of *Paenibacillus*, *Paenibacillus borealis* (Élo et al., 2001), *Paenibacillus brasiliensis* (von der Weid et al., 2002), *Paenibacillus graminis* and *Paenibacillus odorifer* (Berge et al., 2002), have been described. Some *Paenibacillus* strains also produce antibacterial (e.g. Martin et al., 2003; Piuri et al., 1998) or antifungal compounds (e.g. Beatty & Jensen, 2002; Chung et al., 2000).
Sporogenesis enables members of *Bacillus*, *Paenibacillus* and related organisms to withstand environmentally harsh conditions, allowing long-term survival (Setlow, 1994). The resulting omnipresence of *Bacillus* and its relatives favours contamination of many foods. The resistance of their endospores, which may be associated with psychrophilic or acidophilic properties of the vegetative cells, causes specific problems to the food industry (Andersson et al., 1995). Incidences of paenibacilli in food products such as pasteurized puréed vegetables have been reported previously (Berge et al., 2002; Guinebretière et al., 2001). The most common spore-forming bacteria (55% of the isolates) found in the production process of refined paper products are not predominant, their presence has been recognized (for an overview, see Heyndrickx & Scheldeman, 2002). Although *Paenibacillus* spores in raw and pasteurized milk are not predominant, their presence has been recognized (for an overview, see Heyndrickx & Scheldeman, 2002). Feeds for dairy cattle appear to be a significant source of contamination of raw milk with spores. *Paenibacillus* species are present in both silage (te Giffel et al., 2002) and feed concentrate for dairy cattle (Vaerewijk et al., 2001). To our knowledge, there have been no previous reports of *Paenibacillus* spores surviving industrial sterilization or UHT processing of milk. Nevertheless, te Giffel et al. (2002) reported that some *Paenibacillus* spores were able to withstand temperatures above 120 °C.

In this study, we elucidated the taxonomic position of 19 spore-formers isolated from contaminated industrial UHT milk and from the dairy farm. In addition to molecular typing, we used extensive phenotypic characterization, 16S rRNA gene sequencing and DNA–DNA reassocation to study these isolates.

Details of the strains included in this study are listed in Supplementary Table A available in IJSEM Online. All strains, except for LMG 11157T and SB150-2B, were isolated after a 30-min 100 °C heat treatment of the samples, either from a number of dairy farms (Scheldeman et al., 2002) or from contaminated UHT milk from different processing lines in a dairy plant. All MB-numbered strains were grown on brain heart infusion (BHI; Oxoid) supplemented with bacteriological agar no. 1 (Oxoid) and filter-sterilized vitamin B₁₂ (1 mg l⁻¹; Sigma). The type strain of *Paenibacillus laitutus*, LMG 11157T, was grown according to the recommendations of the BCCM/LMG Bacteria Culture Collection.

Whole-cell DNA for PCR amplification was extracted from pure cultures by the method described by Pitcher et al. (1989). For DNA–DNA reassociation experiments, high-quality total genomic DNA was purified on a larger scale as described by Logan et al. (2000), except that lysozyme was dissolved in TE buffer with an increased EDTA concentration (1:21 g Tris, 6 ml 0.5 M EDTA, pH 8.0, in 1 l MilliQ water) and that the cell suspensions were incubated with lysozyme until the viscosity increased (usually after 10–15 min).

In this study, the term rep-PCR is used as an overall term to refer to molecular typing based on repetitive elements [including REP+, (GTG)_5-, BOX- and ERIC-PCR], whereas REP-PCR refers to one specific method using the primers REP 1R-I and REP 2-I (Versalovic et al., 1994). The conditions for REP-PCR were described previously by Herman et al. (1998). In addition, the (GTG)_3 primer (Versalovic et al., 1994) was used under the following PCR conditions. Amplification reactions were performed in a final volume of 25 μl containing PCR buffer (10 mM Tris/HCl, pH 8.3, and 50 mM KCl) (Applied Biosystems), 1.5 mM MgCl₂, 0.2 mM of each dNTP (Pharmacia), 0.3 μg primer, 1 U Goldstar DNA polymerase (Eurogentec) and 25 ng DNA template. The PCR program (Versalovic et al., 1994) was run on a DNA thermal cycler (Perkin Elmer 9600). To avoid inter-PCR differences, all samples were included in one single PCR run.

The rep-PCR amplicons were separated in a 1.5% LSI LE agarose gel (Life Science International) (20 × 25 cm) for 4 h at a constant voltage of 4 V cm⁻¹ and 1× TBE (100 mM Tris/HCl, 100 mM boric acid, 2 mM EDTA, pH 8.0). rep-PCR profiles were visualized after staining with ethidium bromide, followed by digital image capturing using a GelDoc 2000 camera (Bio-Rad). A mixture of molecular mass markers VIII, IX and X (Roche) was used as references for intra- and inter-gel comparison. Numerical analysis of the resulting fingerprints was done using the GelCompar II version 2.0 software package (Applied Maths). Similarity among digitized profiles was calculated using Pearson’s correlation coefficient and an average linkage (UPGMA) dendrogram was derived. Finally, both the REP- and (GTG)_3-PCR patterns were linearly combined, assigning the same weight to each analysis.

Amplified rDNA restriction analysis (ARDRA) of 16S rDNA was performed as described previously (Heyndrickx et al., 1996).

The 16S rRNA genes of strains MB 1871T (=LMG 21940T = DSM 15596T), MB 1928 and MB 2035 were amplified by PCR using conserved primers pA (5′-AGAGTTTGATCCT-GGCTCAG-3′) and pH (5′-AAGGAGGTGATCAGCCGGA-3′). The PCR products were purified and subsequently sequenced using an ABI 310 sequencer (Applied Biosystems) as described previously (Scheldeman et al., 2002). A combination of primers pA, pD, o, rR, gamma and pH (Coenye et al., 1999) was used to obtain a continuous stretch of the 16S rRNA gene sequence. Sequence assembly was implemented using the GeneBase software (Applied Maths). The BioNumerics 3.0 software package (Applied Maths) was used for construction of a phylogenetic tree based on the neighbour-joining method.

DNA–DNA hybridizations were performed with photo-biotin-labelled probes in microplate wells as described by Ezaki et al. (1989). For the fluorescence measurements, a Bio Assay Reader (HTS7000, Perkin Elmer) was used as described in detail by Willems et al. (2001). A hybridization
species was only 96 % to **B. sporothermodurans** (AF395029), a strain isolated from an ice core in Sajama, Bolivia. The highest similarity to a recognized strain: 1, **P. lactis** sp. nov. MB 1871T; 2, **Paenibacillus** sp. MB 2039; 3, **P. laetus** NRRL NRS-666T; 4, **P. glucanolyticus** DSM 5162T; 5, **P. macearans** JCM 2500T; 6, **P. polymyxa** JCM 2507T. Values are percentages of total fatty acids. Data for reference strains (columns 3–6) were taken from Shida et al. (1997). ND, Not detected.

### Table 1. Cellular fatty acid composition of strain MB 1871T and some other species of the genus *Paenibacillus*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.9</td>
<td>0.8</td>
<td>1.1</td>
<td>0.8</td>
<td>3.7</td>
<td>0.4</td>
</tr>
<tr>
<td>C15:0</td>
<td>2.7</td>
<td>6.6</td>
<td>0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>9.7</td>
<td>9.2</td>
<td>15.6</td>
<td>11.2</td>
<td>17.9</td>
<td>9.3</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.6</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated fatty acids</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1ω7c alcohol</td>
<td>0.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C16:1ω11c</td>
<td>4.2</td>
<td>ND</td>
<td>2.0</td>
<td>0.5</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Branched fatty acids</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>iso-C14:0</td>
<td>1.2</td>
<td>1.7</td>
<td>0.8</td>
<td>1.4</td>
<td>7.9</td>
<td>0.5</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>11.5</td>
<td>8.4</td>
<td>1.5</td>
<td>3.2</td>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>6.7</td>
<td>13.5</td>
<td>7.4</td>
<td>13.9</td>
<td>16.4</td>
<td>5.6</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>5.6</td>
<td>5.4</td>
<td>1.2</td>
<td>2.0</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
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<td>44.5</td>
<td>57.3</td>
<td>56.5</td>
<td>36.1</td>
<td>62.9</td>
</tr>
<tr>
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<td>8.3</td>
<td>9.7</td>
<td>8.2</td>
<td>12.2</td>
<td>16.9</td>
</tr>
<tr>
<td>iso-C17:0ω10c</td>
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<td>ND</td>
<td>0.2</td>
<td>0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

The DNA base composition was determined by HPLC using further specifications given by Logan et al. (2000).

The culture conditions for fatty acid methyl ester (FAME) analysis were as described by Scheldeman et al. (2002). The methods used for fatty acid extraction, methyl ester preparation and separation by GC were described by Vancanneyt et al. (1996).

Phenotypic tests were performed as described by Logan & Berkeley (1984); other characters were determined and the data numerically analysed as described by Logan et al. (2000). For observations on sporangia, cells were grown on TSA MnSO₄ at 30 °C.

During a period of obstinate contamination of the UHT-milk production in a dairy company, two colony types were isolated from nine individual packages from different processing lines (both direct and indirect UHT). The first colony type, present in only two samples, resembled *Bacillus sporothermodurans* and was indeed identified as such by the PCR-detection method described by Herman et al. (1997) (data not shown). Isolates of the second colony type, present in all nine samples, reacted negatively both to the latter PCR detection method and to the more universal PCR test of Scheldeman et al. (2002), based on the 16S rDNA of *B. sporothermodurans*. The contamination of the UHT-milk packages was therefore due to another spore-forming organism, present in co-contamination with *B. sporothermodurans* and probably also surviving the UHT process.

The 16S rRNA gene sequence of one of the nine non-*B. sporothermodurans* UHT isolates, MB 1928, was determined and the highest similarity in the EMBL database found was 97·0 % (1432 nt overlap) to *Paenibacillus* sp. SB-150-2B (=MB 2328; AF395029), a strain isolated from an ice core in Sajama, Bolivia. The highest similarity to a recognized species was only 96·6 %, to *P. laetus* NRRL NRS-666T (D78473; 1396 nt overlap). However, the 16S rRNA gene of strain MB 1928 did show a pairwise similarity of 99·1 % to MB 2035 (=R-6472), a strain isolated from raw milk during a survey for very heat-resistant spore-forming bacteria on dairy farms in 1999 (Scheldeman et al., 2002).

In addition to MB 2035 and MB 2328 (=SB-150-2B), more close relatives of the fresh-UHT isolate MB 1928 were sought among a large collection of strains on the basis of their whole-cell FAME profiles (data not shown) and a selection of representative strains was subsequently subjected to ARDRA (data not shown). In ARDRA, the raw-milk strain MB 2035 grouped together with three other strains, MB 1868 (=R-6435), MB 1871T (=R-7191T) and MB 1879 (=R-7193), with a similarity of 95 % (data not shown). Based on the FAME data, 15 other potentially related *Paenibacillus* strains from dairy farms (see Table 1), grouping within an Euclidian distance of 15 from the above-mentioned strains, were selected for polyphasic characterization.

Repetitive element-based genomic fingerprinting (REP-PCR) was used to assess the diversity of the selected strains mentioned in Supplementary Table A. From a cluster analysis based on combined REP and (GTG)₅ fingerprints, combined with a visual examination of the banding patterns, five clusters (designated A–E in Fig. 1) could be delineated. The nine UHT isolates and one farm strain isolated from a cluster of the milking apparatus (MB 1868) all grouped together in cluster B at an internal similarity of 92·1 %. Interestingly, all these isolates showed virtually identical banding patterns and could thus be regarded as of clonal origin. A group of nine farm isolates grouped together in a more diverse cluster A, at a similarity level of 62·4 %. The remainder of the strains grouped together in three smaller clusters. Cluster C, with an internal similarity of 56·9 %, contained four raw milk strains, whereas cluster D consisted of a raw milk strain and a farm strain grouping together at 63·6 %. Three strains originating from the milking apparatus of the same farm grouped together in cluster E at a similarity level of 72·7 %. Finally, the ice-core strain MB 2328 took a single position in the dendrogram.

Although clusters A and B showed a mutual similarity of only 34·4 %, common bands between these two groups could be observed [particularly in the (GTG)₅ fingerprints].
This observation fitted with the observation that strains MB 2035 from cluster A and MB 1928 from cluster B showed over 99 % similarity in their 16S rRNA gene sequences. An additional 16S rDNA sequence of another strain from the more heterogeneous cluster A, MB 1871T, was determined and found to be 99-5 and 99-6 % similar to the 16S rDNA of MB 1928 and MB 2035, respectively. In contrast, the 16S rDNA sequence from a raw milk strain from cluster C (Fig. 1), MB 2039, showed only 91-1 % similarity to the 16S rDNA of MB 1928. As a result, the strains of cluster C cannot be considered as phylogenetically closely related to strains of clusters A and B.

Comparison of the 16S rDNA sequences of strains MB 1871T, MB 1928 and MB 2035 with those of all recognized species of the genus Paenibacillus is given in a phylogenetic neighbour-joining tree (Fig. 2; see also Supplementary Fig. A). The three strains MB 1871T, MB 1928 and MB 2035 showed mutual 16S rDNA similarities of over 99 % and they appeared as a well-separated branch in the phylogenetic tree (Fig. 2), with P. lautus JCM 9073T (approx. 97 % 16S rDNA similarity), Paenibacillus sp. SB-150-2B (approx. 97 %) and Paenibacillus glucanolyticus DSM 5162T (approx. 96 %) as their closest relatives.

The status of the three strains MB 1871T, MB 1928 and MB 2035 was further confirmed by genomic DNA–DNA reassociation
studies (for detailed results, see Supplementary Table B). Strain MB 1871\textsuperscript{T} showed 90 and 79 % DNA–DNA relatedness with strains MB 2035 and MB 1928, respectively. The latter two strains showed 79 % mutual DNA–DNA relatedness. Moreover, the DNA–DNA relatedness of these three strains with \textit{P. lautus} LMG 11157\textsuperscript{T}, as well as with strain MB 2328, was generally low (20–25 %). The ice-core strain MB 2328 is clearly related to \textit{P. lautus}, as evidenced by a high DNA reassociation value (62 %; Supplementary Table B) as well as a high 16S rDNA sequence similarity of 99-6 % with the \textit{P. lautus} type strain. The representative strains MB 1871\textsuperscript{T}, MB 1928 and MB 2035 and the other 16 strains of the REP and (GTG)\textsubscript{3} clusters A and B can therefore be considered as belonging to a novel species of the genus \textit{Paenibacillus} (Wayne \textit{et al}., 1987; Stackebrandt \textit{et al}., 2002). The usefulness of rep-PCR for delineating groups of strains for taxonomic purposes was also demonstrated in previous studies (e.g. Rademaker \textit{et al}., 2000; Goris \textit{et al}., 2002). The status of the novel strains is also corroborated by their DNA base composition (Supplementary Table B), which falls within the range 45–54 mol% G + C, as reported for the genus \textit{Paenibacillus} (Shida \textit{et al}., 1997).

In the cellular fatty acid analysis (Table 1), a predominance of anteiso-C\textsubscript{15:0}, typical of paenibacilli (Shida \textit{et al}., 1997), was also observed for MB 1871\textsuperscript{T} (45.9 % of total cellular fatty acids). The fatty acid profile of MB 1871\textsuperscript{T} resembles those of other type strains of \textit{Paenibacillus} species, but there are significant differences in the proportions of anteiso-C\textsubscript{15:0} and iso-C\textsubscript{15:0} (Table 1).

The strains were found to be Gram-variable or Gram-negative, aerobic and motile rods. They formed ellipsoidal or cylindrical spores that lay paracentrally or subterminally in slightly swollen sporangia (Supplementary Fig. B). On TSA plates, the strains formed discrete colonies and occasionally formed spreading growth reminiscent of \textit{Paenibacillus alvei}. Most of the strains clustered together at 87.5 % similarity in a UPGMA cluster analysis (not shown) based upon their biochemical reactions, and this cluster merged with other species of \textit{Paenibacillus}, including \textit{P. lautus}, \textit{P. maccarans} and \textit{P. polymyxa}, at 80 % similarity. Two strains, MB 1868 and MB 2044, gave weak reactions in the biochemical tests and did not group with other \textit{Paenibacillus} species, but with the type strain of \textit{Bacillus lentus}; this misallocation could be attributed entirely to the weakness of their reactions in the API 50CHB gallery, rather than to them having different patterns of carbohydrate utilization. These results indicate that, although the majority of the milk isolates form a group phenotypically distinct from established species of \textit{Paenibacillus}, weakly reacting strains may be misidentified if phenotypic tests alone are relied upon for identification (see Table 2).

The genotypic and phenotypic data showed that the 19 novel isolates form a homogeneous group that is different from all other related species within the genus \textit{Paenibacillus}. Therefore, they represent a novel species of the genus \textit{Paenibacillus}, for which we propose the name \textit{Paenibacillus lactis} sp. nov.

### Table 2. Characters that distinguish \textit{P. lactis} sp. nov. from related species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Spore position*</td>
<td>s/C</td>
<td>s</td>
<td>T</td>
<td>s/T</td>
<td>s/C</td>
</tr>
<tr>
<td>Growth at 50 °C</td>
<td>+</td>
<td>−</td>
<td>T</td>
<td>s/T</td>
<td>s/C</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein hydrolysis</td>
<td>−</td>
<td>−</td>
<td>( + )</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Citrate utilization</td>
<td>−</td>
<td>−</td>
<td>( + )</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
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<td>+</td>
<td>−</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>−</td>
<td>−</td>
<td>( + )</td>
<td>V</td>
<td>−</td>
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<td>Acid from:</td>
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<tr>
<td>D-Arabinose</td>
<td>V</td>
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<td>−</td>
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<td>+</td>
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<td>( + )</td>
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<td>+</td>
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<td>V</td>
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<td>V</td>
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<tr>
<td>Rhamnose</td>
<td>−</td>
<td>−</td>
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<td>−</td>
<td>V</td>
</tr>
</tbody>
</table>

* C, Central/paracentral; s, subterminal; T, terminal.

Strains of this novel species were isolated from both raw and heat-treated milk. Moreover, we detected a clonal relationship between a raw-milk strain (MB 1868) and isolates from a contaminated lot of UHT milk. A possible contamination route from raw milk to the dairy farm to heat-treated milk in the dairy is therefore not implausible. The fact that some of these strains were present jointly with \textit{B. sporothermodurans} spores, known for its extreme heat resistance (Huemer \textit{et al}., 1998), in several UHT-milk packages from different processing lines indicates that, by analogy, spores of the UHT isolates of this novel species might be capable of surviving severe heat treatment and, as a result, affecting the keeping quality or safety of food products.

### Description of \textit{Paenibacillus lactis} sp. nov.

\textit{Paenibacillus lactis} [lac’tis. L. gen. n. lactis from milk, referring to milk (and its environment on the dairy farm) as the principal isolation source].
Cells are single, straight and round-ended, motile rods, that may occasionally be slightly tapered and curved. Gram-negative or Gram-variable. Cell diameter is 0.6–0.9 μm and cell length is 3–6 μm. Endospores are ellipsoidal or cylindrical, are located subterminally and occasionally paracentrally and usually swell the sporangia. Colonies cylindrical, are located subterminally and occasionally spreading transparent edges and with eggshell surface texture. Motile microcolonies may spread across the surface of the agar and rotate in a clockwise direction. Colony diameter is 1–2 mm. Aerobic. Maximum temperature for growth lies between 50 and 55°C and the optimum temperature lies between 30 and 40°C. Optimum pH for growth is 7-0; the minimum pH is between 5-0 and 6-0 and the maximum pH between 10-5 and 11. Casein is not hydrolysed. In the API 20E strip, o-nitrophenyl β-D-galactopyranoside hydrolysis is positive. Nitrate reduction is variable and Voges-Proskauer reaction is negative or weak, while reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrogen sulphide production, urease, tryptophan deaminase, indole production and hydrolysis of gelatin are negative. In the API 50CH gallery using the CHB suspension medium, hydrolysis of aesculin is positive and acid is produced without gas from the following carbohydrates: amygdalin, L-arabinose, arbutin, D-cellobiose, D-fructose, D-glucose, glycogen, lactose, maltose, mannitol, D-mannose, D-melobiose, D-melibiose, D-manno-ribose, starch, sucrose, D-trehalose, D-turanose and D-xyllose. Occasional strains give weak reactions with the following substrates: amygdalin, L-arabinose, arbutin, D-cellobiose, D-fructose, D-glucose, lactose, maltose, mannitol, D-mannose, D-melobiose, D-melibiose, sucrose, D-turanose and D-xyllose.

Production of acid without gas is strain-dependent for D-arabinose, L-fucose, galactose, gentiobiose, gluconate, D-melezitose, methyl D-glucoside, methyl xylloside, N-acetylgalactosamine and salicin. Acid is not produced from adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, glycerol, inulin, 2-keto-D-gluconate, 5-keto-D-glucurate, D-lyxose, meso-inositol, methyl D-mannoside, rhamnose, sorbitol, L-sorbose, D-tagatose, D-xyllose or xylobitol. Of the variable results, the type strain was positive for D-arabinose, L-fucose, galactose, gentiobiose, gluconate, D-melezitose, methyl xylloside, N-acetylglucosamine and salicin and was weakly positive for methyl D-glucoside. Nitrate reduction is weak and Voges-Proskauer reaction is negative. The major cellular fatty acids are (means of 10 strains): anteisomer-C15:0 (38·5±5·3% of total cellular fatty acids), C16:0 (23·9±6·7%), iso-C15:0 (11·1±1·4%), anteisomer-C17:0 (5·4±1·9%), iso-C16:0 (5·4±1·4%), iso-C17:0 (4·8±1·2%), C16:1ω11c (4·3±1·3%), C14:0 (3·1±1·4%), iso-C14:0 (2·0±0·6) and C15:0 (1·0±0·7%). Fatty acids present at less than 1% are not listed. The DNA G+C content varies from 51·6 mol% for MB 1871 T to 51·7 mol% for strains MB 1928 and MB 2035.

The type strain is MB 1871 T (=LMG 21940 = DSM 15596 T ).

Acknowledgements

The authors are grateful to B. Christner for providing strain SB150-2B (MB 2328). We wish to thank Petra Vannol, Liestbeth Lebbe and Elly Engels for excellent technical assistance. We are most grateful to bioMérieux SA for providing API materials and for supporting M.R.-D. P.D.V. is indebted to the National Fund for Scientific Research (Belgium) for financial support by grant G.0156.02.

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