Reclassification of *Lactobacillus catenaformis* (Eggerth 1935) Moore and Holdeman 1970 and *Lactobacillus vitulinus* Sharpe *et al.* 1973 as *Eggerthia catenaformis* gen. nov., comb. nov. and *Kandleria vitulina* gen. nov., comb. nov., respectively

Elisa Salvetti, Giovanna E. Felis, Franco Delliglio, Anna Castioni, Sandra Torriani and Paul A. Lawson

1Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy
2Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA

The development of molecular tools and in particular the use of 16S rRNA gene sequencing has had a profound effect on the taxonomy of many bacterial groups. Gram-positive organisms that encompass the genera *Lactobacillus* and *Clostridium* within the *Firmicutes* are examples of taxa that have undergone major revisions based on phylogenetic information. A consequence of these reorganizations is that a number of organisms are now recognized as being misclassified. Previous studies have demonstrated that *Lactobacillus catenaformis* and *Lactobacillus vitulinus* are phylogenetically unrelated to *Lactobacillus sensu stricto* and should be reclassified in two new genera, named respectively *Eggerthia* gen. nov., with the type species *Eggerthia catenaformis* gen. nov., comb. nov. (type strain DSM 20559T = ATCC 25536T = CCUG 48174T = CIP 104817T = JCM 1121T) and *Kandleria* gen. nov., with the type species *Kandleria vitulina* gen. nov., comb. nov. (type strain LMG 18931T = ATCC 27783T = CCUG 32236T = DSM 20405T = JCM 1143T).

The genus *Lactobacillus* and the genera *Paralactobacillus* and *Pediococcus* belong to the family *Lactobacillaceae*, which is a member of the order ‘*Lactobacillales*, a major phylogenetic group of the *Firmicutes*. Numerous studies using 16S rRNA gene sequencing have demonstrated that the species *Lactobacillus catenaformis* and *Lactobacillus vitulinus* are phylogenetically unrelated to *Lactobacillus sensu stricto* (Collins *et al.*, 1991; Pot *et al.*, 1994; Schleifer & Ludwig, 1995). Furthermore, Collins and co-workers demonstrated that these species were phylogenetically placed within the *Clostridia* rRNA cluster XVII. Recently, two novel genera have been described, namely *Catenibacterium* (Kageyama & Benno, 2000) and *Sharpea* (Morita *et al.*, 2008) and were phylogenetically located in cluster XVII of the clostridia. In particular, in the latest edition of the *Bergey’s Manual of Systematic Bacteriology* (available online at http://www. bergeys.org/outline.html), *Catenibacterium mitsuokai* is placed within the family *Erysipelotrichaceae* together with other species described as members of genera not assigned to this family, including *L. catenaformis* and *L. vitulinus*. Furthermore, it has also been suggested that *L. catenaformis* and *L. vitulinus* be included in this phylogenetic grouping (Stackebrandt, 2009).

Based on our present study and the observations of others, *L. catenaformis* and *L. vitulinus* are discussed with respect to their taxonomic status and their reclassification as *Eggerthia* gen. nov., with the type species *Eggerthia catenaformis* gen. nov., comb. nov. and *Kandleria* gen. nov., with the type species *Kandleria vitulina* gen. nov., comb. nov.

The 16S rRNA gene sequence of *L. catenaformis* DSM 20559T located in EMBL/GenBank was submitted in 1989, and contained a number of sequencing ambiguities originating from the reverse transcriptase sequencing method used. To ensure as accurate results as possible, this strain was resequenced using present day methods incorporating direct sequencing of the 16S rRNA gene PCR product. The most recent published sequence of

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of *Lactobacillus catenaformis* DSM 20559T is AJ821549. A supplementary table is available with the online version of this paper.
L. vitulinus was from a recent study (Morita et al., 2008) and was therefore used in the present study. L. catenaformis DSM 20559<sup>T</sup> was grown in an optimized peptone medium and in MRS + 0.5 g Cys-HCl<sup>T</sup>, respectively, both at 37 °C in anaerobiosis. The composition of the peptone medium was the following (l<sup>-1</sup>): 20 g BHI broth, 15 g tryptone, 20 g phytone peptone, 20 g special peptone, 20 g Bacto peptone, 20 g meat extract, 20 g proteose peptone, 20 g peptone, 5 g yeast extract, 5 g K<sub>2</sub>HPO<sub>4</sub>, 7.5 ml freshly prepared yeast extract and 0.5 g Cys-HCl.

The partial 16S rRNA gene sequence for L. catenaformis DSM 20559<sup>T</sup> was obtained using primers Lac16S-for (5′-AATGAGAGTTTGATCCTGGCT-3′) and Lac16S-rev (5′-GAGGTTGATCCAGCGCAGTTT-3′). The reaction mixture (20 µl) contained 30 ng template DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 1 µM each primer and 1 U Taq DNA polymerase (Polymed) in a standard reaction buffer. After an initial denaturation of 4 min at 94 °C, 25 cycles of 1 min at 94 °C, 1.5 min at 50 °C, 2 min at 72 °C and a final extension at 72 °C for 7 min were performed. The 1.6 kb amplification product was extracted from the agarose gel (Promega elution kit) and sequenced at the Biomolecular Research (BMR) Center at Padua University (Italy). The cells harvested were checked for purity and DNA was extracted by the procedure of Marmur (1961).

To establish the closest relatives, 16S rRNA gene sequences were used to check the phylogenetic placement of the two species as obtained by online resources such as BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and SEQMATCH (http://rdp.cme.msu.edu/index.jsp). BLAST searches and SEQMATCH analyses showed a relationship between the two species and the type sequences and those of Sharpea azabuensis ST18<sup>T</sup> and Catenibacterium mitsuokai JCM 10609<sup>T</sup>, respectively, which are included in Clostridium subphylum cluster XVII.

16S rRNA gene sequences of the type species of family Lactobacillaceae, L. catenaformis, L. vitulinus, S. azabuensis and Catenibacterium mitsuokai, as well as other related organisms of the family Erysipelothricaceae, which belong to other Clostridium clusters, were aligned. Unknown bases were disregarded and 1320 positions were included in a phylogenetic analysis. Phylogenetic trees were constructed using the Jukes and Cantor method as the distance formula and the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood methods as suggested by Kämpfer and co-workers (Kämpfer et al., 2003) as implemented in the MEGA v4 software package (Tamura et al., 2007). The statistical reliability of the topology of the phylogenetic trees was evaluated using bootstrapping with 1000 replicates (Felsenstein, 1985). All the major branching nodes were confirmed by maximum-parsimony analysis (data not shown).

Phylogenetic analyses employing 16S rRNA gene sequences showed that the species of the family Lactobacillaceae included in the analysis formed a separate clade while both L. catenaformis and L. vitulinus were placed within the Clostridium subphylum cluster XVII, related to S. azabuensis and Catenibacterium mitsuokai, respectively (Fig. 1).

Although this placement is within clostridia-like organisms, phylogenetic studies based on the 16S rRNA gene have been used by a number of authors to show the need for a revision of the genus Clostridium (Lawson et al., 1993; Stackebrandt et al., 1999; Wiegel et al., 2006). Indeed, Collins et al. (1994) proposed a possible hierarchical framework for future classification of clostridia with a proposal to restrict the

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**Fig. 1.** The phylogenetic relationship of Kandleria vitulina gen. nov., comb. nov. and Eggerthia catenaformis gen. nov., comb. nov. with respect to representative species of the Lactobacillaceae based on the 16S rRNA gene sequences. The tree was constructed using Jukes and Cantor’s method and the minimum evolution algorithm. Bootstrap values (1000 replicates) are reported in percentage at nodes (only values higher than 60% are represented). Bar, 0.02 nucleotide substitutions.

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genus *Clostridium* to organisms that formed a distinct cluster in the 16S rRNA tree (cluster I) and that these should be regarded as the true representatives of the genus *Clostridium* (i.e. *Clostridium sensu stricto*).

The phylogenetic analysis undertaken in this study confirmed previous findings that *L. catenaformis* and *L. vitulinus* formed a loose association with *S. azabuensis* and *Catenibacterium mitsuokai* respectively. Pairwise sequence alignments revealed that *L. vitulinus* showed low sequence similarities with *Catenibacterium mitsuokai* (91.2 %), *L. catenaformis* (87.1 %), and *S. azabuensis* (90.6 %). Similarly, *L. catenaformis* showed equally low values with *Catenibacterium mitsuokai* (84.8 %), and *S. azabuensis* (88.9 %). These phylogenetic depths (almost higher than 10 %) of *L. catenaformis* and *L. vitulinus* with their respective nearest relatives suggest that each of these misplaced lactobacilli forms the nucleus of a novel genus. The separateness of these organisms was supported by low DNA–DNA hybridization values (Morita *et al.*, 2008) (see Supplementary Table S1 in IJSEM Online) in addition to phenotypic and chemotaxonomic information (Table 1).

However, it is clear that this cluster of organisms share many similar traits with only a few of them useful as diagnostic markers. Thus an accurate identification, especially at the laboratory bench, is becoming ever more reliant on rapid molecular genetic techniques such as 16S rRNA gene sequence comparisons. These high throughput methodologies are becoming increasingly automated and the costs are declining, making them accessible to the routine laboratory or available as services offered by commercial facilities. These methods allow accurate identification of hitherto unknown taxa with a turnaround time of only a matter of hours or days. It is becoming increasingly common for isolates to be identified by 16S rRNA gene sequencing first before biochemical and chemotaxonomic analyses are undertaken. Such changes are reflected in the latest edition of *Bergey’s Manual of Systematic Bacteriology* where the format now follows a phylogenetic basis for the classification of micro-organisms and many examples exist of organisms being grouped on phylogenetic information but which possess few differential phenotypic traits (Rainey, 2009).

Based on phenotypic, chemotaxonomic and phylogenetic evidence that demonstrates their separateness from the genus *Lactobacillus* and placement in family *Erysipelotrichaceae* (*Clostridium* subphylum cluster XVII) within the class *Erysipelotrichia*, we consider that the species *L. catenaformis* and *L. vitulinus* merit classification in two new genera, for which the names *Eggerthia* gen. nov., and *Kandleria* gen. nov. are proposed, respectively.

Table 1. Physiological characteristics of *Catenibacterium mitsuokai, L. catenaformis, L. vitulinus* and *S. azabuensis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Metabolic end products*</td>
<td>A, L, ib</td>
<td>L, a, (f)</td>
<td>L, a</td>
<td>L</td>
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<tr>
<td>Lactic acid isomer(s)</td>
<td>DL</td>
<td>D</td>
<td>D</td>
<td>D</td>
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<tr>
<td>Fermentation style†</td>
<td>HO</td>
<td>HO</td>
<td>HO</td>
<td>HE</td>
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<td>Fermentation of:</td>
<td></td>
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<td></td>
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<tr>
<td>Salcin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Melibiose</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
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<td>−</td>
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<td>Starch</td>
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</tr>
<tr>
<td>D-Ribose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>36.6</td>
<td>34.8</td>
<td>34.4</td>
<td>37.4</td>
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<td>Cell wall murein</td>
<td>A1γ (L-Ala)–D-Glu–m-Dpm</td>
<td>A3α l-Lys–l-Ala3</td>
<td>A1γ m-Dpm-direct</td>
<td>A1γ (L-Ala)–D-Glu–m-Dpm</td>
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<tr>
<td>Source</td>
<td>Human faeces</td>
<td>Human faeces, intestinal and pleural infections</td>
<td>Bovine rumen</td>
<td>Horse faeces</td>
</tr>
</tbody>
</table>

*From glucose.
†HE, heterofermentative; HO, homofermentative.
Description of *Eggerthia* gen. nov.

*Eggerthia* (Eg ger’thi.a. N.L. fem. n. *Eggerthia* named after Arnold H. Eggerth, who first identified the species *Lactobacillus catenaformis*).

Cells are Gram-positive, non-spore-bearing anaerobic rods that often occur in short chains. Lactic acid is the main product from glucose. The cell wall contains the Lys–Ala peptidoglycan type and the G+C content of the DNA is 31–33 mol%. The genus *Eggerthia* is a member of the family *Erysipelotrichaceae* (*Clostridium* subphylum cluster XVII) and exhibits a close phylogenetic association with *Catenibacterium mitsuokai*, *L. vitulinus* (to be reclassified as *Kandleria vitulina* gen. nov., comb. nov., see below) and *S. azabuensis*. The type species is *Eggerthia catenaformis*.

Description of *Eggerthia catenaformis* comb. nov.

*Eggerthia catenaformis* [c.a.te.na.for’mis. L. n. *catena* chain; L. suff. -formis (from L. n. forma figure, shape, appearance) -like, in the shape of; N.L. fem. adj. *catenaformis* chain-shaped].


Displays the following properties in addition to those described for the genus. Cells are small, slightly irregular bacilli catenaformis, *Kandleria vitulina* gen. nov., comb. nov., see below) and *Catenibacterium mitsuokai*, *L. vitulinus* (to be reclassified as *Kandleria vitulina* gen. nov., comb. nov., see below) and *S. azabuensis*. The type species is *Eggerthia catenaformis*.

The type strain, LMG 18931T (=ATCC 27783T=CCUG 32236T=DSM 20405T=JCM 1143T), was isolated from the rumen of a six-week-old calf. Members of this species have also been isolated from bovine runen.

Description of *Kandleria vitulina* comb. nov.


Displays the following properties in addition to those described for the genus. Cells are rod-shaped with rounded ends (0.5–0.7 × 2.4 μm) and occur singly and in pairs. Catalase-negative and non-motile. Good growth in freshly boiled MRS broth supplemented with 0.05% (w/v) cysteine-HCl. Grows at 45 °C, but not at 15 °C. Arginine is not hydrolysed. Acid is produced from galactose, glucose, fructose, mannose, cellobiose, maltose, lactose, sucrose, melibiose, starch, amygdalin, aesculin and salicin. Acid is not produced from arabinose, ribose, xylose, rhamnose, trehalose, melezitose, raffinose, mannitol or sorbitol. The type strain metabolizes glucose homofermentatively producing (−)-d-lactic acid. No gas is produced from glucose. The peptidoglycan type is meso-diaminopimelic acid.

The type strain, LMG 18931T (=ATCC 27783T=CCUG 32236T=DSM 20405T=JCM 1143T), was isolated from the rumen of a six-week-old calf. Members of this species have also been isolated from bovine runen.

References


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