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

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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 cateniformis* and *Lactobacillus vitulinus* are phylogenetically unrelated to *Lactobacillus sensu stricto*, being placed within the *Clostridia* rRNA cluster XVII. Based on the phenotypic, chemotaxonomic and phylogenetic data presented, it is proposed that *L. cateniformis* and *L. vitulinus* be reclassified in two new genera, named respectively *Eggerthia* gen. nov., with the type species *Eggerthia cateniformis* gen. nov., comb. nov. (type strain DSM 20559^T=ATCC 25536^T=CCUG 48174^T=CIP 104817^T=JCM 1121^T) and *Kandleria* gen. nov., with the type species *Kandleria vitulina* gen. nov., comb. nov. (type strain LMG 18931^T=ATCC 27783^T=CCUG 32236^T=DSM 20405^T=JCM 1143^T).

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 cateniformis* 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/outlines.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. cateniformis* and *L. vitulinus*. Furthermore, it has also been suggested that *L. cateniformis* and *L. vitulinus* be included in this phylogenetic grouping (Stackebrandt, 2009).

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

The 16S rRNA gene sequence of *L. cateniformis* DSM 20559^T 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/DDBJ accession number for the 16S rRNA gene sequence of *Lactobacillus cateniformis* DSM 20559^T is AJ621549.

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^T was grown in an optimized peptone medium and in MRS+0.5 g Cys-HCl l⁻¹, respectively, both at 37 °C in anaerobiosis. The composition of the peptone medium was the following (l⁻¹): 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 K₂HPO₄, 7.5 ml freshly prepared yeast extract and 0.5 g Cys-HCl.

The partial 16S rRNA gene sequence for *L. catenaformis* DSM 20559^T was obtained using primers Lac16S-for (5'-AATGAGAGTTTGATCCTGGCT-3') and Lac16S-rev (5'-GAGGTGATCCAGCCGAGGTT-3'). The reaction mixture (20 µl) contained 30 ng template DNA, 1.5 mM MgCl₂, 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 *L. catenaformis* DSM 20559^T and *L. vitulinus* LMG 18931^T sequences and

those of *Sharpea azabuensis* ST18^T and *Catenibacterium mitsuokai* JCM 10609^T, 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 minimum evolution (Nei & Kumar, 2000) 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; Wiegand *et al.*, 2006). Indeed, Collins *et al.* (1994) proposed a possible hierarchical framework for future classification of clostridia with a proposal to restrict the

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.

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. cateniformis* 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. cateniformis* (87.1%), and *S. azabuensis* (90.6). Similarly, *L. cateniformis* showed equally low values with *Catenibacterium mitsuokai* (84.8%), and *S. azabuensis* (88.9%). These phylogenetic depths (almost higher than 10%) of *L. cateniformis* 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. cateniformis* 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. cateniformis*, *L. vitulinus* and *S. azabuensis*

Taxa: 1, *Catenibacterium mitsuokai* JCM 10609^T; 2, *L. cateniformis* JCM 1121^T; 3, *L. vitulinus* JCM 1143^T; 4, *S. azabuensis* ST18^T. +, Positive; –, negative; ND, no data available; Dpm, diaminopimelic acid; A, acetate; L, lactate; B, butyrate; F, formate; IB, iso-butyrate. Upper case letters represent a major product, lower case letters represent minor products. Values in parentheses show end products present only in some strains. All strains were positive for the fermentation of glucose, galactose, fructose, mannose, cellobiose and lactose. All strains were negative for aerobic growth, growth at 15 °C, catalase activity and fermentation of rhamnose, mannitol, sorbitol and melezitose. Data from Sharpe *et al.*, 1973; Moore & Holdeman, 1970; Kageyama & Benno, 2000 and Morita *et al.*, 2008.

Characteristic	1	2	3	4
Metabolic end products*	A, L, B, ib	L, a, (f)	L, a	L
Lactic acid isomer(s)	DL	D	D	D
Fermentation style†	HO	HO	HO	HE
Fermentation of:				
Salicin	+	+	+	–
Melibiose	ND	–	+	+
Trehalose	–	–	–	–
Raffinose	–	–	–	–
Starch	–	+	+	+
D-Xylose	–	+	–	–
D-Arabinose	–	+	–	–
D-Ribose	–	+	–	–
DNA G + C content (mol%)	36.6	34.8	34.4	37.4
Cell wall murein	A1γ (L-Ala)–D-Glu–m-Dpm	A3α L-Lys–L-Ala ₃	A1γ m-Dpm-direct	A1γ (L-Ala)–D-Glu–m-Dpm
Source	Human faeces	Human faeces, intestinal and pleural infections	Bovine rumen	Horse faeces

*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 [ca.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].

Basonym: *Lactobacillus catenaformis* corrig. (Eggerth 1935) Moore and Holdeman 1970.

Displays the following properties in addition to those described for the genus. Cells are small, slightly irregular rods and occur often in chains. Catalase-negative and non-motile. Good growth occurs at 37–45 °C. Negative in tests for arginine dihydrolase. Acid is produced from galactose, glucose, fructose, mannose, cellobiose, lactose, sucrose, starch, amygdalin, aesculin and salicin. Acid is not produced from arabinose, ribose, xylose, rhamnose, trehalose, melibiose, melezitose, raffinose, mannitol or sorbitol. The type strain metabolizes glucose homofermentatively to produce (–)-D-lactic acid. No gas is produced from glucose. The structure of the cell-wall murein is L-Lys-L-Ala₃ (A3α).

The type strain is DSM 20559^T (=ATCC 25536^T=CCUG 48174^T=CIP 104817^T=JCM 1121^T). Strains of this species have been isolated from human faeces and intestinal and pleural infections.

Description of *Kandleria* gen. nov.

Kandleria (Kand.le'ri.a. N.L. fem. n. *Kandleria* named after Otto Kandler, for his outstanding contribution to the study of lactic acid bacteria and for the first identification of *Lactobacillus vitulinus*).

Cells are Gram-positive, non-spore-forming, anaerobic rods. The cell wall contains peptidoglycan of the D-meso-diaminopimelic acid type and G+C content of the DNA is 34–37 mol%. The genus *Kandleria* is a member of the family *Erysipelotrichaceae* (*Clostridium* subphylum cluster XVII) and exhibits a close phylogenetic association with *Catenibacterium mitsuokai*, *Eggerthia catenaformis* and *S. azabuensis*. The type species is *Kandleria vitulina*.

Description of *Kandleria vitulina* comb. nov.

Kandleria vitulina (vi.tu.li'na. L. fem. adj. *vitulina* of a calf).

Basonym: *Lactobacillus vitulinus* Sharpe *et al.* 1973.

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

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