NOTE

***Lactobacillus coleohominis* sp. nov., isolated from human sources**

¹ Culture Collection,
Department of Clinical
Bacteriology, University of
Göteborg, Göteborg,
Sweden

² Russian Peoples'
Friendship University,
Moscow, Russia

³ Departamento de
Alimentos and
Biotecnologia, Facultad de
Química, Universidad
Nacional Autónoma de
México, México

⁴ School of Food Biosciences,
University of Reading,
Reading, UK

Natalia Nikolaitchouk,^{1,2} Carmen Wachter,³ Enevold Falsen,¹
Björn Andersch,¹ Matthew D. Collins⁴ and Paul A. Lawson⁴

Author for correspondence: Paul A. Lawson. Tel: +44 118 935 7000. Fax: +44 118 935 7222.
e-mail: p.a.lawson@reading.ac.uk

Four strains of a hitherto unrecognized Gram-positive, catalase-negative, facultatively anaerobic, rod-shaped bacterium isolated from human sources were characterized using phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing demonstrated that the bacterium represents a new subspecies within the *Lactobacillus casei*/*Pediococcus* rRNA group of the genus *Lactobacillus*. The unknown bacterium was readily distinguished from all other described *Lactobacillus* species and related taxa by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Lactobacillus coleohominis* sp. nov. The type strain of *Lactobacillus coleohominis* is CCUG 44007^T (= CIP 106820^T).

Keywords: *Lactobacillus coleohominis*, 16S rRNA, taxonomy, phylogeny

The genus *Lactobacillus* represents one of the largest genera within the *Clostridium* subphylum of the Gram-positive bacteria, comprising over 60 species. *Lactobacilli* are widespread in nature and are found in fermented foods and beverages, vegetable material and sewage. *Lactobacillus* species are also found in association with the intestinal tracts and mucous membranes of humans and many animals, and, except in very rare circumstances, are considered non-pathogenic (Aguirre & Collins, 1993; Hammes *et al.*, 1992; Roos *et al.*, 2000; Wibowo *et al.*, 1985). During the course of an investigation of Gram-positive, catalase-negative, rod-shaped organisms from human sources referred to the Culture Collection of the University of Göteborg (CCUG) for identification, we have characterized four strains of an unknown *Lactobacillus*-like organism. Preliminary phenotypic tests indicated that the isolates were possibly related to the genus *Lactobacillus*, but they could not be assigned to any recognized species. In this article, we report the results of a polyphasic taxonomic study on this previously unknown lactic acid bacterium from humans. On the basis of the findings presented, a new member of the genus *Lactobacillus*, namely *Lactobacillus coleohominis* sp. nov., is described.

Four catalase-negative, facultatively anaerobic, Gram-positive, rod-shaped isolates were referred to the CCUG for identification. Strains CCUG 44007^T and CCUG 44087 both originated from the vaginas of healthy women aged 32 and 29 years, respectively. Strains CCUG 30840 and CCUG 44174 were recovered from the urine of a 45-year-old woman and from the cervix of a 34-year-old woman, respectively. The four strains were biochemically characterized by using the API rapid ID 32S and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Biochemical tests were performed in duplicate. The ability to produce gas from glucose was determined using Gibson's semi-solid medium as described by Harrigan & McCance (1976). The diamino acid content of the cell-wall murein was determined as described by Schleifer & Kandler (1972). To assess the overall phenotypic resemblance of the isolates to each other and to reference *Lactobacillus* species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. PAGE analysis of whole-cell proteins was carried out as described by Pot *et al.* (1994) and Vandamme *et al.* (1998). For densitometric analysis, normalization and interpretation of protein patterns, the GCW 4.1 software package (Applied Maths) was used. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted, for convenience, to a percentage similarity. The profiles

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CCUG 44007^T is AJ292530.

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *Lactobacillus coleohominis* sp. nov. and some other *Lactobacillus* species. Levels of correlation are expressed as percentages of similarity, for convenience.

generated were compared with those of an extensive library maintained by the CCUG. The type strains and other reference strains used in the investigation were all maintained by the CCUG. Phylogenetic analysis was performed by comparative 16S rRNA gene sequence analyses. The 16S rRNA genes of the isolates

were amplified by a PCR and directly sequenced using a *Taq* dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. These

Fig. 2. Unrooted tree showing the phylogenetic relationships of *L. coleohominis* sp. nov. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as a percentages of 500 replications, are given at branching points. Bar, 1% sequence divergence.

sequences and those of other known related strains were retrieved from the EMBL or Ribosomal Database Project data libraries and aligned with the newly determined sequences using the program DNATOOLS (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually using the program GENEDOC (Nicholas *et al.*, 1997) and a distance matrix was calculated using the program DNADIST (using the Kimura two-parameter model). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The isolates from human sources consisted of Gram-positive, non-spore-forming, rod-shaped cells. All isolates were facultatively anaerobic and catalase- and oxidase-negative. When grown on Columbia horse-blood agar, their colonies were small, entire, non-pigmented and did not produce an odour. Growth was observed at 45 °C but not at 15 °C in MRS broth. Acid but no gas was produced from glucose. In the API rapid ID 32S system, three out of the four strains produced acid from D-ribose, and a single isolate produced acid from maltose. However, with the exception of these sugars, no other carbohydrate was fermented by any of the strains in this test system. All of the isolates showed activity for acid phosphatase (weak reaction), β -galactosidase, β -glucuronidase and phosphoamidase (weak reaction). Three strains gave positive reactions for leucine arylamidase, and two strains displayed activity for α -glucosidase. Different results were obtained for β -glucosidase and α -galactosidase reactions. With the API rapid ID 32S system, all of the isolates were β -glucosidase-negative but a single isolate produced a positive reaction with

the API ZYM system. Similarly, with the API rapid ID 32S system, a single strain displayed α -galactosidase activity, but, with the API ZYM kit, all strains gave negative reactions. None of the isolates showed activity for alanine phenylalanine proline arylamidase, alkaline phosphatase, cystine arylamidase, *N*-acetyl- β -glucosaminidase, chymotrypsin, ester lipase C8, lipase C14, α -fucosidase, glycyl tryptophane arylamidase, α -mannosidase, β -mannosidase, pyroglutamic acid arylamidase, valine arylamidase, trypsin or urease. The organisms failed to hydrolyse aesculin, gelatin or hippurate. They all gave negative results in the Voges-Proskauer test and did not reduce nitrate to nitrite. An examination of the wall-murein amino acid composition of a representative strain (CCUG 44007^T) revealed *meso*-diaminopimelic acid as the dibasic amino acid. This is consistent with the presence of a directly cross-linked *meso*-diaminopimelic acid (variation A1 γ)-type murein. On the basis of the above cultural and biochemical characteristics, the isolates resembled the genus *Lactobacillus* but did not correspond to any of the currently recognized species. To clarify the overall phenotypic resemblance of the unidentified isolates and their relationship to known members of this genus, whole-cell protein profiles were determined. A numerical analysis of PAGE protein patterns of the four strains together with some reference species of *Lactobacillus* is shown in Fig. 1. Protein profiling confirmed the high overall phenotypic homogeneity of the isolates and showed that they represent a tight cluster (intracluster correlation level > 83%) that is separate from other *Lactobacillus* species. To ascertain the phylogenetic relationships of the unknown isolates, their 16S rRNA genes were sequenced and subjected to a comparative analysis. The almost complete gene sequences (> 1450 nucleotides) of the four strains were determined and pair-wise analysis showed them to be identical (100% sequence

Table 1. Characteristics that are useful in differentiating *Lactobacillus coleohominis* sp. nov. from its nearest phylogenetic relatives

Biochemical tests were performed using the API rapid ID32S system. +, Positive test result; –, negative test result; + (w), weakly positive reaction.

Test	4 <i>L. coleohominis</i> strains	<i>L. oris</i> CCUG 37396 ^T	<i>L. panis</i> CCUG 37482 ^T	<i>L. pontis</i> CCUG 33456 ^T	<i>L. vaginalis</i> CCUG 31452 ^T
Acid from:					
Lactose	–	+(w)	–	–	+(w)
Melibiose	–	+	–	–	+
D-Raffinose	–	+	–	–	+
Production of:					
β-Glucuronidase	+	–	–	–	–
β-Glucosidase	–	–	+	–	–
Voges–Proskauer	–	+(w)	+	–	–
Murein type	A1γ	A4α	A4α	A4β	A4β

similarity), thereby demonstrating the high degree of genetic relatedness of the isolates. Sequence database searches showed that the unknown bacterium was most closely related to members of rRNA group II (*Lactobacillus casei*/*Pediococcus* group) of the genus *Lactobacillus* (see Collins *et al.*, 1991). The results of neighbour-joining analysis are shown in Fig. 2 and confirmed the placement of the unknown bacterium (as exemplified by strain CCUG 44007^T) within rRNA group II of the genus *Lactobacillus*. The unknown bacterium formed a distinct subline within a small cluster of species that included *Lactobacillus oris*, *Lactobacillus panis*, *Lactobacillus pontis* and *Lactobacillus vaginalis* (94.8–95.8% 16S rRNA sequence similarity).

It is clear from the investigation that the *Lactobacillus*-like isolates recovered from human sources represent a homogeneous group of organisms which are phenotypically and phylogenetically distinct from currently recognized members of the genus *Lactobacillus*. Phylogenetically, the unknown bacterium represents a hitherto unknown subline within the genus *Lactobacillus*, and the rRNA sequence divergence values of > 4% with respect to other lactobacilli unequivocally demonstrates that it warrants classification as a new species. Biochemically, the novel bacterium from humans can be readily distinguished from all described species of the genus. Therefore, on the basis of the findings presented, we propose that the unidentified bacterium be classified in the genus *Lactobacillus*, as *Lactobacillus coleohominis* sp. nov. Characteristics which are useful in distinguishing *L. coleohominis* from its closest phylogenetic relatives are shown in Table 1.

Description of *Lactobacillus coleohominis* sp. nov.

Lactobacillus coleohominis (co.le.o.ho'mi.nis. Gr. n. koleos vagina; L. gen. n. hominis of humans; N.L. gen. n. coleohominis of the vagina of humans).

Cells consist of Gram-positive, non-spore-forming rods. On Columbia horse-blood agar its colonies are small, entire, non-pigmented and do not produce an odour. Facultatively anaerobic and catalase- and oxidase-negative. There is no growth at 15 °C but growth is observed at 45 °C. L(+)-Lactic acid and D(–)-lactic acid are produced from glucose without gas. Ammonia is not produced from arginine. In the API rapid ID 32S system, most strains produce acid from ribose; acid may be produced from maltose. Acid is not produced from D-arabitol, L-arabinose, cyclo-dextrin, glycogen, lactose, mannitol, melibiose, mel-ezitose, methyl β-D-glucopyranoside, pullulan, sorbitol, sucrose, D-raffinose, tagatose or trehalose. Activity is detected for acid phosphatase (weak reaction), β-galactosidase, β-glucuronidase and phosphoamidase (weak reaction). Activity is not detected for alanine phenylalanine proline arylamidase, alkaline phosphatase, cystine arylamidase, N-acetyl-β-glucosaminidase, chymotrypsin, ester lipase C8, lipase C14, α-fucosidase, glycyl tryptophan arylamidase, α-mannosidase, β-mannosidase, pyroglutamic acid arylamidase, valine arylamidase, trypsin or urease. The reactions are variable for arginine dihydrolase, esterase C-4, α-glucosidase, β-glucosidase and leucine arylamidase. Aesculin, gelatin and hippurate are not hydrolysed. Voges–Proskauer-negative. Nitrate is not reduced. meso-Diaminopimelic acid is present in the cell-wall murein. Isolated from human sources. The habitat is not known. The type strain is CCUG 44007^T (= CIP 106820^T).

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