**Lactobacillus ceti** sp. nov., isolated from beaked whales (Ziphius cavirostris)

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Biochemical and molecular genetic studies were performed on three isolates of an unknown Gram-positive, catalase-negative and rod-shaped organism isolated from the lungs and liver of two beaked whales. The organisms were tentatively identified as *Lactobacillus* spp. based on cellular morphology and biochemical tests. 16S rRNA gene sequencing studies confirmed the provisional identification of the novel isolates as members of the genus *Lactobacillus*, but the isolates did not correspond to any recognized species of this genus. The novel strains shared the same phenotypic characteristics and exhibited 100% 16S rRNA gene sequence similarity. The nearest phylogenetic relatives of the novel isolates were *Lactobacillus satsumensis* DSM 16230T (94.2% 16S rRNA gene sequence similarity), *Lactobacillus salivarius* JCM 1047 (94.0%), *Lactobacillus nagelii* ATCC 700692T (94.0%) and *Lactobacillus saerimneri* DSM 16049T (93.8%). The novel isolates could be distinguished from these species and other related species of the genus *Lactobacillus* by physiological and biochemical tests. On the basis of these phenotypic, physiological and phylogenetic findings, it is proposed that the new isolates from whales be classified as a novel species of the genus *Lactobacillus*, *Lactobacillus ceti* sp. nov. The type strain is 142-2T (=CECT 7185T=CCUG 53626T).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 142-2T is AM292799.
minimized biochemical kits, the novel isolates displayed identical phenotypic profiles. The lactate isomer was determined enzymically using the DL-lactate test kit (Boehringer Mannheim). A detailed description of the morphological, physiological and biochemical characteristics of the isolates is given in the species description and in Table 1.

Analysis of the peptidoglycan structure of one isolate (strain 142-2<sup>T</sup>) was performed as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was used instead of paper chromatography. Analysis of the cell-wall composition of strain 142-2<sup>T</sup> revealed the presence of lysine and serine, indicating the presence of the A3<sub>a</sub>-Lys–D-Ser peptidoglycan type (DSMZ, 2001).

To establish the phylogenetic affinities of the novel isolates, the 16S rRNA gene sequences were determined as described previously (Vela et al., 2005) and were subjected to a comparative analysis. The almost complete sequences (2006 nucleotides) of the three novel isolates were determined and pairwise analysis revealed that the isolates were phylogenetically identical (100 % gene sequence similarity). Sequence searches of GenBank using the FASTA program (Pearson, 1994) revealed that the novel isolates had <95 % gene sequence similarity with any recognized species. The novel bacteria were phylogenetically closely related to Lactobacillus satsumensis DSM 16230<sup>T</sup> (94.2 % 16S rRNA gene sequence similarity), Lactobacillus salivarius JCM 1047 (94.0 %), Lactobacillus nagelii ATCC 700692<sup>T</sup> (94 %) and Lactobacillus saerimneri DSM 16049<sup>T</sup> (93.8 %). These sequences, and those of other known related strains, were retrieved from GenBank and aligned with the newly determined sequences using the DNAnalyze program (Rasmussen, 1995). Phylogenetic trees were constructed according to three different methods, a neighbour-joining algorithm (Saitou & Nei, 1987), performed with the DNAnalyze and TREEVIEW (Page, 1996) programs, maximum-likelihood analysis conducted using PHYLML software (Guindon & Gascuel, 2003) and the maximum-parsimony method carried out using the MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 software package (Kumar et al., 2004). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated by the Kimura two-parameter method (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions=100) was applied in maximum-parsimony analysis. The stability of the groupings was estimated by bootstrap analysis (1000 replications). Phylogenetic trees obtained using the neighbour-joining (Fig. 1) and the other two methods revealed a clear affiliation of the novel strains (as exemplified by strain 142-2<sup>T</sup>) to the genus Lactobacillus, as they were positioned as a separate branch close to L. saerimneri DSM 16049<sup>T</sup>, Lactobacillus aviaris ATCC 43234<sup>T</sup>, L. salivarius JCM 1047 and Lactobacillus acidipiscis JCM 10692<sup>T</sup>. Although bootstrap resampling analysis did not reveal the affiliation between strain 142-2<sup>T</sup> and the aforementioned species to be statistically significant (50 % value), the branching position of strain 142-2<sup>T</sup> within this clade was relatively stable according to the three tree-making algorithms used in this study (Fig. 1). These data together with 16S rRNA gene sequence divergence values of >4 % between the novel isolates and other species of the genus Lactobacillus suggest they represent a separate species

Table 1. Characteristics that differentiate Lactobacillus ceti sp. nov. from other members of the L. salivarius subgroup of lactobacilli

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Table 1. Characteristics that differentiate Lactobacillus ceti sp. nov. from other members of the L. salivarius subgroup of lactobacilli

| Taxa: 1, L. ceti sp. nov.; 2, L. saerimneri; 3, L. nagelii; 4, L. mali; 5, L. salivarius; 6, L. aviarius; 7, L. acidipiscis; 8, L. ruminis; 9, L. agilis; 10, L. equi; 11, L. animalis; 12, L. murinus; 13, L. vinii; 14, L. satsumensis; 15, L. apodermis. Data for Lactobacillus species were obtained from Kandler & Weiss (1986); Hammes et al. (1992); Edwards et al. (2000); Tanasupawat et al. (2000); Morotomi et al. (2002); Pedersen & Roos (2004); Endo & Okada (2005); Osawa et al. (2006) and Rodas et al. (2006). +, ≥90 % strains positive; −, ≥90 % strains negative; d, 11–89 % strains positive; NA, no data available. |
In order to differentiate the three novel isolates, pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Sma* I (MBI Fermentas) was performed as described previously (Vela et al., 2003). Strains 159-2 and 142-2T isolated from different whales generated distinguishable PFGE fingerprint profiles that revealed their genotypic differences at the strain level. On the other hand, strains 160-1 and 159-2, isolated from different organs of the same animal, exhibited undistinguishable PFGE profiles (data not shown).

Overall, the results of the present study show that the novel isolates from whales constitute a distinct branch and do not display a close relationship with any recognized organism (Fig. 1). Moreover, the novel isolates could be distinguished from their closely phylogenetic relatives on the basis of phenotypic characteristics (Table 1). Therefore, based on both phylogenetic and phenotypic criteria, it is evident that the new isolates merit classification as a novel species of the genus *Lactobacillus*, for which the name *Lactobacillus ceti* sp. nov. is proposed. Strains 142-2T, 159-2 and 160-1 showed identical phenotypic and physiological properties; these are given in the species description. Characteristics that are useful in differentiating *Lactobacillus ceti* sp. nov. from other members of the *L. salivarius* group of lactobacilli are summarized in Table 1.

**Description of Lactobacillus ceti** sp. nov.

*Lactobacillus ceti* (ce.ti. L. gen. n. *ceti* of a whale).

Cells are Gram-positive, 0.5 μm wide and 2.7 μm long, catalase-negative, non-spore-forming and non-motile rods. Cells are found singly, in pairs and in short chains. Facultatively anaerobic. Colonies are non-haemolytic, circular, smooth, entire and approximately 1 mm diameter on Columbia blood agar after 2 days incubation at 37 °C. Growth does not occur on MRS agar after 48 h. Grows at 22, 30 and 37 °C after 2 days incubation and at 15 °C after 5 days. Growth is not detected at 42 or 4 °C. Growth occurs in broth containing 3 % NaCl (w/v), but not with 6.5 % NaCl. L-Lactate is exclusively produced as the end product from hexoses and pentoses. Aesculin and urea are not hydrolysed.

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of *Lactobacillus ceti* sp. nov. with other species of the genus *Lactobacillus*. *Oenococcus oeni* ATCC 23279T was used as an outgroup. Bootstrap values (expressed as a percentage of 1000 replications) >50 % are given at the branching points. Filled circles indicate that the corresponding nodes (groupings) were also obtained in maximum-likelihood trees. Open circles indicate that the corresponding nodes (groupings) were also obtained in maximum-likelihood and maximum-parsimony trees. Bar, 1 % sequence divergence.](http://ijs.sgmjournals.org)
Gelatin is hydrolysed after 2 days incubation. Nitrate is not reduced. Acidification is detected from 5-ketoglucunate and ribose after 48 h. Acid is not produced from D-glucose, D- or L-xyllose, D-mannitol, maltose, D-lactose, sucrose, N-acetyl-β-glucosamine, glycerol, erythritol, L-arabinose, D-adonitol, methyl β-sylopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, cellobiose, melibiose, trehalose, inulin, melize, maltose, raffinose, glycerogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol or 2-ketogluconate. Activity is detected for acid phosphatase, naphthol-AS-Bl-phosphohydrolase, alkaline phosphatase, esterase C4 (weak), ester lipase C8 (weak) and leucine arylamidase (weak). Pyrazinamidase, lipase C14, valine arylamidase pyrrolidonyl arylamidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, α-chymotrypsin, trypsin and cystine arylamidase are not produced. The peptidoglycan type is A3α L-Lys–D-Ser.

The type strain, 142-2T (=CECT 7185T =CCUG 53626T), was isolated from the lungs of a beaked whale.

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References


