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Note

Lactobacillus apodemi sp. nov., a new tannase producing *Lactobacillus* species isolated from wild mouse feces

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Subject categorie: New Taxa; Subsection : Gram-positive

The EMBL accession number of the 16S rRNA gene sequence of strain DSM 16634^T *Lactobacillus apodemi* sp. nov. (= CIP 108913) is AJ871178

SUMMARY

A Gram-positive, rod-shaped, non-endospore forming bacterium, strain ASB1^T (DSM 16634^T), able to degrade tannin, was isolated from feces of the Japanese large wood mice, *Apodemus speciosus.* Comparative analysis of the 16S rRNA gene sequence revealed that the strain could be assigned as a member within the genus Lactobacillus. Nearest phylogenetic neighbors were determined as *Lactobacillus animalis* (98.9%) and *Lactobacillus murinus* (98.9%). Subsequent polyphasic analysis, including automated ribotyping and DNA:DNA hybridization experiments, confirmed that the isolate represents a novel species for which the name *Lactobacillus apodemi* is proposed. The DNA base composition of the strain is 38.5 mol % G+C. The peptidoglycan type is A4α L-Lys-D-Asp. The type strain is ASB1^T (= DSM 16634^T = CIP 108913^T)

In recent years, several studies have been reported the presence of bacterial species with tannase activity in the guts or feces of different animals (Osawa et al. 1995a,b). The Japanese large wood mouse (Apodemus specious) inhabits various types of forests in Japan it feeds on acorns in autumn and winter, which may contain considerable amounts of tannins (Sasaki et al. 2005). Fresh fecal pellets were collected from wild wood mice from the forests of Kyotanabe, Japan.

Bacterial strains were isolated from spread plates (tannic acid treated brain heart infusion agar, prepared as described by Osawa et al. 1990) of serial diluted fecal samples incubated under anaerobic conditions at 37°C. For further testing, the strains were grown in MRS medium (Difco).Colony and cell morphology was determined after 2 days of incubation. The ability to grow at 15℃ or 45℃ was tested in MRS broth (Difco). Anaerobic growth was tested on MRS plates incubated by use of the Anaerocult A mini system (Merck). Determination of catalase activity was performed by using reagent droppers (Becton Dickinson). Lactic acid configuration was determined using the D-lactic acid / L-lactic acid test kit (Boehringer Mannheim/R-Biopharm) based on the UV-method according to the manufacturer’s instructions. Determination of phenotypic properties was

done due to the characterization with the API 50 CHL Kit (Biomerieux, France). Genomic DNA was extracted from bacterial cells and purified using a protocol as described previously (Pukall *et al*., 1998). The primer pair 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1527r (5´-AGAAAGGAGGTGATCCAGCC-3´) was used for amplification of the 16S rRNA gene (Lane, 1991). PCR-amplification of 16S rRNA encoding gene by PCR was done as described earlier (Pukall *et al*., 1999). Amplicons were sequenced by using the Dye labelled dideoxy terminator cycle sequencing (DTCS) Quick start kit and the Ceq 8000 Genetic Analysis System from Beckman Coulter. Sequences were manually aligned and compared to sequences published previously. These were stored in the DSMZ-internal database consisting of more than 6000 16S rRNA gene sequence entries, including those from the Ribosomal Database Project (Maidak *et al*., 2001) and EMBL. Similarity values were transformed into genetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The neighbor-joining method contained in the PHYLIP package (Felsenstein, 1993) was used in the construction of the phylogenetic dendrogram. In addition the DNAml algorithm for maximum-likelihood analysis (Felsenstein 1993) was applied, but revealed in nearly the same clusters for closely related species as shown within the neighbor joining dendrogramm. All analyses were done on a SUN SparcII workstation.

Automated ribotyping was carried out on strains ASB1^T (= DSM 16634^T), ASB 2 (= DSM16635), ASB 6 (= DSM16648), ASB 8 (= DSM16649), and ASB 7 (= DSM16748) that had been isolated from faeces of Japanese large wood mice and found to be able to produce tannase as described previously (Sasaki *et al*., 2005). Fingerprints were generated by using the RiboPrinter microbial characterization system (Qualicon) The RiboPrinter system combines molecular processing steps for ribotyping in a stand-alone, automated instrument. Steps include cell lysis, digestion of chromosomal DNA with a restriction enzyme, separation of fragments by electrophoresis, transfer of DNA fragments to a nylon membrane, hybridization to a *E. coli rrn*B probe, chemiluminescent detection of the probe to the fragments containing rrn operon sequences, image detection

and computerized analysis and storage of RiboPrint patterns. Sample preparation and analysis was performed according the manufacturer's instructions, using the *Eco*RI restriction enzyme to generate restriction fragments. The band patterns were compared using the BioNumerics software (Applied Maths, Ghent, Belgium). Clustering was performed by the unweighted pair group method with arithmetic averages (UPGMA) method based on the Pearson correlation coefficient, using an optimization coefficient of 1.2%.

Analysis of the peptidoglycan structure was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. Strain ASB 1 (DSM 16634^T) possesses peptidoglycan of type A4α, L-Lys-D-Asp (type A11.31) according to page 617 of DSMZ catalogue of strains, seventh edition 2001 also available at http://www.dsmz.de/species/murein.htm).
Estimatation of guanine-plus-cytosine (G+C) content of strain DSM 16634^T followed the procedure described by Sasaki et al. 2005.
Levels of total DNA:DNA hybridization of genomic DNA extracted from ASB strains and type strains of *Lactobacillus animalis* and *Lactobacillus murinus* were determined as published by Sasaki et al. 2005.

16S rRNA gene sequence analysis indicated that DSM 16634^T is affiliated to the genus *Lactobacillus*. Species *Lactobacillus murinus* and *Lactobacillus animalis* were determined as next phylogenetic neighbors, showing sequence similarity values of 98.9%. The position of strain DSM 16634^T relative to its phylogenetic neighbors is shown by neighbor-joining analysis (Fig. 1). In addition, a second algorithm, the maximum-likelihood method included within the DNAml program (Felsenstein, 1993) was applied to confirm the phylogenetic position of strain *Lactobacillus apodemi* DSM 16634^T. Most closely related species grouped together in both dendrograms. Strain *Lactobacillus apodemi* formed a stable cluster together with it's nearest phylogenetic neighbors *Lactobacills animalis* and *Lactobacillus murinus*. Both algorithms applied have placed the Lactobacillus apodemi cluster nearby to the *Lactobacillus mali* goup and the

Lactobacillus salivarius group (Hammes & Hertel, 2003). Partial sequences for all ASB strains tested revealed in nearly identical stretches. Further, close relationship of the ASB strains could be confirmed by automated riboprinting (Fig. 2) Similarity of riboprint pattern obtained for the different ASB strains, compared to strain DSM 16634^T, ranged between 87.5 and 93.9 % and similarity values of fingerprints derived from type strains of *Lactobacillus animalis* (DSM 20602^T) and *Lactobacillus murinus* (DSM 20452^T) were determined as to be low (< 50%). Finally, DNA:DNA hybridization experiments performed (Sasakai et al. 2005) have confirmed that all ASB strains tested could been affiliated to the same species, showing a relative binding with DNA from strain DSM 16634^T between 73.8 and 97.3 %. In contrast, relative binding of DNA from strain DSM 16634T compared to those originated from the type strains of *Lactobacillus animalis* and *Lactobacillus murinus* revealed in values < 15% (Sasakai et al. 2005)
Phenotypic properties differentiating strain *Lactobacillus apodemi* DSM 16634T from type strains of *Lactobacillus animalis* and *Lactobacillus murinus* are summarized in Table 1.

The above evidence and the phenotypic characteristics described previously (Sasaki *et al.,* 2005) and summarized within the species description confirmed that the ASB strains represent a novel species, for which the name *Lactobacillus apodemi* sp. nov., is proposed. The type strain is strain ASB1 (= DSM 16634^T = CIP 108913^T).

Description *of Lactobacillus apodemi* sp. nov.
Lactobacillus apodemi (a.po.de.mi. N.L. gen. n. apodemi, because the organism was first isolated from a species of the field mice, Apodemus specious, feeding on tannin rich acorns).

Gram-positive, non-motile, non-endospore-forming, catalase-negative rods, 0.5~1 μm x 5 ~ 6 μm in size and occurring as single cells or in pairs. After anaerobic growth at 37 ・C for 48 h, colonies on MRS agar are 2 to 3 mm in diameter; they are white with an opaque border, smooth and convex. Growth on MRS agar also occurs under microaerophilic conditions and reduced growth

under aerobic conditions are detected. Grows best at mesophilic temperatures between 25-37℃. In MRS broth growth occurs at 45℃ , but not at 15℃. Produces gallic acid from tannic acid (tannase positive) but not converting gallic acid further to pyrogallol (Sasaki et al. 2005). Only L-lactate is produced. Gas is not produced from glucose as tested in MRS broth with Durham tube. Acid is produced from galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, arbutin, maltose, lactose, melibiose, sucrose, trehalose and D-raffinose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, methyl -D-xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl -D-mannoside, methyl-D-glucoside, amygdalin, inulin, melezitose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Ribose, salicine and cellobiose variable. Aesculin is hydrolysed. The DNA G+C content of strain DSM 16634^{T} is 38.5 mol% and the peptidoglycan type is A4α, L-Lys-D-Asp (type A11.31). The type strain is DSM 16634^{T}.

REFERENCES

Felsenstein, J. (1993). Phylip (phyogenetic inference package) version 3.5.1. Department of Genetics, University of Washington, Seattle, WA, USA.

Hames W.P & Hertel, C. (2003) The genera *Lactobacillus* and *Carnobacterium*, in M. Dworkin et al., eds., The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, 3rd edition, release 3.15, Springer-Verlag, New York, http://link.springer-ny.com/link/service/books/10125/.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein* Metabolism, pp. 21-132. Edited by N. Munro. New York: Academic Press.

Lane, D. J. (1991). 16S-23S rRNA sequencing. In *Nucleic Acid Techniques in*

Bacterial Systematics, pp. 125-175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Maidak, B.L., Cole, J. R., Lilburn, T.G., Parker, C.T. jr., Saxman, P.R., Farris, R.J., Garrity, G.M., Olsen, G.J., Schmidt, T.M. & Tiedje, J.M. (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173-174.

Pukall, R., Brambilla, E. & Stackebrandt, E. (1998). Automated fragment length analysis of fluorescently-labeled 16S rDNA after digestion with 4-base cutting restriction enzymes. *J Microbiol Methods* **32**, 55-64.

Pukall, R., Buntefuss, D., Fruhling, A., Rohde, M., Kroppenstedt, R. M., Burghardt, J., Lebaron, P., Bernard, L., & Stackebrandt, E. (1999). *Sulfitobacter mediterraneus* sp. nov., a new sulfite-oxidizing member of the alpha-Proteobacteria. *Int J Syst Bacteriol* **49,** 513-519.

Sasaki, E., Shimada, T., Osawa, R., Nishitani, Y., Spring, S and E. Lang (2005). Isolation of tannin-degrading bacteria isolated from feces of the Japanese large wood mouse, *Apodemus speciosus*, feeding on tannin rich acorns. *System Appl Microbiol* **28,** 358-365.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123-156.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglyca types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407-477.

Table 1:

Comparison of phenotypic properties of *Lactobacillus apodemi* DSM 16634^{T} and its nearest phylogenetic neighbors

Strain/species: 1, *Lactobacillus apodemi* DSM 16634^{T}; 2, *Lactobacillus animalis* JCM 5670^{T}; 3, *Lactobacillus murinus* JCM 1717^{T}.

Characteristic	**1**	**2**	**3**
Tannase activity	+	-	-
Mannitol	-	-	+
Trehalose	+	-	-
Gentiobiose	-	-	+

Legends to the figures:

Figure 1.

Neighbor-joining tree showing the next phylogenetic neighbors as determined for strain DSM 16634^{T}. Bootstrap values (percentage of 1000 replications) with greater than 70% confidence are shown at branching points. The scale bar represents 1 nucleotide substitutions per 100 sequence positions.

Figure 2:

Ribotype pattern obtained from ASB strains compared to those derived from type strains of *Lactobacillus animalis* and *Lactobacillus murinus*. Cluster analysis was performed by the unweighted pair group method with arithmetric averages (UPGMA) based on the Pearson correlation coefficient using an optimization coefficient of 1.2. Abbreviation VCA indicates a standard *Eco*RI batch.

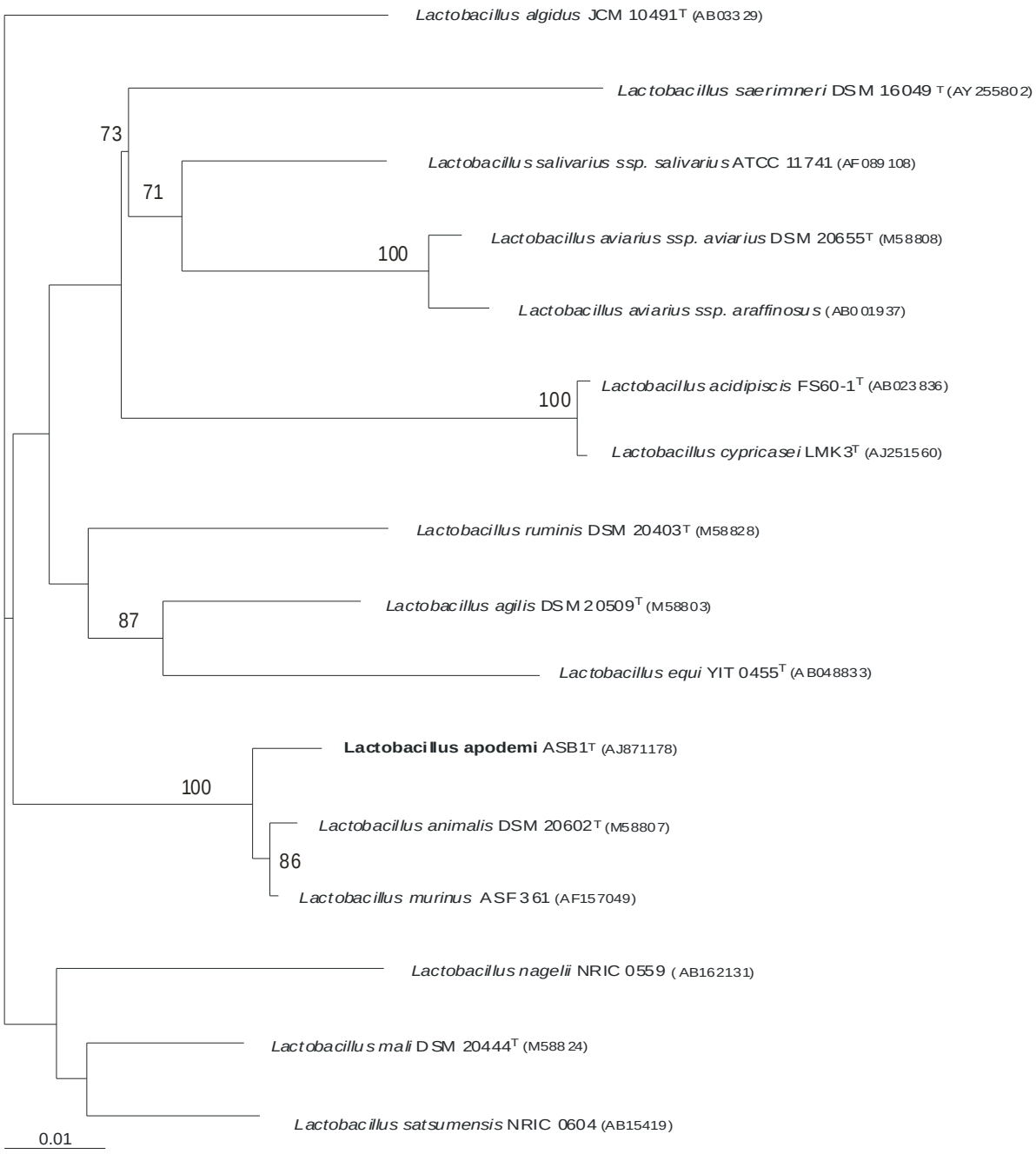

Lactobacillus algidus JCM 10491T (AB033329)
Lactobacillus saerimneri DSM 16049 T (AY255802)
73
Lactobacillus salivarius ssp. salivarius ATCC 11741 (AF089108)
71
Lactobacillus aviarius ssp. aviarius DSM 20655T (M58808)
100
Lactobacillus aviarius ssp. araffinosus (AB001937)
Lactobacillus acidipiscis FS60-1T (AB023836)
100
Lactobacillus cypricasei LMK3T (AJ251560)
Lactobacillus ruminis DSM 20403T (M58828)
Lactobacillus agilis DSM 20509T (M58803)
87
Lactobacillus equi YIT 0455T (AB048833)
Lactobacillus apodemi ASB1T (AJ871178)
100
Lactobacillus animalis DSM 20602T (M58807)
86
Lactobacillus murinus ASF361 (AF157049)
Lactobacillus nagelii NRIC 0559 (AB162131)
Lactobacillus mali DSM 20444T (M58824)
Lactobacillus satsumensis NRIC 0604 (AB15419)
0.01

Fig. 1

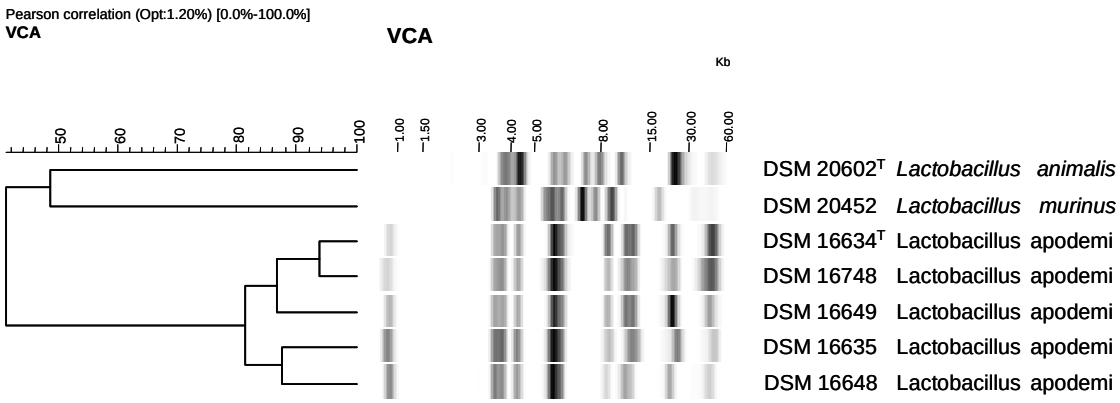
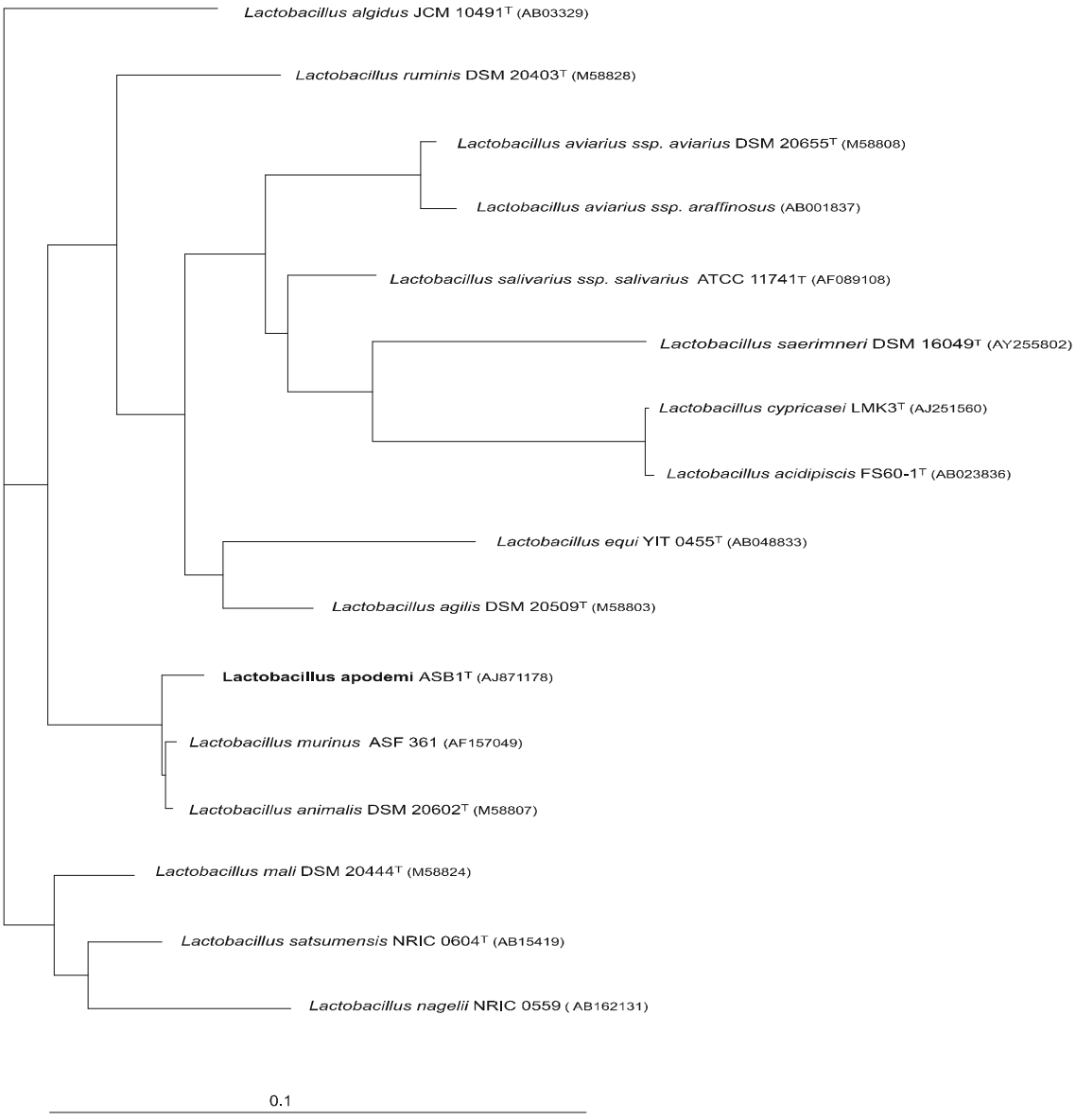
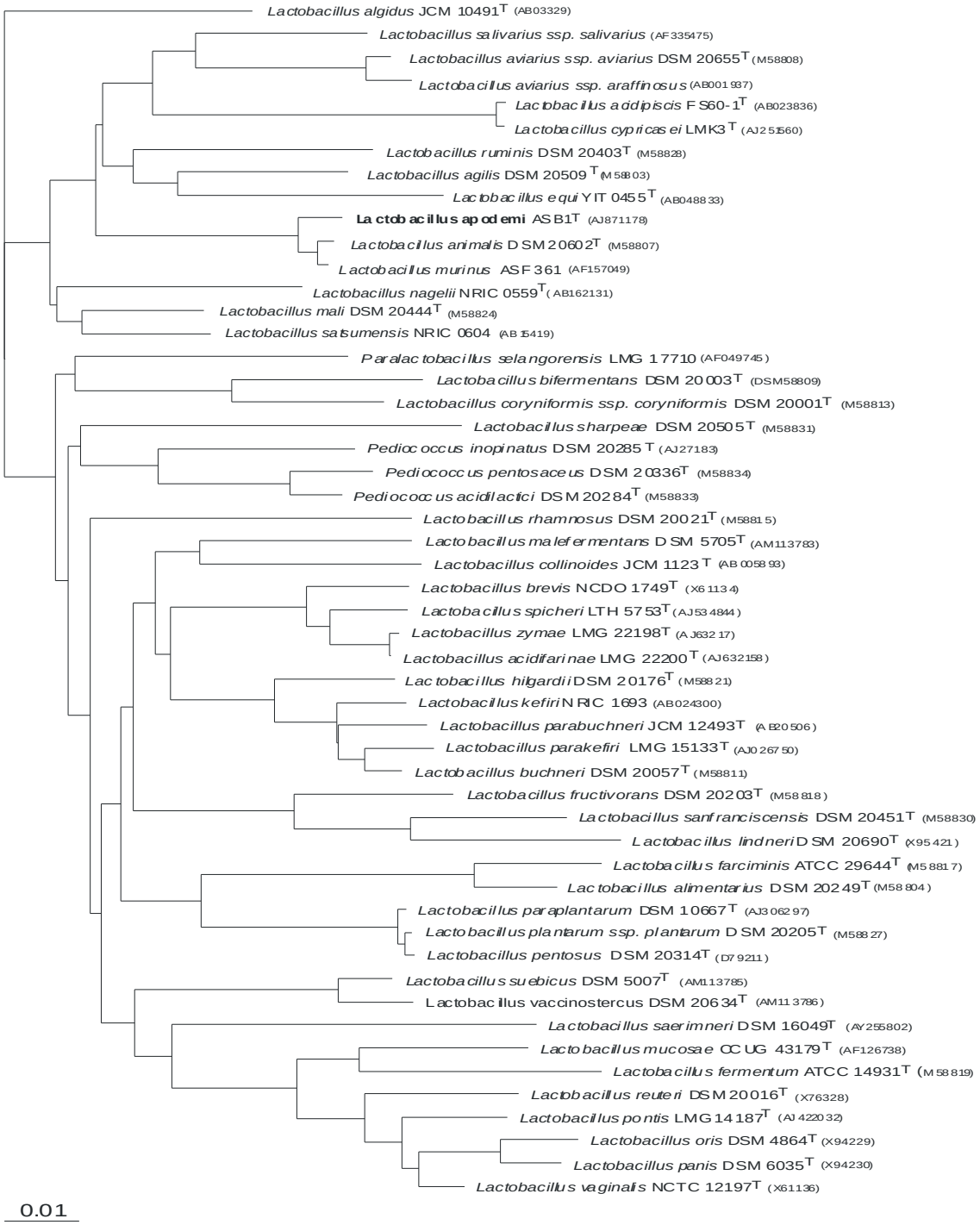

Pearson correlation (Opt:1.20%) [0.0%-100.0%]
VCA
VCA
Kb
50
60
70
80
90
100
1.00
1.50
3.00
4.00
5.00
8.00
15.00
30.00
60.00
DSM 20602T Lactobacillus animalis
DSM 20452 Lactobacillus murinus
DSM 16634T Lactobacillus apodemi
DSM 16748 Lactobacillus apodemi
DSM 16649 Lactobacillus apodemi
DSM 16635 Lactobacillus apodemi
DSM 16648 Lactobacillus apodemi

Fig.2

Supplementary data for reviewer's and editor:



DNAml maximum-likelihood based tree confirming the phlogenetic position of Lactobacillus apodemi



Neighbor-joining tree showing same clustering of L. apodemi between the L.

mali and L. salivarius group