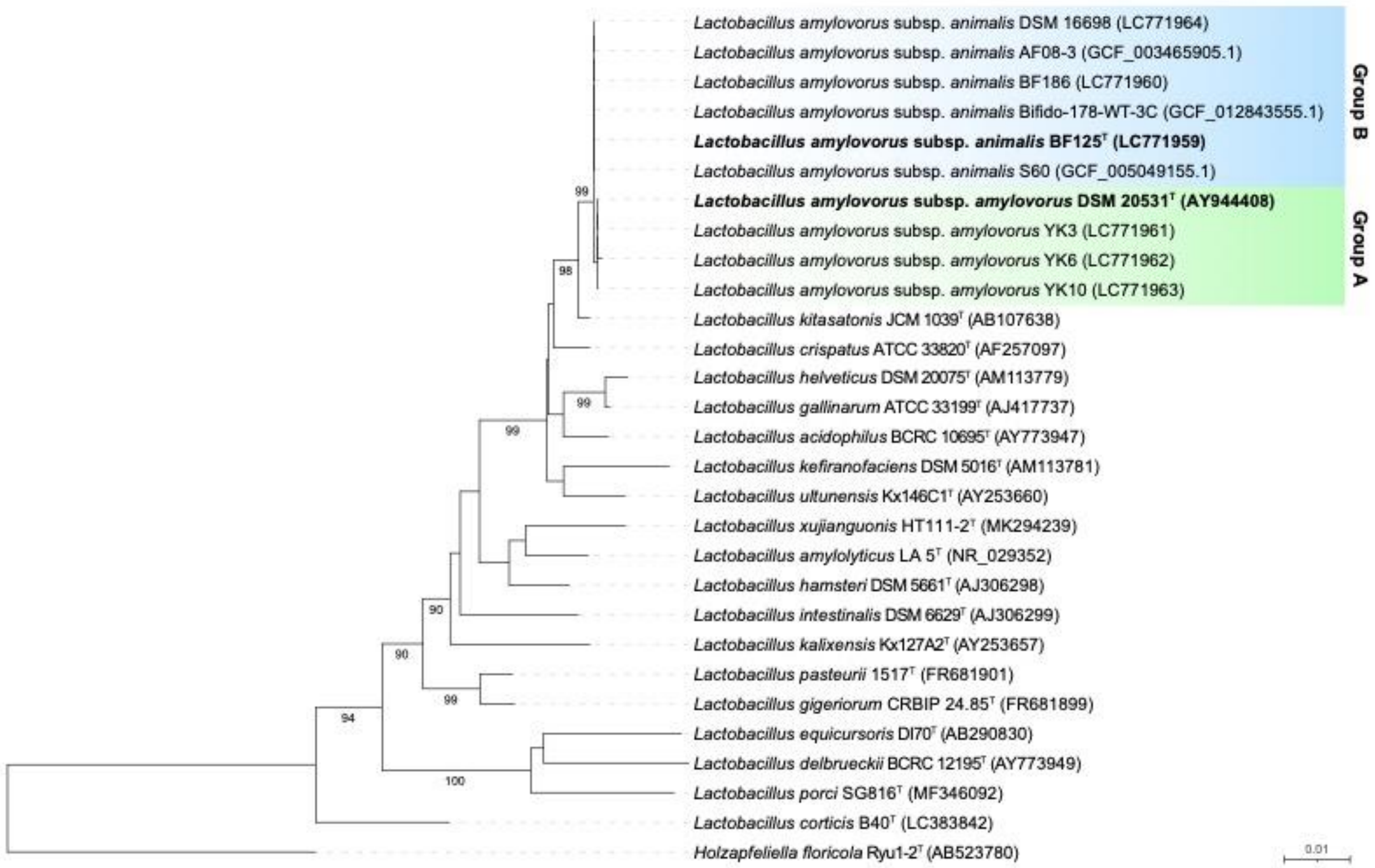
Recognition of two subspecies of *Lactobacillus amylovorus*, with proposal of *Lactobacillus amylovorus* subsp. *animalis* subsp. nov., isolated from bovine feces and *Lactobacillus amylovorus* subsp. *amylovorus*, and an emended description of *Lactobacillus amylovorus*

Author names

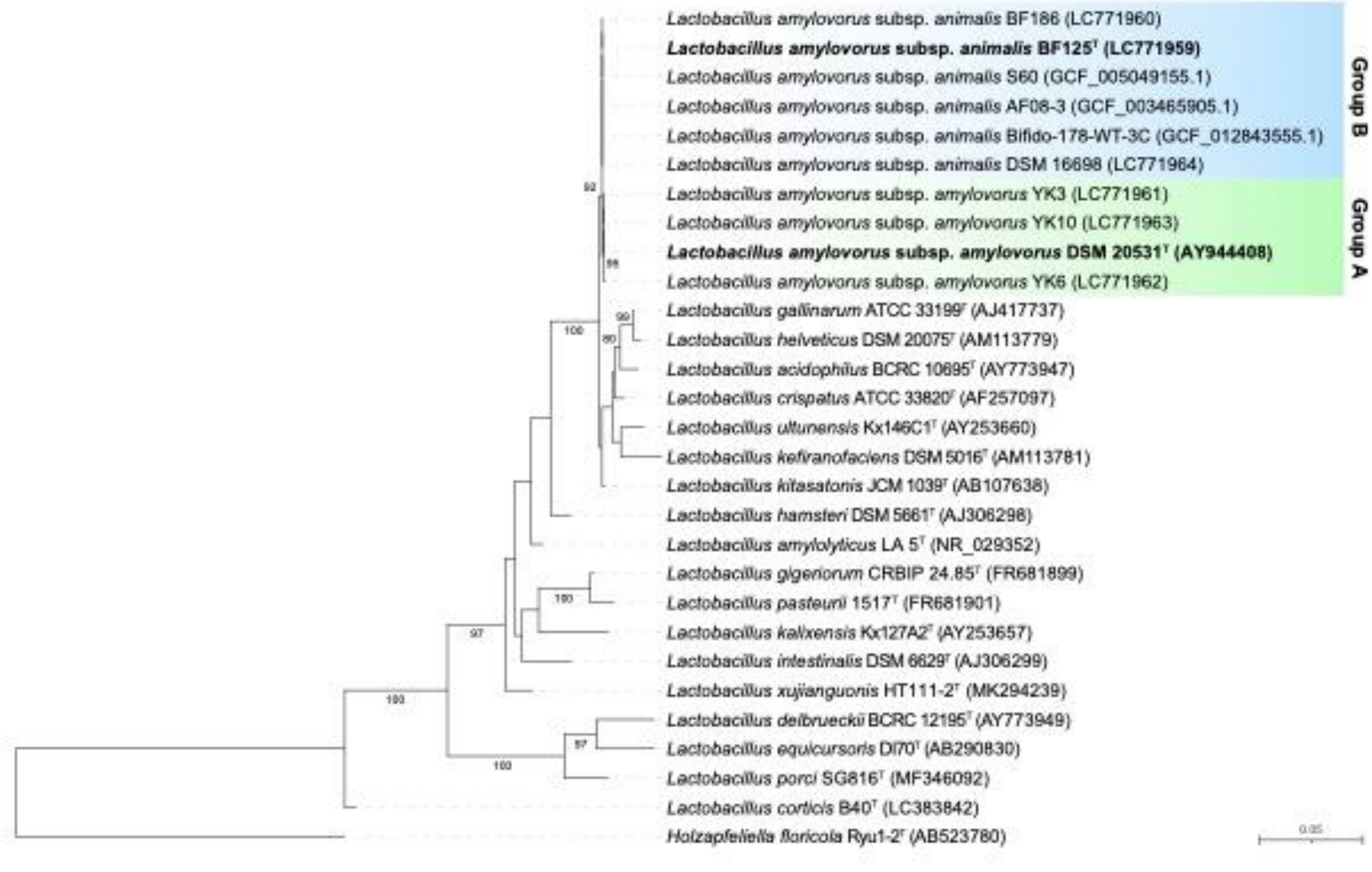
Kenji Yamane[1,2], Yasuhiro Tanizawa[3], Hisami Kobayashi[4], Tomomi Kamizono[4], Yoichiro Kojima[4], Hiroki Takagi[2] and Masanori Tohno[1,4,5]

Affiliation

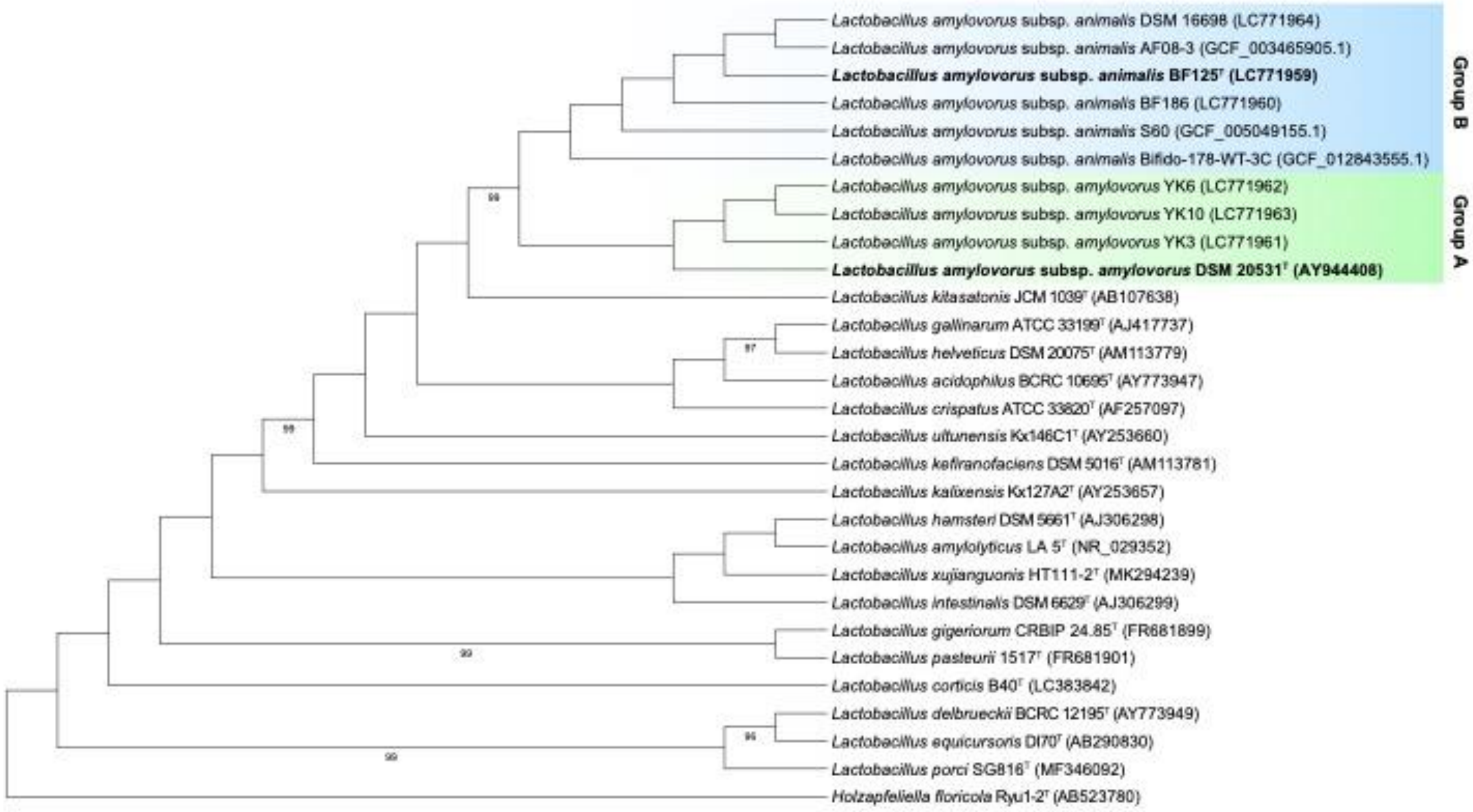
[1]Innovative Animal Production System, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan; [2]Nihon Shokuhin Kako Co., LTD, Research Institute, 30, Tajima, Fuji, Shizuoka 417-8530, Japan; [3]Department of Informatics, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan; [4]Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Nasushiobara, Tochigi 329-2793, Japan; [5]Research Center of Genetic Resources, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305-8602, Japan.



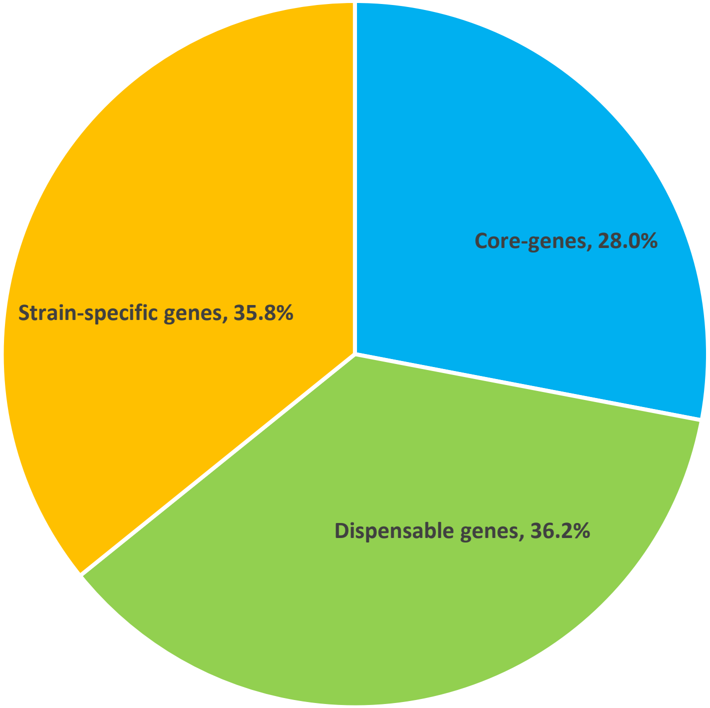
Supplementary Fig. S1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of five isolates BF125^{T}, BF186, YK3, YK6 and YK10 within the closely related species of the genus *Lactobacillus*. Strains DSM 20531^{T} (representative of group A) and BF125^{T} (representative of group B) are highlighted in boldface type. The sequence of *Holzapfeliella floricola* Ryu1-2^{T} is used as an outgroup. Bootstrap percentages greater than 80% (based on 1,000 replications) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.



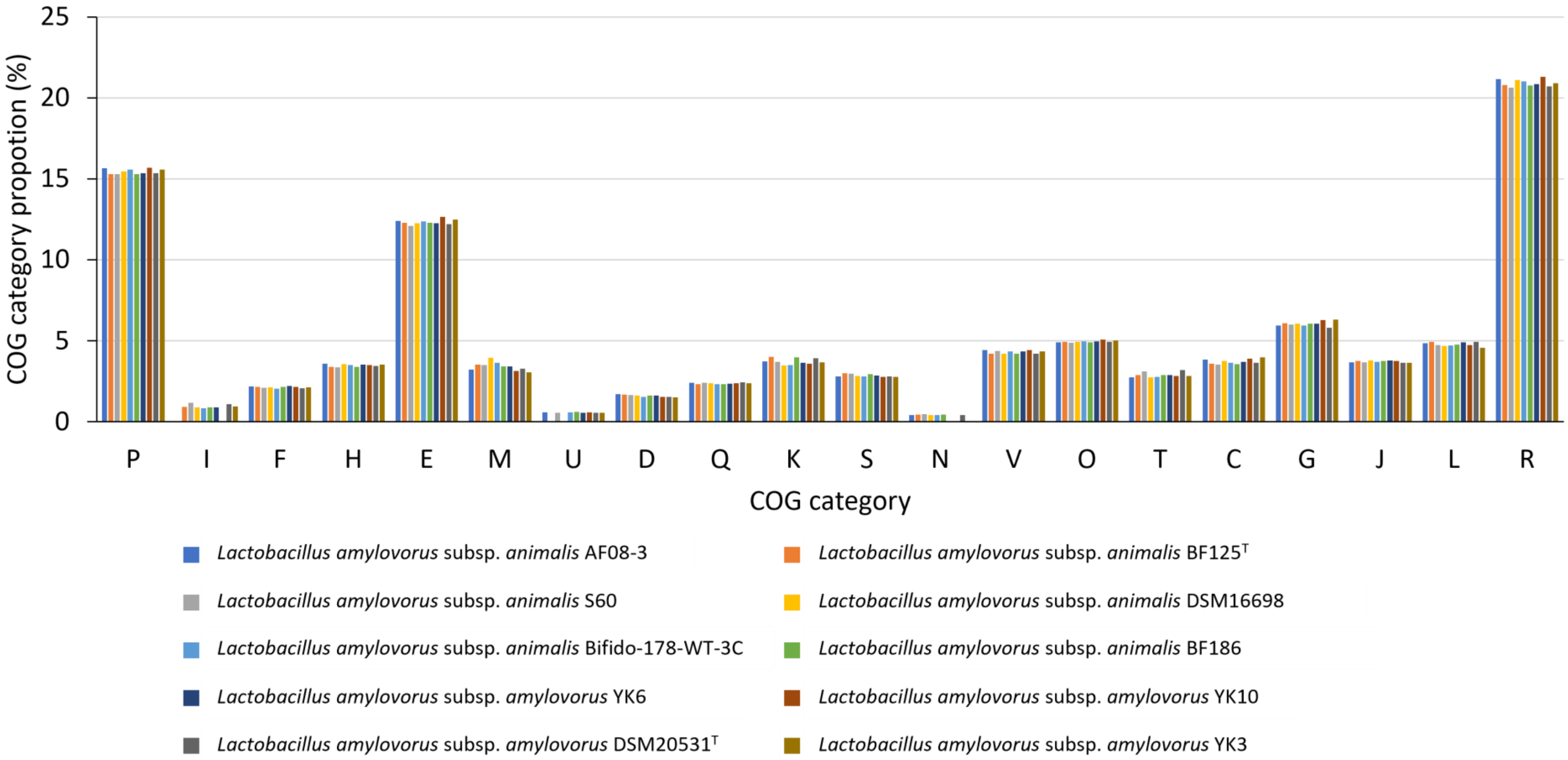
Supplementary Fig. S2. Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationship of five isolates BF125^T, BF186, YK3, YK6 and YK10 within the closely related species of the genus *Lactobacillus*. Strains DSM 20531^T (representative of group A) and BF125^T (representative of group B) are highlighted in boldface type. The sequence of *Holzapfeliella floricola* Ryu1-2^T is used as an outgroup. Bootstrap percentages greater than 80% (based on 1,000 replications) are shown at branch points. Bar, 0.05 substitutions per nucleotide position.



Supplementary Fig. S3. Maximum-parsimony tree based on 16S rRNA gene sequences showing the phylogenetic relationship of five isolates BF125^{T}, BF186, YK3, YK6 and YK10 within the closely related species of the genus *Lactobacillus*. Strains DSM 20531^{T} (representative of group A) and BF125^{T} (representative of group B) are highlighted in boldface type. The sequence of *Holzapfeliella floricola* Ryu1-2^{T} is used as an outgroup. Bootstrap percentages greater than 80% (based on 1,000 replications) are shown at branch points.



Supplementary Fig. S4. The pan-genome pie chart showing gene percentages of *L. amylovorus* in 10 strains visualized with the use of Pan-genome Explorer. Core genes accounted for 28.0%, dispensable genes for 36.2%, and strain specific genes for 35.8%.



Supplementary Fig. S5. Proportions of genes associated with each clusters of orthologous groups (COG) category of *L. amylovorus* in 10 strains. J, Translation, ribosomal structure and biogenesis; G, Carbohydrate transport and metabolism; ; U, Intracellular trafficking, secretion, and vesicular transport; D, Cell cycle control, cell division, chromosome partitioning; L, Replication, recombination and repair; I, Lipid transport and metabolism; Z, Cytoskeleton; M, Cell wall/membrane/envelope biogenesis; C, Energy production and conversion; Q, Secondary metabolites biosynthesis, transport and catabolism; B, Chromatin structure and dynamics; Y, Nuclear structure; W, Extracellar structures; N, Cell motility; R, General function prediction only; E, Amino acid transport and metabolism; K, Transcription; T, Signal transduction mechanisms; A, RNA processing and modification; O, Posttranslational modification, protein turnover, chaperones; S, Function unknown; F, Nucleotide transport and metabolism; ; H, Coenzyme transport and metabolism; P, Inorganic ion transport and metabolism; V, Defense mechanisms;

Table S1. Statistics of the genome sequences obtained in this study.

Strains	Number of contigs	Total length (bp)	N50 (bp)	G+C ratio (%)	Gap ratio (%)	Depth coverage	Number of coding sequences	Completeness (%)	Contamination (%)	INSDC accession number
BF125^T	33	1,984,003	133,636	37.8	0.0	298x	2,018	99.03	0.16	BTFR01
BF186	77	1,969,667	91,131	37.8	0.0	306x	1,981	99.03	0.16	BTFQ01
YK3	55	1,859,039	62,523	38.0	0.0	624x	1,903	98.92	0.0	BTFS01
YK6	32	1,885,203	117,657	38.0	0.0	617x	1,911	97.63	0.16	BTFT01
YK10	72	1,835,711	54,982	38.0	0.0	536x	1,855	98.92	0.0	BTFU01

Table S2. Cellular fatty acid compositions of *Lactobacillus amylovorus* subsp. *animalis* subsp. nov. and its closest phylogenetic relatives

Strains: 1, DSM 20531^T; 2, YK10; 3, BF125^T; 4, DSM 16698. Each value shown is expressed as a percentage of the total fatty acids. tr, trace (<0.5 %); -, not detected; DMA, dimethylacetal; ECL, equivalent chain-length. All tested fatty acid profiles were determined under identical conditions in the present study.

Fatty acid		**1**	**2**	**3**	**4**
Saturated:					
	$C_{10:0}$	tr	tr	tr	0.7
	$C_{12:0}$	2.3	tr	tr	tr
	$C_{14:0}$	**17.1**	tr	tr	tr
	$C_{16:0}$	**39.8**	**24.0**	**22.7**	**23.8**
	$C_{16:0}$ 3OH	tr	-	-	-
	$C_{18:0}$	2.0	3.1	3.4	3.3
	$C_{18:0}$ 12OH	1.7	2.1	1.7	3.4
Unsaturated:					
	$C_{16:1}$ ω7*c*	1.3	2.6	2.6	3.2
	$C_{18:1}$ ω9*c*	**14.0**	**25.9**	**31.3**	**27.6**
	$C_{18:1}$ ω7*c* DMA	1.2	2.0	2.0	2.5
Cyclopropane acids:					
	$C_{17:0}$ cyclo	-	-	-	-
	C_{19} cyclo 9,10	**11.2**	**24.0**	**16.7**	**18.1**
Summed features*:					
	5	tr	-	-	-
	8	-	-	tr	-
	10	7.0	**12.4**	**15.0**	**12.9**
	12	0.5	1.0	1.2	1.0
Unknown ECL 18.199		1.2	2.3	2.2	2.8

* Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 5: $C_{15:0}$ DMA/$C_{14:0}$ 3OH; summed feature 8: $C_{18:1}$ ω8*c*/$C_{17:2}$ at 16.801 ; summed feature 10: $C_{18:1}$ ω7*c*/ unknown 17.834 ; summed feature 12: unknown ECL 18.622/$C_{19:0}$ iso.