

# EMbaRC

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<b>Authors</b>	Christiane Bouchier
<b>Abstract</b>	The purpose of this task was to furnish sequences to improve identification of lactobacilli and to improve identification scheme for 4 genera of interest were selected by partners : <i>Lactobacillus</i> , <i>Propionibacterium</i> , <i>Acetobacter</i> and <i>Campylobacter</i> .
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## Abbreviation keys

CIP            Collection de l'Institut Pasteur

MLSA/T       Multi Locus Sequence Analysis/Typing

# 1 Background and Objectives

Identification and classification of bacterial strains depends on DNA sequences retrieved from standardized genetic markers. 16S rRNA gene sequence plays a central role in molecular studies of microbial diversity. However gene markers with higher substitution rates than that of the 16S rRNA gene are sometimes needed to improve the resolution of typing and/or subtyping strains.

## 2 Methods and Results

in this deliverable the aim was to apply to a collection of strains the MLSA schemes designed by partners, i) in particular for genera of interest *Lactobacillus*, *Acetobacter*, *Campylobacter*, ii) to validate the set of primers designed for *L. delbrueckii* in JRA2.3.2 iii) to confront partial MLST analysis to non molecular method (mass spectrometry analysis by MALDI-TOF) for delineation. **Sequences obtained in this subtask were deposited into the GenBank just before the submission of the manuscripts.**

### 2.1 MLSA approach

#### 2.1.1 *Lactobacillus* genus

The 16S rRNA gene sequences of type strains for 31 species of the genus *Lactobacillus* were determined according to the method described in the paper Clermont *et al.*, (2009). Three genes, heat-shock protein 60 (*hsp60*, 537 bases), phenylalanine tRNA synthetase alpha subunit (*pheS*, 453 bases) and RNA polymerase alpha subunit (*rpoA*, 666 bases), were used to perform MLSA studies on the nucleotide and amino acid levels. PCR and sequencing were performed using the primers and methods described by Goh *et al.*, (2000), Blaiotta *et al.*, (2008) and Naser *et al.*, (2007).

**See Annexe 1 of this document for the detailed list of sequences deposited into GenBank.**

#### 2.1.2 *Acetobacter* genus

A MLSA scheme was published for *Gluconacetobacter* and related taxa based on partial sequences of the genes *dnaK*, *groEL* and *rpoB* (Cleenwerck *et al.*, 2010). This scheme was proven useful for accurate identification of the acetic acid bacteria investigated. However, as a general tool for identification of acetic acid bacteria at large, its use was limited, because the genus *Acetobacter*, which is the largest one within the family of the acetic acid bacteria (*Acetobacteraceae*).

At present, the family Acetobacteraceae comprises 14 genera and ca 70 species, of which

*Acetobacter* (20 species), *Asaia* (8 species), *Gluconacetobacter* (17 species) and *Gluconobacter* (13 species) are the largest ones.

It was investigated whether the genes used for the existing MLSA scheme, were suitable for the *Acetobacter* species and the additional remaining species recently described. A set of ca. 100 *acetic acid bacteria* was selected and analysed. For 77 strains, partial sequences of the 3 genes could be obtained after optimization of the annealing temperatures for amplification (where necessary) and after design of a new primer for amplification of the *groEL* gene. The sequences of these 77 strains were added to the existing MLSA identification scheme, that was hereby successfully expanded with 17 *Acetobacter* species (43 strains), 5 *Asaia* species (10 strains), 1 *Gluconacetobacter* species (2 strains), 5 *Gluconobacter* species (13 strains) and 9 additional strains of already covered species.

The MLSA data of *Acetobacter*, generated in the subtask 2.1.2 are not yet published, but the data will be integrated in a paper draft on the description of a novel *Acetobacter* species ("*Acetobacter sicerae*") that will be submitted to the International Journal of Systematic and Evolutionary Microbiology in September 2013.

### **2.1.3 Campylobacter genus**

Several MLSA schemes were established and expanded for typing of *Campylobacter*. Housekeeping genes coding for proteins with higher substitution rates than that of the 16S rRNA gene were selected for MLST. Presently the genus *Campylobacter* comprises 30 species and subspecies, which can be differentiated by *hsp60* (*groEL*) gene sequencing. Progress in whole genome studies of *C. jejuni* was led to the application of additional housekeeping genes as potential markers to study the population biology of this species. Three housekeeping genes (*accD*, *thiC* and *recA*) were selected to improve the resolution of MLST for subtyping *C. jejuni*. Partial sequences of the 3 genes were performed for 24 *Campylobacter* strains. A dendrogram constructed after concatenation of partial sequences of the 3 genes was compared with those of obtained by the PstI-generated RiboPrint patterns or by MALDI-TOF MS. Contrary to PstI RiboPrinting but comparable to MALDI-TOF MS, MLSA was able to separate *C. coli* clearly from *C. jejuni* strains, but could even group the various *C. jejuni subsp. jejuni* strains into different subclusters.

The characterization of the *Campylobacter* strains was included into the following paper: P. Schumann and R. Pukall. (2013) The discriminatory power of ribotyping as automatable technique for differentiation of bacteria. Systematic and Applied Microbiology Volume 36, Issue 6, September 2013, Pages 369–375. Volume 36, Issue 6, September 2013, Pages 369–375]

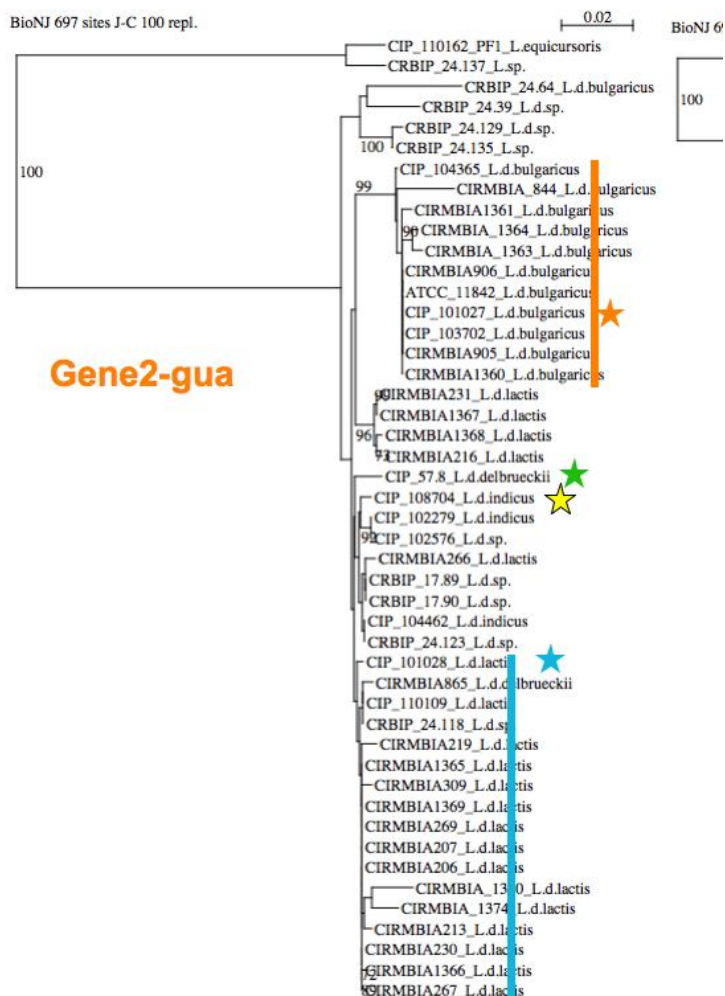
## 2.2 *Lactobacillus delbrueckii* subspecies differentiation by MLST approach

Housekeeping genes of *L. delbrueckii* provided in the annex 1 are known from the literature to be unable to separate subspecies in this cluster. Other candidates genes were thus used issued from the whole genome sequencing performed in the JRA 2.3.2 after comparative genomics (dnaC, guaB/guaA, mfd, uvrB/uvrA, ftsW, comEC, tfp, relA, addB, prtB, glyS, hrcA, smc, polA, ldb1525, mut2, thrC) . The sequence of these 17 genes was extracted from high-throughput sequencing data of *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp *lactis*, *L. delbrueckii* subsp. *indicus*, *L. equicursoris* or/and strain 66C. Then alignments of genes were performed with BLAST program (v2.2.5) using the 17 genes of *L. delbrueckii* subsp. *bulgaricus* ATCC 11842<sup>T</sup> strain. Sets of primers were designed to cover these 17 genes, two of them did not generate satisfying amplification and were suppressed from the identification scheme (gene 3, mfd and gene 16, mut) . **Primers (indicated table 1 below) were used for both PCR amplification and cycle sequencing of purified PCR products to develop a MLST scheme for 59 strains of *L. delbrueckii* (21 strains from CRBIP and 38 strains from INRA collections).** Interesting results were obtained in the separation of *L. delbrueckii* subspecies and a publication is up to be submitted. For each primer, a phylogenetic tree was elaborated with the obtained sequences (exemple below). The main results were: that all the genes of *L. delbrueckii bulgaricus* stood in the *L. delbrueckii lactis* cluster; new promising gene markers were found for subspecies and/or species description (ie. genes *gua*).

**Table 1** : primers used in the MLST Scheme of the 59 strains

Oligo Name	Sequence 5' to 3' (include modification codes if applicable)
gene1-dnaC-f	TGCCTTTCACGCTAAGGACT
gene1-dnaC-r	GACAACCTGCGACAGGGAAAT
gene2-gua-f	GATCCGTGACTTTGGCATCT
gene2-gua-r	CATGAAGCGGTCTTAGCAT
gene3-mfd-f-a	TGGAGGAATTGGAGGAAGTG
gene3-mfd-r-a	GGTTAAAAAGCTCCCGTTCC
gene3-mfd-f-b	CTCAAGCCTGGCGACTATGT
gene3-mfd-r-b	GCTTGAGCTTTTCCTTGTGG
gene4-uvr-f	GATGACCGGCTGGAAGAATA
gene4-uvr-r	GGTCCAAAACCTGGCTTGAGA
gene5-fts-f	TCGGCCAGTTCAGAAATCTT
gene5-fts-r	AGCCAACCTTCTTCGGCAATA
gene6-comEC-f-a	CCTGCTCTTTTGCTCAAGG
gene6-comEC-r-a	GATCCTCCGGCTCTTAAAGG
gene6-comEC-f-b	CAGGTCAATGTCTGCACGAT
gene6-comEC-r-b	GGCAGGTAAGCGACAAAGAG
gene7-dfp-f	ATGTGGGAAAATCCTGCTGT
gene7-dfp-r	GCCTAGCCAAAGTCAGTTCCG
gene8-relA-f	GACCATCAAGTGGGAAGTGG
gene8-relA-r	CCCCGTTTTTCAGCTTGTAG

gene9-addB-f	AAGAACCTGCACGTCCTGTC
gene9-addB-r	GCCACCTCGTCATAGATGGT
gene10-prtB-f	GTTTGGTGTTCCTGGTCTT
gene10-prtB-r	AAGGCTGATGGGGAAGTTCT
gene11-gly-f	AGGGACCAGTCTTCGTTCAA
gene11-gly-r	AGAGCCACAACCTGGCAAATC
gene12-hrcA-f	CAGCCGTTACCAGGCTAAAA
gene12-hrcA-r	AAGGTGTCCAGTGCACAAT
gene13-smc-f	GTACAGCTGGTCGGCTTGTT
gene13-smc-r	GCCAGTTAAATGGGCAAATC
gene14-polA-f-a	AACAGCCTGGTCCATGTAGG
gene14-polA-r-a	ATGGAATGAACGGGATCAA
gene14-polA-f-b	ACTCTTCCCCTTCCCGTAAA
gene14-polA-r-b	TCATCGACATGAAGGCTTTG
gene15-Ldb1525-f	CCTTGGTGGCAAACCTTGTTG
gene15-Ldb1525-r	AAGCCTTGGGCAAACAACT
gene16-mut-f	CACCCGAAATCAGTTTGAC
gene16-mut-r	TTGACATTTTGGTCCCAGGT
gene17-thrC-f	GCTTAATGAAGACGGCCTTG
gene17-thrC-r	TTGATTCGCCTTCGATTC



Exemple of phylogenetic tree obtained with the gene gua



Strains used for the validation of the primers

strain name	collection nb	isolation origin	Country/city*	year
<b><i>L. delbru. bulgaricus</i></b>	<b>CIP 101027</b>	<b>Bulgarian yogurt</b>		<b>1919</b>
<i>L. delbru. bulgaricus</i>	CIP 103702	Blood, leukemia		1993
<i>L. delbru. bulgaricus</i>	CIP 104365	Bioghurt	Belgium	1960
<i>L. delbru. bulgaricus</i>	CRBIP 24.64	Urine	France	1963
<i>L. delbru. bulgaricus</i>	<b>CIRM-BIA 658</b>	Bulgarian yogurt	NR	1919
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1358	yogurt, lactic yeast	Bulgarie	NR
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1359	lactic yeast	USA	<1974
<i>L. delbru. bulgaricus</i>	CIRM-BIA 879	Tarag (yogurt)	Mongolie/Oulan-Bator	1974
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1360	yogurt traditional of sheep milk	Grece/Vrysses	1987
<i>L. delbru. bulgaricus</i>	CIRM-BIA 844	yogurt traditional	Grece/Hania	1987
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1361	yogurt traditional	Grece/Heraclion	1991
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1362	yogurt (Bionic yogurt sweetened)	Indonesie/Bali	1990
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1363	fermented milk	France	1995
<i>L. delbru. bulgaricus</i>	CIRM-BIA 1364	fermented milk	France	2001
<i>L. delbru. bulgaricus</i>	CIRM-BIA 905	Cheese, boulette of Avesnes	France/Maroilles	1998
<i>L. delbru. bulgaricus</i>	CIRM-BIA 906	yak milk	Nepal	1996
<b><i>L. delbru. delbrueckii</i></b>	<b>CIP 57.8</b>	<b>Sour grain mash</b>		<b>1957</b>
<i>L. delbru. delbrueckii</i>	CIRM-BIA 865	most (grape, apple); must (hop, barley) wort	Royaume-Uni	NR
<i>L. delbru. delbrueckii</i>	<b>CIRM-BIA 675</b>	Distillery sour grain mash incubated at 45°C	USA	NR
<b><i>L. delbru. indicus</i></b>	<b>CIP 108704</b>	<b>Dairy fermented product</b>	<b>India</b>	<b>2005</b>
<i>L. delbru. indicus</i>	CIP 102279	Urine from human	France/Paris	1986
<i>L. delbru. indicus</i>	CIP 104462	Urine from human	France/Saint-Etienne	1965
<b><i>L. delbru. lactis</i></b>	<b>CIP 101028</b>	<b>Cheese, Emmental Swiss</b>		
<i>L. delbru. lactis</i>	CIP 110109	Urine from human	France/Nice	1976
<i>L. delbru. lactis</i>	CIP 203077	Urine from human	France/Charny	1982
<i>L. delbru. lactis</i>	CIP 203080	Urine from human	France/Paris	1982
<i>L. delbru. lactis</i>	CIRM-BIA 1365	Cheese starter	Finlande	NR
<i>L. delbru. lactis</i>	CIRM-BIA 269	lactic yeast traditional, Emmental	Finlande	early 1950
<i>L. delbru. lactis</i>	CIRM-BIA 206	NR	Finlande	NR
<i>L. delbru. lactis</i>	CIRM-BIA 219	NR	Finlande	NR
<i>L. delbru. lactis</i>	CIRM-BIA 230	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 231	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 265	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 266	<b>Kefir</b>	Russie/Moscou	1971
<i>L. delbru. lactis</i>	CIRM-BIA 267	lactic yeast, Emmental	Finlande/Helsinki	NR
<i>L. delbru. lactis</i>	CIRM-BIA 277	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 309	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 1366	Emmental	Finlande	1968
<i>L. delbru. lactis</i>	CIRM-BIA 1367	Cheese, Caciocavallo Ragusano	Italie	NR
<i>L. delbru. lactis</i>	CIRM-BIA 1368	lactic yeast commercial, Grana Padano	Italie/Novara	1986
<i>L. delbru. lactis</i>	CIRM-BIA 1369	Lactoserum of cheese dairy, Emmental	Suisse/Berne	1974
<i>L. delbru. lactis</i>	CIRM-BIA 1370	Lactoserum of cheese dairy, Emmental	Suisse/Berne	1974
<i>L. delbru. lactis</i>	CIRM-BIA 1371	NR	Finlande	NR
<i>L. delbru. lactis</i>	CIRM-BIA 1372	yak milk	Nepal	1996
<i>L. delbru. lactis</i>	CIRM-BIA 304	lactic yeast traditional, Gruyere of Comte	France/Franche-Comte	1960
<i>L. delbru. lactis</i>	CIRM-BIA 207	lactic yeast traditional, Gruyere of Comte	France/Mignovillard	1963
<i>L. delbru. lactis</i>	CIRM-BIA 213	lactic yeast traditional, Gruyere of Comte	France/Lemuy	1959
<i>L. delbru. lactis</i>	CIRM-BIA 216	lactic yeast traditional, Gruyere of Comte	France/Monnet la Ville	1964
<i>L. delbru. lactis</i>	CIRM-BIA 1374	recuite (fabrication Comte)	France/Jura	1993
<i>L. delbru. lactis</i>	CIRM-BIA 1375	Morbier from farm	France/Jura	1993
<b><i>L. equicursoris</i></b>	<b>CIP 110162</b>	<b>horse feces</b>	<b>Ikee stable/Japan</b>	<b>2006</b>
<i>L. sp.</i>	CRBIP 24.137	Urine from human	France	1966
<i>L. delbru.</i>	CIP 102576	Urine from human	France/Avignon	1986
<i>L. delbru.</i>	CRBIP 17.90	Urine from human	France/Seine-et-Marne	~ 2004
<i>L. delbru.</i>	CRBIP 17.89	Urine from human	France/Yonne	~ 2005
<i>L. delbru.</i>	CRBIP 24.123	Urine	France	1965
<i>L. delbru.</i>	CRBIP 24.39	Urine	France	1960
<i>L. delbru.</i>	CRBIP 24.135	Urine	France	1967
<i>L. delbru.</i>	CRBIP 24.118	intestinal strain	France	1967

### **2.3 *Propionibacterium* identification by partial MLST and MALDI-TOF analysis**

For two genera of *Propionibacterium*, the partial sequencing of genes coding for proteins (Adk, RpoB, GyrB, and RecA)(Dalmaso et al., Multi locus séquence typing of *Propionibacterium freudenreichii* IJFM 2011. ...) in addition to a mass spectrometric analysis of proteins (MALDI-TOF, MS) were applied to 20 clinical strains of *Propionibacterium acnes* and 20 dairy strains of *Propionibacterium freudenreichii* ( methods described in the D21.3). The degree of molecular diversity of these strains at species level was determined and ranking of the strains compared to the one obtain by MALDI-TOF, MS in order to evaluate possible application of mass spectrometry for the *Propionibacterium* species delineation. Results allowed the validation of this triple-locus sequence analysis (genes *adK*, *rpoB*, *gyrB*) versus to the MLST analyses usually used which incorporated a greater number of genes.

Results of the work were presented at WFCC meeting in September 2010 and at HPA mass spectrometry meeting in London in April 2011. A scientific publication is in progress.

## **Conclusion**

All the sequences presented in the annex 1 are in Genbank and can be used by scientists involved in lactobacilli identification. .in parallel, MLST shemes involving new genes were tested to improve the differentiation within *Lactobacillus*, *Acetobacter*, *Propionibacterium* and *Campylobacter* genus.

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215.

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## Annexes

### 1. 1- Molecular gene markers presents in the GenBank database for *Lactobacillus* species (type strains) Partial 16S rRNA gene

1. *Lactobacillus helveticus* partial 16S rRNA gene, type strain DSM 20075T - 1,516 bp linear DNA  
FR683085.1 GI:323669184
2. *Lactobacillus gallinarum* partial 16S rRNA gene, type strain ATCC 33199T - 1,518 bp linear DNA  
FR683086.1 GI:323669185
3. *Lactobacillus acidophilus* partial 16S rRNA gene, strain VPI 6032 - 1,531 bp linear DNA  
FR683087.1 GI:323669186
4. *Lactobacillus crispatus* partial 16S rRNA gene, type strain DSM 20584T - 1,516 bp linear DNA  
FR683088.1 GI:323669187
5. *Lactobacillus amylovorus* partial 16S rRNA gene, type strain DSM 20531T - 1,516 bp linear DNA  
FR683089.1 GI:323669188
6. *Lactobacillus kitasatonis* partial 16S rRNA gene, type strain DSM 16761T - 1,445 bp linear DNA  
FR683090.1 GI:323669189
7. *Lactobacillus ultunensis* partial 16S rRNA gene, type strain CCUG 48460T - 1,502 bp linear DNA  
FR683091.1 GI:323669190
8. *Lactobacillus kefiranofaciens* subsp. *kefiranofaciens* partial 16S rRNA gene, type strain ATCC 43761T - 1,549 bp linear DNA  
FR683092.1 GI:323669191
9. *Lactobacillus kefiranofaciens* subsp. *kefirgranum* partial 16S rRNA gene, type strain JCM 8572T - 1,448 bp linear DNA  
FR683093.1 GI:323669192
10. *Lactobacillus hamsteri* partial 16S rRNA gene, type strain JCM 6256T - 1,494 bp linear DNA  
FR683094.1 GI:323669193
11. *Lactobacillus amyolyticus* partial 16S rRNA gene, type strain CCUG 39901T - 1,443 bp linear DNA  
FR683095.1 GI:323669194
12. *Lactobacillus kalixensis* partial 16S rRNA gene, type strain CCUG 48459T - 1,513 bp linear DNA  
FR683096.1 GI:323669195
13. *Lactobacillus intestinalis* partial 16S rRNA gene, strain TH4 - 1,448 bp linear DNA  
FR683097.1 GI:323669196
14. *Lactobacillus jensenii* partial 16S rRNA gene, strain Gasser 62G - 1,500 bp linear DNA  
FR683098.1 GI:323669197
15. *Lactobacillus delbrueckii* subsp. *delbrueckii* partial 16S rRNA gene, type strain NCIB 8130T - 1,514 bp linear DNA  
FR683100.1 GI:323669199
16. *Lactobacillus delbrueckii* subsp. *indicus* partial 16S rRNA gene, type strain NCC 725T - 1,473 bp linear DNA  
FR683101.1 GI:323669200
17. *Lactobacillus delbrueckii* subsp. *bulgaricus* partial 16S rRNA gene, type strain ATCC 11842T - 1,475 bp linear DNA  
FR683102.1 GI:323669201
18. *Lactobacillus delbrueckii* subsp. *lactis* partial 16S rRNA gene, type strain DSM 20072T - 1,445 bp linear DNA

FR683103.1 GI:323669202

19. *Lactobacillus gigeriorum* partial 16S rRNA gene, strain CRBIP 24.85 - 1,448 bp linear DNA

FR681899.1 GI:313848509

20. *Lactobacillus sp.* 66c partial 16S rRNA gene, strain 66c - 1,617 bp linear DNA

FR681900.1 GI:313848510

21. *Lactobacillus acetotolerans* partial 16S rRNA gene, type strain JCM 3825T - 1,503 bp linear DNA

FR683099.1 GI:323669198

22. *Lactobacillus pasteurii* partial 16S rRNA gene, strain 1517 - 1,426 bp linear DNA

FR681901.1 GI:313848511

23. *Lactobacillus hominis* partial 16S rRNA gene, strain CRBIP 24.179 - 1,718 bp linear DNA

FR681902.1 GI:313848512

24. *Lactobacillus amylophilus* partial 16S rRNA gene, type strain CIP 102988T - 1,530 bp linear DNA

HE573913.1 GI:341599787

25. *Lactobacillus gasseri* partial 16S rRNA gene, type strain CIP 102991T - 1,529 bp linear DNA

HE573914.1 GI:341599788

26. *Lactobacillus johnsonii* partial 16S rRNA gene, type strain CIP 103620T - 1,543 bp linear DNA

HE573915.1 GI:341599789

27. *Lactobacillus iners* partial 16S rRNA gene, type strain CIP 109878T - 1,560 bp linear DNA

HE573916.1 GI:341599790

28. *Lactobacillus amylophilus* partial 16S rRNA gene, type strain CIP 109878T - 1,560 bp linear DNA

HE573917.1 GI:341599791

29. *Lactobacillus taiwanensis* partial 16S rRNA gene, type strain CIP 110030T - 1,509 bp linear DNA

HE573918.1 GI:341599792

30. *Lactobacillus plantarum subsp. plantarum* partial 16S rRNA gene, type strain CIP 103151T - 1,527 bp linear DNA

FR775893.1 GI:341599557

31. *Lactobacillus equicursoris* partial 16S rRNA gene, type strain CIP 110162T - 1,548 bp linear DNA

FR775894.1 GI:341599558

## 2. Partial hsp60 gene

1. *Lactobacillus equicursoris* partial hsp60 gene for heat shock protein 60, type strain CIP 110162T 598 bp linear DNA

FR775917.1 GI:341599591

2. *Lactobacillus sp.* LEM 202 partial hsp60 gene for heat shock protein 60, type strain CIP 24.85T 593 bp linear DNA

FR775914.1 GI:341599585

3. *Lactobacillus plantarum subsp. plantarum* partial hsp60 gene for heat shock protein 60, type strain CIP 103151T - 622 bp linear DNA

FR775922.1 GI:341599601

4. *Lactobacillus delbrueckii subsp. delbrueckii* partial hsp60 gene for heat shock protein 60, type strain CIP 57.8T - 599 bp linear DNA

FR775921.1 GI:341599599  
5. *Lactobacillus delbrueckii* subsp. *lactis* partial hsp60 gene for heat shock protein 60, type strain CIP 101028T - 599 bp linear DNA  
FR775920.1 GI:341599597  
6. *Lactobacillus delbrueckii* subsp. *indicus* partial hsp60 gene for heat shock protein 60, type strain CIP 108704T - 607 bp linear DNA  
FR775919.1 GI:341599595  
7. *Lactobacillus delbrueckii* subsp. *bulgaricus* partial hsp60 gene for heat shock protein 60, type strain CIP 101027T - 604 bp linear DNA  
FR775918.1 GI:341599593  
8. *Lactobacillus jensenii* partial hsp60 gene for heat shock protein 60, type strain CIP 69.17T  
607 bp linear DNA  
FR775916.1 GI:341599589  
9. *Lactobacillus hamsteri* partial hsp60 gene for heat shock protein 60, type strain CIP 103365T  
607 bp linear DNA  
FR775915.1 GI:341599587  
10. *Lactobacillus acetotolerans* partial hsp60 gene for heat shock protein 60, type strain CIP 103180T - 600 bp linear DNA  
FR775913.1 GI:341599583  
11. *Lactobacillus crispatus* partial hsp60 gene for heat shock protein 60, type strain CIP 102990T  
604 bp linear DNA  
FR775912.1 GI:341599581  
12. *Lactobacillus ultunensis* partial hsp60 gene for heat shock protein 60, type strain CIP 109908T  
602 bp linear DNA  
FR775911.1 GI:341599579  
13. *Lactobacillus kefiranofaciens* partial hsp60 gene for heat shock protein 60, type strain CIP 104241T - 603 bp linear DNA  
FR775910.1 GI:341599577  
14. *Lactobacillus kefiranofaciens* subsp. *kefirgranum* partial hsp60 gene for heat shock protein 60, type strain CIP 104241T - 607 bp linear DNA  
FR775909.1 GI:341599575  
15. *Lactobacillus amylovorus* partial hsp60 gene for heat shock protein 60, type strain CIP 102989T - 601 bp linear DNA  
FR775908.1 GI:341599573  
16. *Lactobacillus kitasatonis* partial hsp60 gene for heat shock protein 60, type strain CIP 108218T  
607 bp linear DNA  
FR775907.1 GI:341599571  
17. *Lactobacillus acidophilus* partial hsp60 gene for heat shock protein 60, type strain CIP 76.13T  
606 bp linear DNA  
FR775906.1 GI:341599569  
18. *Lactobacillus kalixensis* partial hsp60 gene for heat shock protein 60, type strain CIP 109909T  
542 bp linear DNA  
FR775905.1 GI:341599567  
19. *Lactobacillus intestinalis* partial hsp60 gene for heat shock protein 60, type strain CIP 104793T  
606 bp linear DNA  
FR775904.1 GI:341599565  
20. *Lactobacillus gallinarum* partial hsp60 gene for heat shock protein 60, type strain CIP 103611T

601 bp linear DNA

FR775903.1 GI:341599563

21. *Lactobacillus helveticus* partial hsp60 gene for heat shock protein 60, type strain CIP 103146T

606 bp linear DNA

FR775902.1 GI:341599561

22. *Lactobacillus amylolyticus* partial hsp60 gene for heat shock protein 60, type strain CIP 107338T - 607 bp linear DNA

FR775901.1 GI:341599559

23. *Lactobacillus taiwanensis* partial hsp60 gene for heat shock protein, type strain CIP 110030T

593 bp linear DNA

HE573896.1 GI:341599753

24. *Lactobacillus johnsonii* partial hsp60 gene for heat shock protein, type strain CIP 103620T

607 bp linear DNA

HE573895.1 GI:341599751

25. *Lactobacillus gasserii* partial hsp60 gene for heat shock protein, type strain CIP 102991T

602 bp linear DNA

HE573894.1 GI:341599749

26. *Lactobacillus iners* partial hsp60 gene for heat shock protein, type strain CIP 105923T

606 bp linear DNA

HE573893.1 GI:341599747

27. *Lactobacillus amylotrophicus* partial hsp60 gene for heat shock protein, type strain CIP 109878T - 604 bp linear DNA

HE573892.1 GI:341599745

28. *Lactobacillus amylophilus* partial hsp60 gene for heat shock protein, type strain CIP 102988T

607 bp linear DNA

HE573891.1 GI:341599743

### 3. Partial rpoA gene

1. *Lactobacillus equicursoris* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 110162T - 712 bp linear DNA

FR775960.1 GI:341599677

2. *Lactobacillus* sp. LEM 202 partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 24.85T - 721 bp linear DNA

FR775956.1 GI:341599669

3. *Lactobacillus plantarum* subsp. *plantarum* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103151T - 705 bp linear DNA

FR775965.1 GI:341599687

4. *Lactobacillus delbrueckii* subsp. *delbrueckii* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 57.8T - 722 bp linear DNA

FR775964.1 GI:341599685

5. *Lactobacillus delbrueckii* subsp. *lactis* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 101028T - 722 bp linear DNA

FR775963.1 GI:341599683

6. *Lactobacillus delbrueckii* subsp. *bulgaricus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 101027T - 709 bp linear DNA

FR775962.1 GI:341599681

7. *Lactobacillus delbrueckii* subsp. *indicus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 108704T - 712 bp linear DNA

FR775961.1 GI:341599679

8. *Lactobacillus acetotolerans* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103180T - 740 bp linear DNA

FR775959.1 GI:341599675

9. *Lactobacillus ultunensis* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 109908T - 730 bp linear DNA

FR775958.1 GI:341599673

10. *Lactobacillus jensenii* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 69.17T - 712 bp linear DNA

FR775957.1 GI:341599671

11. *Lactobacillus kalixensis* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 109909T - 696 bp linear DNA

FR775955.1 GI:341599667

12. *Lactobacillus hamsteri* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103365T - 693 bp linear DNA

FR775954.1 GI:341599665

13. *Lactobacillus amylolyticus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 107338T - 688 bp linear DNA

FR775953.1 GI:341599663

14. *Lactobacillus intestinalis* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 104793T - 699 bp linear DNA

FR775952.1 GI:341599661

15. *Lactobacillus amylovorus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 102989T -707 bp linear DNA

FR775951.1 GI:341599659

16. *Lactobacillus kitasatonis* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 108218T - 700 bp linear DNA

FR775950.1 GI:341599657

17. *Lactobacillus helveticus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103146T - 704 bp linear DNA

FR775949.1 GI:341599655

18. *Lactobacillus gallinarum* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103611T - 711 bp linear DNA

FR775948.1 GI:341599653

19. *Lactobacillus acidophilus* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 76.13T - 729 bp linear DNA

FR775947.1 GI:341599651

20. *Lactobacillus kefiranofaciens* subsp. *kefiranofaciens* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 103307T - 671 bp linear DNA

FR775946.1 GI:341599649

21. *Lactobacillus kefiranofaciens* subsp. *kefirgranum* partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 104241T - 683 bp linear DNA

FR775945.1 GI:341599647

22. *Lactobacillus crispatus* partial poA gene for partial rpoA gene for RNA polymerase, alpha subunit, type strain CIP 102990T - 694 bp linear DNA

FR775944.1 GI:341599645



23. *Lactobacillus gasseri* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 102991T - 727 bp linear DNA  
HE573912.1 GI:341599785
24. *Lactobacillus johnsonii* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 103620T - 723 bp linear DNA  
HE573911.1 GI:341599783
25. *Lactobacillus taiwanensis* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 110030T - 697 bp linear DNA  
HE573910.1 GI:341599781
26. *Lactobacillus iners* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 105923T - 706 bp linear DNA  
HE573909.1 GI:341599779
27. *Lactobacillus amylophilus* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 102988T - 688 bp linear DNA  
HE573908.1 GI:341599777
28. *Lactobacillus amylotrophicus* partial rpoA gene for RNA polymerase alpha subunit, type strain CIP 109878T - 717 bp linear DNA  
HE573907.1 GI:341599775

#### 4. Partial pheS gene

1. *Lactobacillus equicursoris* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 110162T - 466 bp linear DNA  
FR775939.1 GI:341599635
2. *Lactobacillus* sp. LEM 202 partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 24.85T - 460 bp linear DNA  
FR775937.1 GI:341599631
3. *Lactobacillus kitasatonis* partial pheS gene for partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 108218T - 463 bp linear DNA  
FR775923.1 GI:341599603
4. *Lactobacillus plantarum* subsp. *plantarum* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103151T - 462 bp linear DNA  
FR775943.1 GI:341599643
5. *Lactobacillus delbrueckii* subsp. *bulgaricus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 101027T - 467 bp linear DNA  
FR775942.1 GI:341599641
6. *Lactobacillus delbrueckii* subsp. *indicus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 108704T - 470 bp linear DNA  
FR775941.1 GI:341599639
7. *Lactobacillus delbrueckii* subsp. *lactis* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 101028T - 477 bp linear DNA  
FR775940.1 GI:341599637
8. *Lactobacillus jensenii* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 69.17T - 462 bp linear DNA  
FR775938.1 GI:341599633
9. *Lactobacillus kalixensis* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 109909T - 464 bp linear DNA  
FR775936.1 GI:341599629

10. *Lactobacillus amyolyticus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 107338T - 464 bp linear DNA  
FR775935.1 GI:341599627
11. *Lactobacillus intestinalis* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 104793T - 461 bp linear DNA  
FR775934.1 GI:341599625
12. *Lactobacillus kefiranofaciens subsp. kefiranofaciens* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103307T - 462 bp linear DNA  
FR775933.1 GI:341599623
13. *Lactobacillus kefiranofaciens subsp. kefirgranum* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 104241T - 462 bp linear DNA  
FR775932.1 GI:341599621
14. *Lactobacillus hamsteri* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103365T - 462 bp linear DNA  
FR775931.1 GI:341599619
15. *Lactobacillus crispatus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 102990T - 464 bp linear DNA  
FR775930.1 GI:341599617
16. *Lactobacillus helveticus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103146T - 459 bp linear DNA  
FR775929.1 GI:341599615
17. *Lactobacillus gallinarum* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103611T - 464 bp linear DNA  
FR775928.1 GI:341599613
18. *Lactobacillus acetotolerans* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 103180T - 461 bp linear DNA  
FR775927.1 GI:341599611
19. *Lactobacillus acidophilus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 76.13T - 463 bp linear DNA  
FR775926.1 GI:341599609
20. *Lactobacillus ultunensis* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 109908T - 464 bp linear DNA  
FR775925.1 GI:341599607
21. *Lactobacillus amylovorus* partial pheS gene for phenylalanine tRNA synthetase, alpha subunit, type strain CIP 102989T - 465 bp linear DNA  
FR775924.1 GI:341599605
22. *Lactobacillus amylophilus* partial pheS gene for phenylalanine tRNA synthetase alpha subunit, type strain CIP 109878T - 464 bp linear DNA  
HE573904.1 GI:341599769
23. *Lactobacillus amylophilus* partial pheS gene for phenylalanine tRNA synthetase alpha subunit, type strain CIP 102988T - 466 bp linear DNA  
HE573903.1 GI:341599767
24. *Lactobacillus taiwanensis* partial pheS gene for phenylalanine tRNA synthetase alpha subunit, type strain CIP 110030T - 463 bp linear DNA  
HE573902.1 GI:341599765
25. *Lactobacillus gasseri* partial pheS gene for phenylalanine tRNA synthetase alpha subunit, type strain CIP 102991T - 462 bp linear DNA  
HE573901.1 GI:341599763
26. *Lactobacillus johnsonii* partial pheS gene for phenylalanine tRNA synthetase alpha subunit,

type strain CIP 103620T - 462 bp linear DNA

HE573900.1 GI:341599761

27. *Lactobacillus iners* partial pheS gene for phenylalanine tRNA synthetase alpha subunit, type strain CIP 105923T - 458 bp linear DNA

HE573899.1 GI:341599759

## Significance of this deliverable

*D.JR2.1.2: integration into CRBIP and GenBank database of molecular markers sequences for prokaryotes*

More than 110 sequences of molecular markers for lactobacilli species were entered in Genbank as well as new molecular markers for acetobacter, and campylobacter This represents a significant progress for the identification of about 80 species.

In parallel, a new MLST approach (from genes provided in the D. JRA2.3.2) applied to *L. delbrueckii* gave promising results for the identification at a subspecies level.