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Abstract	Bacterial strains must be deposited in public culture collections to make them available for comparisons and re-examinations of published information. As most strains had been deposited in more than one culture collection, the identity of equivalent strains from different collections needs to be confirmed by efficient high-throughput techniques. MALDI-TOF mass spectrometry was used in the present study to examine 451 equivalent strains representing 81 species and 15 subspecies of the genus *Lactobacillus*, 25 species and 3 subspecies of the family "*Leuconostocaceae*" (containing the genera *Leuconostoc, Fructobacillus, Oenococcus* and *Weissella*) and 22 species and 2 subspecies of the genus *Pseudomonas* from DSMZ, IP and UVEG-CECT. 12 strains were detected which were either misclassified or the mass spectra of which were mislabelled in databases. The study allowed not only the examination of the identity of strains maintained in the participating collections, but offered also the possibility to compare and to harmonize the quality of spectra generated by different laboratories and to perform a quality control of existing databases of spectra.
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Contents

Abbreviation key

ISSR	Inter Simple Sequence Repeats
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-of-Flight
MS	Mass Spectrometry
VNTR	Variable Number Tandem Repeats

1 Background and Objectives

Strains must be deposited in public culture collections to make them available for comparisons and re-examinations of published information. This applies in particular to the authentication of taxonomic descriptions by type strains which must be deposited for this purpose in at least two recognized collections in different countries. Due to this regulation and due to the exchange of strains between collections, a strain is usually maintained in more than one culture collection. While recently deposited strains are examined according to quality assurance criteria under inclusion of modern identification approaches, the identity of strains deposited in the "pre-molecular era" remains to be confirmed by current techniques. Even under conditions of strict quality management it can not be excluded that strains could be mixed up or get contaminated and the question is legitimate whether "equivalent" strains from different collections can always be considered identical. For the examination of the identity of equivalent strains from different collections automated high trough-put techniques of sufficient taxonomic resolution are needed. In a recent study (Cundell *et al.*, 2010) the identity of quality control strains from different culture collections was investigated by using RiboPrinting, MALDI-TOF MS, 16S rRNA gene sequencing, rep-PCR, ISSR, VNTR, serotyping and physiological tests. In preparation of the sub-task JRA 2.1.3 (Application of a mass-spectroscopic method (MALDI-TOF) to the identification of prokaryotes) of the EMbaRC project, 47 type strains acquired by IP between 1950 and 1960 which have not been exchanged with DSMZ were chosen as examples and were compared to the corresponding type strains maintained at the DSMZ on the basis of their MALDI-TOF mass spectra. This preliminary study revealed that MALDI-TOF MS is an appropriate and efficient tool to detect misclassified strains in collection holdings (Schumann *et al.*, 2009).

The participants of the sub-task JRA2.1.3 of the EMbaRC project (DSMZ, UVEG-CECT, IP, UGent, INRA) decided at the meeting in Paris (October 2009, Minutes Paris) to focus on the genus *Lactobacillus* and the not yet validly named family "*Leuconostocaceae*" (containing the genera *Leuconostoc, Fructobacillus, Oenococcus* and *Weissella*, http://www.bacterio.cict.fr/) in order to examine the identity of equivalent strains deposited in their collections. Several strains of the genus *Pseudomonas* held at UVEG-CECT and DSMZ were additionally compared on the basis of their MALDI-TOF mass spectra. The project was hampered by the fact that two of the participants (UGent and INRA) are using ABI mass spectrometers while the other participants are equipped with Bruker instruments and that both types of instruments generate spectra of incompatible formats. For this reason this part of Deliverable D.JRA2.1.3 reports only about the results obtained with Bruker instruments. In order to compare collection holdings between the partners working with Bruker and ABI instruments, UGent will send ethanol-suspended biomass of selected LMG strains to DSMZ for measurement in parallel to this carried out with the ABI instrument at UGent by using the same protocol for sample preparation. The Bruker-format spectra of LMG strains recorded at

DSMZ will be compared by the BioTyper software with those of equivalent DSM, CIP and CECT strains. UGent will try to compare spectra obtained at INRA with another model of ABI instrument with those of equivalent LMG strains by the BioNumerics software. In the case that a common BioNumerics dendrogram of spectra of equivalent LMG and INRA strains can be calculated, its topology will be compared with this of the BioTyper dendrogram. Due to the problem of incompatibility of ABI and Bruker formatted spectra, the part of the deliverable D.JRA2.1.3 concerning comparison of collection holdings between the “Bruker-“ and “ABI-communities” can be submitted not before November 2010.

DSMZ, UVEG-CECT, IP and UGent compiled lists of equivalent *Lactobacillus*, “*Leuconostocaceae”* and *Pseudomonas* strains which were held in their collections (Tables 1, 2 and 3). The focus of this study was on the type strains but some other strains which are often less intensively characterized despite of their practical relevance were included as well. DSMZ and UVEG-CECT recorded the spectra of their strains on own mass spectrometers. IP measured 44 % of the spectra with an in-house mass spectrometer but cooperated with DSMZ for the rest of the CIP strains included in this study due to technical problems with this instrument.

2 Methods

2.1 Strains

A total of 98 strains of the family “*Leuconostocaceae*” (containing the genera *Leuconostoc, Fructobacillus, Oenococcus* and *Weissella*, http://www.bacterio.cict.fr/) and 309 strains of the genus *Lactobacillus* from DSMZ, IP and UVEG-CECT as well as 44 strains of the genus *Pseudomonas* from DSMZ and UVEG-CECT were studied. Strains were cultivated on semisolid agar media under cultivation conditions that ensure the optimal growth of the strains. Only few strains from UVEG-CECT were cultivated in liquid cultures (indicated by “t”) for comparison purposes (see 3.4).

2.2 Sample preparation

Sample preparation for matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) protein analysis was carried out according to the ethanol / formic acid extraction protocol recommended by Bruker Daltonics (http://clinprot.bdal.de; Tóth *et al*., 2008): ca. 10 mg biomass (5-10 mg for IP) from agar cultures was suspended first in 300 µl water by careful mixing. Then the suspension was mixed with 900 µl ethanol. The biomass was collected by centrifugation and the pellet was re-suspended in 50 µl 70% formic acid. The suspension was mixed carefully with 50 µl acetonitrile. Immediately after centrifugation the supernatant was removed and aliquots of 1.5 µl (0.5-1 µl for IP) were placed on each spot of a stainless-steel target plate. After air-drying 1.5 µl matrix solution (1 µl for IP) (saturated solution of α-cyano-hydroxy-cinnaminic acid in 50 % aqueous acetonitrile containing 2.5 % trifluoro acetic acid) per spot were applied.

2.3 Instrumentation

Instrumentation at DSMZ

Microflex L20 mass spectrometer (Bruker Daltonics).

Instrumentation at IP

Autoflex™ speed mass spectrometer (Bruker Daltonics).

Instrumentation at UVEG-CECT

Reflex IV mass spectrometer (Bruker Daltonics).

The instruments are equipped with a N_2 laser. All spectra were recorded in linear, positive ion mode. The acceleration voltage was 20 kV. Spectra were collected as a sum of 250 (240 for UVEG-CECT) shots across a spot. A mass range of 2000–20,000 *m/z* was used for analysis.

2.4 Spectra

It was aimed at the representation of each strain by 18 spectra. The vast majority of strains is represented by 12 to 46 (DSM 20054) spectra. Few main spectra of DSMZ strains (indicated by the extension "_DSM" of the strain number) were taken for comparison from the database of the Bruker BioTyper 2.0. These entries were averaged from at least 24 original spectra per strain. The original spectra recorded by Bruker are not available for the participants of sub-task JRA 2.1.3 (The following *Lactobacillus* spectra recorded at IP were also sent to Bruker as regulated by a contract: *Lactobacillus acidifarinae* CIP 108702, *Lactobacillus apodemi* CIP 108913, *Lactobacillus avarius* subsp. *araffinosus* CIP 103145, *Lactobacillus catenaformis* CIP 104817, *Lactobacillus coleohominis* CIP 106820, *Lactobacillus farciminis* CIP 103136, *Lactobacillus frumenti* CIP 106922, *Lactobacillus gallinarum* CIP 103611, *Lactobacillus iners* CIP 105923, *Lactobacillus manihotivorans* CIP 105851, *Lactobacillus psittaci* CIP 106492, *Lactobacillus rhamnosus* CIP A157, *Lactobacillus zymae* CIP 108703).

The following strains are represented by less than 12 spectra:

Genus *Lactobacillus*

11 spectra: CECT 4149, CECT 4140

10 spectra: CECT 903

9 spectra: CECT 5923

8 spectra: CIP 76.13, CIP 102989, CIP 108913, CIP 103145, CIP 103144, CIP 109926, CIP 109883, CIP 103137, CIP 104817, CIP 103008, CIP 57.8, CIP 109834, CIP 103136, CIP 109567, CIP 102991, CIP 109616, CIP 109885, CIP 103146, CIP 103007, CIP 103141, CIP 109892, CIP 105923, CIP 103307, CIP 102983, CIP 110039, CIP 109911, CIP 105162, CIP 107868, CIP 109568, CIP 109872, CIP 109886, CIP 109884, CIP 108703, DSM 20017

7 spectra: CIP 103620

5 spectra: CIP 106922

4 spectra: CIP A157, CIP 101887, CIP 103133, CIP 101027, DSM 20076

3 spectra: CIP 103042, CIP 104818, CIP 103152, CIP 106820, CIP 57.6

2 spectra: CIP 103023, CIP 109909, CIP 103155, CIP 109498

Family “*Leuconostocaceae*”

8 spectra: CIP 102985, CIP 109309, CIP 103319, CIP 103315, CIP 102422, CIP 103009, CIP 102423, CIP 102305, CIP 103316, CIP 109430, CIP 107376, CECT 5747

Genus *Pseudomonas*

8 spectra: DSM 11124

The following strains were cultivated at IP and their biomasses were sent to DSMZ where the sample preparation was carried out and the spectra were recorded:

Genus *Lactobacillus*

CIP 103595, CIP 103597, CIP 101264, CIP 102988, CIP 103474, CIP 105137, CIP 102806, CIP 103131, CIP 102990, CIP 54.4, CIP 101028, CIP 107499, CIP 53163, CIP 76.19, CIP 103143, CIP 103368, CIP 103918, CIP 105422, CIP 103139, CIP 103140, CIP 103699, CIP 104494, CIP 104793, CIP 109151, CIP 103024, CIP 103134, CIP 103156, CIP 103180, CIP 103367, CIP 103411, CIP 103611, CIP 104241, CIP 104242, CIP 105164, CIP 105851, CIP 107019, CIP 107647, CIP 107931, CIP 108394, CIP 108702, CIP 109172, CIP 109261, CIP 109926, CIP 53.61

Family “*Leuconostocaceae*”

CIP 78.59, CIP 103172, CIP 103005, CIP 102809, CIP 102978, CIP 102810, CIP 103325, CIP 103405, CIP 103889, CIP 108011, CIP 110051, CIP 108081, CIP 108761

2.5 Evaluation of Spectra

The Flexanalysis software (Bruker) was used for baseline subtraction, smoothing of spectra and mass labelling of peaks. Spectra with maximal peak intensities of 2000 or less were deleted.

Dendrograms were calculated with the software BioTyper version 1.1 (Bruker) because this version turned out to be better suited for handling of large data sets than version 2.0. The following options were selected for construction of dendrograms:

Distance measure: Correlation

Linkage: Average

Max. Number of top level nodes: All

Color threshold in relation to 1000: 600

Score oriented dendrogram: Activated

Score threshold value for a single organism: 300

Score threshold value for related organisma: 0

The BioTyper version 2.0 (Bruker) with its integrated identification database consisting of 3370 entries was used for identification of strains. The following options were selected for identification of main spectra of strains:

Frequency threshold for spectra adjusting: 50

Frequency threshold for score calculation: 5

Max. mass error of the raw spectrum: 2000

Des. Mass Tolerance of the Adjusted Spectrum: 250

Furthermore Accepted Mass Tolerance of a Peak: 600

Use Individual Analyse Parameters of MSP: deactivated

Parameter of the Intensity Correction Function: 0.25

3 Results and Discussion

In the course of the present study on identification of accurate and misclassified or mislabelled strains in consortium holdings of DSMZ, IP and UVEG-CECT, 451 equivalent strains of 81 species and 15 subspecies of the genus *Lactobacillus*, 25 species and 3 subspecies of the family "*Leuconostocaceae*" and 22 species and 2 subspecies of the genus *Pseudomonas* have been examined by MALDI-TOF MS. The vast majority of the equivalent strains (439) clustered together. However, their spectra branched at rather different distance levels within these clusters. Measurements in duplicate of identical strains with the same instrument under identical conditions for cultivation and sample preparation may result in distance levels as small as 40 (DSM 20004^T, Fig. 2), 60 (DSM 18933^T, Fig. 4) and 100 (DSM 14060^T, Fig. 2). Spectra recorded with different instruments in different laboratories under standardized conditions for cultivation and sample preparation for the same strain may show a distance level of 130 (DSM 16043T, measured by Bruker and DSMZ, Fig. 4). Spectra obtained under identical conditions for sample preparation and measurement of equivalent strains cultivated in different laboratories may show distance levels of 30 (DSM 20555^T and CIP 101340^T, Fig. 6). Even smaller distance levels were found for spectra of equivalent strains measured in different laboratories, e.g. 20 (DSM 17259^T and CECT 7010^T, Fig. 9). On the other hand, spectra of identical strains measured in duplicate may also show distance levels as large as 500 (DSM 19519^T, not shown; CECT 4131^T, Fig. 1) or even appear unrelated (CECT 4133 ^{T}, Fig. 1) when the cultivation was done either on agar plates or in liquid cultures. The distance levels of equivalent strains are strongly affected from the quality of the spectra (e.g. too low intensity of peaks, insufficient mass calibration and peak resolution), especially when only few spectra are available for averaging (see CIP 109909^T, Fig. 4). The quality of spectra seems to vary between taxa. In the lack of experiences in the definition of a threshold value for maximal distance levels of equivalent strains, it was tried to correlate the branching of the dendrograms with the identification criteria of the BioTyper 2.0 by using examples (Table 4). The reliability of an identification hit of the BioTyper 2.0 is evaluated by so-called “Log Score Values” which may range from 0.000 to 3.000:

2.300 ... 3.000	highly probable species identification
2.000 ... 2.299	secure genus identification, probable species identification
1.700 ... 1.999	probable genus identification
0.000 ... 1.699	no reliable identification

The distance levels within clusters vary with the number of spectra selected for the construction of the dendrograms. For reasons of lucidity, the members of the genus *Lactobacillus* were distributed over 6 dendrograms (Figs. 1 to 6), each with 41 to 75 strains, and the members of the family "*Leuconostocaceae*" over 2 dendrograms (Figs. 7 and 8) with 50 and 51 strains. Table 4 demonstrates that a (highly) probable species identification is possible even for spectra which show a distance level of ca. 300. As even some species (see below) and subspecies (see e.g. *Lactobacillus delbrueckii* subspp., *Lactobacillus paracasei* subspp., *Lactobacillus sakei* subspp.) can not be differentiated unambiguously, the taxonomic resolution of MALDI-TOF MS appears to be insufficient for the resolution at the strains level. It is worth mentioning that the differentiation of *Lactobacillus* subspecies by MALDI-TOF MS is in general possible but requires additional techniques as shotgun mass mapping (Schmidt *et al*., 2009). However, in order to detect misclassified strains in culture collections, the identification and differentiation at the species level by MALDI-TOF MS is already a promising approach. For this reason the search for misclassified strains will be focused in the following on those strains which branch by spectral distance levels > 300 from their equivalent partners and which might belong to another species.

3.1 Differentiation of species

While most of the species investigated were very well separated, the type strains of the following species of the genera *Lactobacillus*, *Leuconostoc, Weissella* and *Pseudomonas* can not be separated by MALDI-TOF MS at distance levels > 300 and confusion of equivalent strains within these clusters could hardly be detected by MALDI-TOF MS:

Cluster 1: *Lactobacillus acidophilus / Lactobacillus amylovorus / Lactobacillus gallinarum*
Cluster 2: *Lactobacillus hordei / Lactobacillus mali*
Cluster 3: *Lactobacillus pentosus / Lactobacillus plantarum*
Cluster 4: *Lactobacillus parakefiri / Lactobacillus parabuchneri / Lactobacillus buchneri*
Cluster 5: *Lactobacillus apodemi / Lactobacillus murinus / Lactobacillus animalis*
Cluster 6: *Lactobacillus kimchii / Lactobacillus bobalius*
Cluster 7: *Lactobacillus gasseri / Lactobacillus johnsonii*
Cluster 8: *Lactobacillus fructivorans / Lactobacillus homohiochii*
Cluster 9: *Lactobacillus rhamnosus / Lactobacillus casei*
Cluster 10: *Leuconostoc inhae / Leuconostoc gelidum / Leuconostoc gasicomitatum*
Cluster 11: *Leuconostoc mesenteroides / Leuconostoc pseudomesenteroides*
Cluster 12: *Weissella confusa* / *Weissella cibaria*
Cluster 13: *Pseudomonas pseudoalcaligenes* / *Pseudomonas oleovorans*
Cluster 14: *Pseudomonas marginalis / Pseudomonas fluorescens*
Cluster 15: *Pseudomonas corrugata / Pseudomonas chlororaphis*

The species of clusters 1 to 12 and 14 fall also into common clusters of the phylogenetic tree defined by 16S rRNA gene sequence analysis (www.arb-silva.de/living-tree). Though representing different 16S rRNA clusters (www.arb-silva.de/living-tree), the pair *Pseudomonas corrugata* / *Pseudomonas chlororaphis* (cluster 15) shows a 16S rRNA gene sequence similarity of 99.0% (EzTaxon Server, Chun *et al.*, 2007). The high similarity of the mass spectra of the type strains of *Pseudomonas pseudoalcaligenes* and *Pseudomonas oleovorans* (cluster 13) cannot be explained by a high 16S rRNA sequence similarity (96.4%; EzTaxon Server, Chun *et al.*, 2007).

3.2 Misclassified strains and confused spectra

The spectra of the following strains did not cluster with the spectrum / spectra of the respective equivalent strains:

Lactobacillus casei CIP 103131

The spectrum fell into the cluster of *Lactobacillus plantarum* (Fig. 5). It was identified as *Lactobacillus plantarum* (log score value to DSM 1055_DSM, 2.002) by the BioTyper 2.0. The identity of strain *Lactobacillus casei* CIP 103131 was examined by 16S rRNA gene sequence analysis, its affiliation to *Lactobacillus plantarum* was confirmed and the name in the catalogue was changed accordingly (Dominique Clermont, July 30, 2010).

***Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T 2009_11_06**

The spectrum branched deeply from this of the equivalent strains (Fig. 3). A new lyophilized ampoule was opened and a new spectrum *Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T was recorded which matched the cluster of *Lactobacillus delbrueckii* subsp. *delbrueckii* (Fig. 3). Obviously the spectrum *Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T 2009_11_06 had been confused within the spectra database of DSMZ.

***Lactobacillus delbrueckii* subsp. *lactis* DSM 20076**

The spectrum fell into the cluster of *Lactobacillus gasseri* (Fig. 3). A new lyophilized ampoule was opened and a new spectrum *Lactobacillus delbrueckii* subsp. *lactis* DSM 20076 2010_07_23 was recorded which matched the cluster of *Lactobacillus delbrueckii* (Fig. 3) including the entry *Lactobacillus delbrueckii* subsp. *lactis* DSM 20076_DSM of the BioTyper 2.0 database. Obviously the spectrum of *Lactobacillus delbrueckii* subsp. *lactis* DSM 20076 had been mixed up with this of a *Lactobacillus gasseri* strain in the spectra database of DSMZ.

***Lactobacillus homohiochii* DSM 20351 and CECT 983 240610**

The spectra of both equivalent strains cluster closely together but far outside of the cluster of the 3 equivalent type strains of *Lactobacillus homohiochii* (Fig. 4). Within the tree of all *Lactobacillus* strains studied, both spectra fell into the cluster of *Lactobacillus acetotolerans* (not shown). The spectra of strains DSM 20351 and CECT 983 240610 were identified as those of *Lactobacillus acetotolerans* (log score values, 2.448 to DSM 20749 and 2.305 to CECT 4019, respectively) by using the database of the BioTyper 2.0 supplemented with *Lactobacillus* spectra recorded in the course of sub-task JRA2.1.3. The RiboPrint pattern of strain DSM 20351 shows a high similarity to this of *Lactobacillus acetotolerans* DSM 20749^T (not shown). It is suggested to examine the identity of strains DSM 20351 and CECT 983 by 16S rRNA gene sequence analysis.

***Lactobacillus lindneri* CECT 5957^T 300310**

The spectrum fell into the cluster of the species *Lactobacillus bobalius* (Fig. 2) (cluster 6 *Lactobacillus kimchii / Lactobacillus bobalius*, see above) and was identified as *Lactobacillus kimchii* (log score value to DSM 13961T_DSM, 1.999) by the BioTyper 2.0. Strain CECT 5957^T

showed a 16S rRNA gene sequence similarity of 99.6% to *Lactobacillus bobalius* (Amparo Ruvira Garrigues, email dated May 24, 2010). A new lyophilized ampoule was opened and a new spectrum *Lactobacillus lindneri* CECT 5957^T 210510 was recorded which matched the cluster of *Lactobacillus lindneri* (Fig. 4)

Lactobacillus malefermentans **CECT 5956 150610**

The spectrum fell into the cluster of *Lactobacillus plantarum* (Fig. 5). It was identified as *Lactobacillus plantarum* (log score value to DSM 20246_DSM, 2.413) by the BioTyper 2.0. It is suggested to examine the identity of strain *Lactobacillus malefermentans* CECT 5956 by 16S rRNA gene sequence analysis.

Lactobacillus ruminis **CECT 4061^T** 300310

The spectrum fell into the cluster of the species *Lactobacillus animalis* (Fig. 1) and was identified as *Lactobacillus animalis* (log score value to DSM 20602T_DSM, 2.051) by the BioTyper 2.0. Strain CECT 4061^T showed 99.9% 16S rRNA gene sequence similarity to the type strain of *L. animalis.* A new ampoule was opened but the recorded spectrum was the same (Amparo Ruvira Garrigues, email dated May 24, 2010). The strain needs to be replaced in the culture collection.

Leuconostoc fallax **CIP 104855^T**

The spectrum fell into the cluster of the species *Lactobacillus hilgardii* (Fig. 4) and was identified as *Lactobacillus hilgardii* (log score value of 2.250 to DSM 20051_DSM) by the BioTyper 2.0. Strain CIP 104855^T showed the highest 16S rRNA gene sequence similarity to *Lactobacillus hilgardii* (Dominique Clermont, email dated June 17, 2010). The strain needs to be replaced in the culture collection.

Pseudomonas alcaligenes **CECT 929^T**

The spectrum branched deeply from this of *Pseudomonas alcaligenes* DSM 50342^T (Fig. 9) and was identified as *Stenotrophomonas maltophilia* (log score value of 2.499 to ATCC 13637 THL) by the BioTyper 2.0. Strain CECT 929^T showed 99.9% 16S rRNA gene sequence similarity to *Stenotrophomonas maltophilia* (Amparo Ruvira Garrigues, email dated July 19, 2010). The strain needs to be replaced in the culture collection.

Weissella confusa **DSM 20196^T**

The spectrum fell into the cluster of *Weissella minor* (Fig. 8). A new lyophilized ampoule was opened and a new spectrum *Weissella confusa* DSM 20196^T 2010_06_16 was recorded which matched the cluster of *Weissella confusa* and *Weissella cibaria* (Cluster 12, see above and Fig. 8).

Weissella minor **DSM 20014^T**

The spectrum fell into the cluster of *Weissella confusa* and *Weissella cibaria* (Cluster 12, see above and Fig. 8). A new lyophilized ampoule was opened and a new spectrum *Weissella minor* DSM 20014^T 2010_06_16 was recorded which matched the cluster of *Weissella minor* (Fig. 8). Obviously the spectra of *Weissella confusa* DSM 20196^T and *Weissella minor* DSM 20014^T had been mixed up in the spectra database of DSMZ.

3.3 Unclassified strains

Lactobacillus **sp. DSM 6265 and CECT 5918 170610**

Both equivalent strains fell into the cluster of *Lactobacillus brevis* (Fig. 2) and were identified as *Lactobacillus brevis* (log score values of DSM 6265 and CECT 5918 170610, 2.244 and 2.418, respectively, to DSM 1267_DSM) by the BioTyper 2.0. This identification appears to be rather reliable due to the high log score values but needs to be confirmed by comparison with the respective type strain using independent methods before classifying both strains as members of the species *Lactobacillus brevis*.

Lactobacillus **sp. DSM 20182 and CECT 5927 170610**

Both equivalent strains fell into the cluster of *Lactobacillus farciminis* (Fig. 3) and were identified as *Lactobacillus farciminis* (log score values of DSM 20182 and CECT 5927 170610, 1.901 and 1.944, respectively, to 103136 CIP) by the BioTyper 2.0. This proposal for identification is based on rather low log score values and needs to be re-examined by independent methods.]

3.4 Influence of cultivation conditions and quality of spectra

Lactobacillus amylophilus CECT 4133^T t 230210
The spectra of strains CECT 4133^T t 230210 and CECT 4133^T p 190210 are very different (Fig. 1). The strain indicated by "t" was cultivated in liquid medium (Amparo Ruvira Garrigues, email dated July 2, 2010). The equivalent strains were cultivated on agar plates and their spectra cluster tightly with this of strain CECT 4133^T p 190210 (Fig. 1). The spectra of *Lactobacillus agilis* CECT 4131T t old liof. 230210 and CECT 4131T new liof. 020310 differ also by a distance level of more than 470 (Fig. 1). These observations demonstrate that it is necessary to cultivate all strains under

comparison on either agar plates or in liquid medium.

The spectra of the following strains fell into the same cluster like those of equivalent strains but the distance level is higher than 300:

Fig. 1:

Lactobacillus aviarius subsp. *aviarius* DSM 20655^T and CIP 103144^T

Lactobacillus aviarius subsp. *araffinosus* DSM 20653^T and CIP 103145^T

Lactobacillus acidophilus CECT 362 and CECT 903^T (the spectrum of the latter strain was confirmed by re-recording after 3 months)

Fig. 2:

Lactobacillus coleohominis CIP 106820^T (only 3 spectra are available)

Lactobacillus buchneri DSM 5987

Fig. 3:

Lactobacillus farraginis DSM 18382^T and CIP 109567^T

Fig. 4:

Lactobacillus jensenii DSM 20557^T and CECT 4306^T (the stability of the spectrum of the latter strain was confirmed by re-recording after 3 months)

Lactobacillus homohiochii DSM 20571^T

Lactobacillus hordei CIP 109892^T

Lactobacillus kalixensis DSM 16043^T and CIP 109909^T
The 16S rRNA gene sequences of both strains agreed to this deposited for the type strain of *Lactobacillus kalixensis* (R. Pukall, personal communication; Dominique Clermont, email dated April 7, 2010)***.*** The spectrum of strain DSM 16043^T clustered with the entry *Lactobacillus kalixensis* DSM 16043^T _DSM of the database of the BioTyper 2.0 (Fig. 4). The spectrum of CIP 109909^T was identified as *Lactobacillus kalixensis* (log score value 2.431 to DSM 16043T_DSM) by the BioTyper 2.0. Because only 2 spectra of strain CIP 109909^T are available (see 2.4), it is suggested to record additional spectra of strain CIP 109909^T.

Fig. 5:

Lactobacillus oris CECT 4021^T

The stability of the spectrum was confirmed by re-recording after 3 months. The identity of strain CECT 4021^T was confirmed on the basis of a new ampoule by 99.8% 16S rRNA gene sequence similarity to the sequence deposited for the type strain of *Lactobacillus oris* (Amparo Ruvira Garrigues, email dated May 24, 2010).

Lactobacillus parabuchneri CECT 5740^T 020310

Lactobacillus plantarum CECT 4180 080610

Lactobacillus oligofermentans DSM 15707^T and CIP 109911^T

The spectrum of strain DSM 15707^T clustered with the entries of additional *Lactobacillus oligofermentans* DSM-strains of the BioTyper 2.0 database (Fig. 5). Both strains CIP 109911^T and DSM 15707^T were identified by the Biotyper 2.0 as "*L. oligofermentans*": CIP 109911^T with a log score value of 2.441 to the Bruker entry for DSM 15708_DSM; DSM 15707^T with a log score value of 2.325 to the Bruker entry for DSM 15704_DSM (2.289 to the Bruker entry for DSM 15708_DSM; DSM 15707^T is not included in the Bruker database).

Fig. 6:

Lactobacillus suebicus CECT 5917^T

The stability of the spectrum was re-recorded after 3 months and the replicated spectra clustered together but showed a distance level of ca. 330. The identity of strain CECT 5917^T was confirmed on the basis of a new ampoule by 99.9% 16S rRNA gene sequence similarity to the sequence deposited for the type strain of *Lactobacillus suebicus* (Amparo Ruvira Garrigues, email dated May 24, 2010).

Lactobacillus uvarum DSM 19971^T and CECT 7335^T

The stability of the spectrum of the latter strain was confirmed by re-recording after 3 months. The identity of strain CECT 7335^T was confirmed on the basis of a new ampoule by 99.9% 16S rRNA gene sequence similarity to the sequence deposited for the type strain of *Lactobacillus uvarum* (Amparo Ruvira Garrigues, email dated May 24, 2010).

Fig. 8:

Oenococcus oeni CECT 217^T 230610

The stability of the spectrum was confirmed by re-recording after 3 months (Amparo Ruvira Garrigues, email dated May 24, 2010; not shown).

Fig. 9:

Pseudomonas agarici DSM 11810^T and CECT 4467^T 290410

It is note-worthy that deviations of spectra within clusters could be reproduced in some cases by replicated spectra (see CECT 4021^T [Fig. 5], CECT 5917^T [Fig. 6]; CECT 217^T, not shown; CECT 283^T, not shown) while replication of measurements lead in other cases to the harmonization of spectra (CECT 5922^T, CECT 5925^T etc., not shown). Differences within clusters of spectra recorded by different laboratories for equivalent strains held in the collections of DSMZ, IP and UVEG-CECT might be explained by the following reasons:

1. Variations in the conditions for cultivation on agar media (composition of media, cultivation time, temperatures etc.)
2. Variations in the conditions of measurement (mass calibration, intensity and resolution of peaks, mass spectrometer model, age-dependent state of the laser, handling of the extracts, etc.)
3. Genetic differences between the equivalent strains

Conclusion

In the course of the study, the identity of 451 equivalent strains representing 81 species and 15 subspecies of the genus *Lactobacillus*, 25 species and 3 subspecies of the family "*Leuconostocaceae*" (containing the genera *Leuconostoc, Fructobacillus, Oenococcus* and *Weissella*) and 22 species and 2 subspecies of the genus *Pseudomonas* from DSMZ, IP and UVEG-CECT was examined by using MALDI-TOF MS. Twelve strains were detected which were either misclassified or the mass spectra of which were mislabelled in databases. Comparison of 16S rRNA gene sequences revealed that some of the strains with unusual MALDI-TOF mass spectra were indeed misclassified and need to be replaced in the collections by new cultures. Some other possibly misclassified strains remain to be studied by 16S rRNA gene sequence analysis and/or other independent methods. Identification proposals for 2 pairs of unclassified equivalent *Lactobacillus* strains were obtained by MALDI-TOF MS but need to be confirmed by 16S rRNA gene sequence analysis and additional methods. The study allowed not only the examination of the identity of strains maintained in the participating collections, but offered also the possibility to compare and to harmonize the quality of spectra generated by different laboratories and to perform a quality control of existing databases of spectra.

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Annexes

Table 1. List of equivalent *Lactobacillus* strains held in the DSMZ, IP, UGent and UVEG-CECT culture collections.

Species and subspecies for which the spectrum of the type strain was not available or was available from only one collection were not considered. Strains with gray numbers were not yet included into the study. Only type strains were considered from UGent.

Species	**DSM**	**CIP**	**CECT**	**LMG**
Lactobacillus acetotolerans	20749^T	103180^T	4019^T	10751^T
Lactobacillus acidifarinae	19394^T	108702^T		22200^T
Lactobacillus acidophilus	9126	108702		
Lactobacillus acidophilus	20242	103595		
Lactobacillus acidophilus	20079^T	76.13^T	903^T	9423^T
Lactobacillus acidophilus			362	
Lactobacillus acidophilus		103597	4179	
Lactobacillus agilis	20509^T	101264^T	4131^T	9186^T
Lactobacillus alimentarius	20181	105163		
Lactobacillus alimentarius	20249^T	102986^T	570^T	9187^T
Lactobacillus amylophilus	20533^T	102988^T	4133^T	6900^T
Lactobacillus amylovorus	16698	109151		
Lactobacillus amylovorus	20531^T	102989^T	4132^T	9496^T
Lactobacillus animalis	20602^T	103152^T	4060^T	9843^T
Lactobacillus apodemi	16634^T	108913^T		
Lactobacillus aquaticus	21051^T		7355^T	
Lactobacillus aviarius subsp. ***aviarius***	20655^T	103144^T		10753^T
Lactobacillus aviarus subsp. ***araffinosus***	20653^T	103145^T		23560^T
Lactobacillus bobalius	19674^T	109926^T	7310^T	
Lactobacillus brevis	1267			
Lactobacillus brevis	1268		216	

Lactobacillus brevis	6235	103474	5172	
Lactobacillus brevis	20556		4669	
Lactobacillus brevis	20054^{T}	102806^{T}	4121^{T}	6906^{T}
Lactobacillus brevis		105137	5354	
Lactobacillus buchneri	20057^{T}	103023^{T}	4111^{T}	6892^{T}
Lactobacillus buchneri			4683	
Lactobacillus camelliae	22697^{T}	109883		
Lactobacillus casei	20011^{T}	103137^{T}	475^{T}	6904^{T}
Lactobacillus casei			4044	
Lactobacillus casei		103131	4180	
Lactobacillus casei			4045	
Lactobacillus catenaformis	20559^{T}	104817^{T}	4140^{T}	23559^{T}
Lactobacillus ceti	22408^{T}	109847^{T}	7185^{T}	
Lactobacillus coleohominis	14060^{T}	106820^{T}		21591^{T}
Lactobacillus collinoides	20515^{T}	103008^{T}	922^{T}	9194^{T}
Lactobacillus coryniformis subsp***. coryniformis***	20001^{T}	103133^{T}	982^{T}	9196^{T}
Lactobacillus coryniformis subsp***. torquens***	20004^{T}	103134^{T}	4129^{T}	9197^{T}
Lactobacillus crispatus	20584^{T}	102990^{T}	4840^{T}	9479^{T}
Lactobacillus curvatus subsp***. curvatus***	20019^{T}	102992^{T}	904^{T}	9198^{T}
Lactobacillus delbrueckii subsp***. bulgaricus***	20080			
Lactobacillus delbrueckii subsp***. bulgaricus***	20081^{T}	101027^{T}	4005^{T}	6901^{T}
Lactobacillus delbrueckii subsp***. bulgaricus***			5036	
Lactobacillus delbrueckii subsp***. delbrueckii***	20074^{T}	57.8^{T}	286^{T}	6412^{T}
Lactobacillus delbrueckii subsp***. delbrueckii***			4685	
Lactobacillus delbrueckii subsp***. lactis***	20076	54.4	287	
Lactobacillus delbrueckii subsp***. lactis***	20355	53.61	282	
Lactobacillus delbrueckii subsp***. lactis***	20072^{T}	101028^{T}		7942^{T}
Lactobacillus dextrinicus	20335^{T}	103407^{T}	4791^{T}	11485^{T}
Lactobacillus diolivorans	14421^{T}	107499^{T}		19667^{T}
Lactobacillus equigenerosi	18793^{T}	109834^{T}		
Lactobacillus farciminis	20184^{T}	103136^{T}	571^{T}	9200^{T}

Lactobacillus farraginis	18382^{T}	109567^{T}		24140^{T}
Lactobacillus fermentum	20055	102981	562	
Lactobacillus fermentum	20391	64.53, 53.163	285	
Lactobacillus fermentum	20052^{T}	102980^{T}	4007^{T}	6902^{T}
Lactobacillus fructivorans	20203^{T}	103042^{T}	4785^{T}	9201^{T}
Lactobacillus frumenti	13145^{T}	106922^{T}		19473^{T}
Lactobacillus gallinarum	10532^{T}	103611^{T}		9435^{T}
Lactobacillus gasseri	20077	103699		
Lactobacillus gasseri	20243^{T}	102991^{T}	4479^{T}	9203^{T}
Lactobacillus ghanensis	18630^{T}	109616^{T}		
Lactobacillus graminis	20720			
Lactobacillus graminis	20721			
Lactobacillus graminis	20719^{T}	105164^{T}	4017^{T}	9825^{T}
Lactobacillus hayakitensis	18933^{T}	109885^{T}		24490^{T}
Lactobacillus helveticus	20075^{T}	103146^{T}	4305^{T}	6413^{T}
Lactobacillus helveticus			541	
Lactobacillus helveticus		76.19	800	
Lactobacillus helveticus		104343	403	
Lactobacillus helveticus			404	
Lactobacillus hilgardii	20176^{T}	103007^{T}	4786^{T}	6895^{T}
Lactobacillus homohiochii	20351		983	
Lactobacillus homohiochii	20571^{T}	103141^{T}	4136^{T}	9478^{T}
Lactobacillus hordei	19519^{T}	109892^{T}		24241^{T}
Lactobacillus iners	13335^{T}	105923^{T}		18914^{T}
Lactobacillus intestinalis	6629^{T}	104793^{T}		14196^{T}
Lactobacillus jensenii	20557^{T}	69.17^{T}	4306^{T}	6414^{T}
Lactobacillus johnsonii		103653	289	
Lactobacillus johnsonii	10533^{T}	103620^{T}		9436^{T}
Lactobacillus kalixensis	16043^{T}	109909^{T}		22115^{T}
Lactobacillus kefiranofaciens subsp. ***kefiranofaciens***	5016^{T}	103307^{T}	5954^{T}	19149^{T}
Lactobacillus kefiranofaciens subsp. ***kefirgranum***	10550^{T}	104241^{T}	5919^{T}	15132^{T}

Lactobacillus kimchii	13961^T	107019^T		19822^T
Lactobacillus lindneri	20690^T	102983^T	5957^T	14528^T
Lactobacillus malefermentans	20177		5956	
Lactobacillus malefermentans	5705^T	103367^T	5928^T	11455^T
Lactobacillus mali	20483	103143	4149	
Lactobacillus manihotivorans	13343^T	105851^T		18010^T
Lactobacillus murinus	20452^T	104818^T	4135^T	14189^T
Lactobacillus nagelii	13675^T	107647^T	5983^T	21593^T
Lactobacillus namurensis	19117^T	109498^T		23583^T
Lactobacillus oeni	19972^T	110039^T	7334^T	
Lactobacillus oligofermentans	15707^T	109911^T		22743^T
Lactobacillus oris	4864^T	105162^T	4021^T	9848^T
Lactobacillus parabuchneri	5707^T	103368^T	5740^T	11457^T
Lactobacillus paracasei	20008		277	
Lactobacillus paracasei subsp. ***paracasei***	4905			
Lactobacillus paracasei subsp. ***paracasei***	20006			
Lactobacillus paracasei subsp. ***paracasei***	20020		981	
Lactobacillus paracasei subsp. ***paracasei***	46331			
Lactobacillus paracasei subsp. ***paracasei***	5622^T	103918^T	4022^T	13087^T
Lactobacillus paracasei subsp. ***paracasei***		107868		
Lactobacillus paracasei subsp. ***paracasei***	20312		4583	
Lactobacillus paracasei subsp. ***tolerans***	20258^T	102994^T /103024 ^{T}	4175^T	9191^T
Lactobacillus paracollinoides	20197		5921	
Lactobacillus paracollinoides	15502^T	108394^T		22473^T
Lactobacillus parafarraginis	18390^T	109568^T		24141^T
Lactobacillus parakefiri	10551^T	104242^T		15133^T
Lactobacillus pentosus	20314^T	103156^T	4023^T	10755^T
Lactobacillus perolens	12744^T		5955^T	18936^T
Lactobacillus plantarum	2601	71.39	749	
Lactobacillus plantarum	20205	A159	220	
Lactobacillus plantarum subsp. ***plantarum***	20174^T	103151^T	748^T	6907^T

Lactobacillus rennini	20254		5923	
Lactobacillus rennini	20253^{T}	109172^{T}	5922^{T}	23902^{T}
Lactobacillus reuteri	20016^{T}	101887^{T}	925^{T}	13557^{T},9213^{T}
Lactobacillus rhamnosus	20245	57.6	288	
Lactobacillus rhamnosus	20247			
Lactobacillus rhamnosus	20021^{T}	A157^{T}	278^{T}	6400^{T}
Lactobacillus ruminis	20403^{T}	103153^{T}	4061^{T}	10756^{T}
Lactobacillus sakei	20494	104494		
Lactobacillus sakei subsp. ***carnosus***	15740	109872		
Lactobacillus sakei subsp. ***carnosus***	15831^{T}	105422^{T}	5766^{T}	17302^{T}
Lactobacillus sakei subsp. ***sakei***	20017^{T}	103139^{T}	906^{T}	9468^{T}
Lactobacillus salivarus	20554^{T}	103155^{T}	4062^{T}	9476^{T}
Lactobacillus salivarus	20555^{T}	103140^{T}	4063^{T}	9477^{T}
Lactobacillus salivarus			4530	
Lactobacillus senmaizukei	21775^{T}	109886^{T}		
Lactobacillus **sp.**	6265		5918	
Lactobacillus **sp.**	20182	102987	5927	
Lactobacillus suebicus	5007^{T}	103411^{T}	5917^{T}	11408^{T}
Lactobacillus thailandensis	22698^{T}	109884^{T}		
Lactobacillus tucceti	20183^{T}	110049^{T}	5920^{T}	
Lactobacillus uvarum	19971^{T}	109959^{T}	7335^{T}	
Lactobacillus vaccinostercus	20634^{T}	102807^{T}	5925^{T}	9215^{T}
Lactobacillus vaginalis	5837^{T}	105932^{T}	4089^{T}	12891^{T}
Lactobacillus versmoldensis	14857^{T}	107931^{T}	5773^{T}	21929^{T}
Lactobacillus vini	20605^{T}	109261^{T}	5924^{T}	23202^{T}
Lactobacillus zymae	19395^{T}	108703^{T}		22198^{T}

Table 2. List of equivalent strains of the family "*Leuconostocaceae*" held in the DSMZ, IP and UVEG-CECT culture collections.

Species and subspecies for which the spectrum of the type strain was not available or was available from only one collection were not considered. Strains with gray numbers were not included into the study.

Name	**DSM**	**CIP**	**CECT**
Fructobacillus durionis	19113^{T}	108761^{T}	7089^{T}
Fructobacillus ficulneus	13613^{T}	107593^{T}	5747^{T}
Fructobacillus fructosus	20349^{T}	102985^{T}	7088^{T}
Fructobacillus pseudoficulneus	15468^{T}	109309^{T}	5759^{T}
Leuconostoc carnosum	5576^{T}	103319^{T}	4024^{T}
Leuconostoc citreum	20188	103405	4018
Leuconostoc citreum	5577^{T}	103315^{T}	4025^{T}
Leuconostoc fallax	10614		
Leuconostoc fallax	10615		
Leuconostoc fallax	20189^{T}	104855^{T}	7087^{T}
Leuconostoc gasicomitatum	15947^{T}		5767^{T}
Leuconostoc gelidum	5578^{T}	103318^{T}	4026^{T}
Leuconostoc inhae	15101^{T}	108081^{T}	7026^{T}
Leuconostoc lactis	8581	103889	5746
Leuconostoc lactis	8582		
Leuconostoc lactis	8583		
Leuconostoc lactis	20192		
Leuconostoc lactis	20202^{T}	102422^{T}	4173^{T}
Leuconostoc mesenteroides subsp***. cremoris***	20200		
Leuconostoc mesenteroides subsp***. cremoris***	20346^{T}	103009^{T}	872^{T}
Leuconostoc mesenteroides subsp***. dextranicum***	20187		
Leuconostoc mesenteroides subsp***. dextranicum***	46216		
Leuconostoc mesenteroides subsp***. dextranicum***	20484^{T}	102423^{T}	912^{T}
Leuconostoc mesenteroides subsp***. mesenteroides***	20240	78.59	394
Leuconostoc mesenteroides subsp***. mesenteroides***	20241		

Leuconostoc mesenteroides subsp***. mesenteroides***	20343^T	102305^T	219^T/891^T
Leuconostoc pseudomesenteroides	5624	103325	
Leuconostoc pseudomesenteroides	5625		4705
Leuconostoc pseudomesenteroides		110051	
Leuconostoc pseudomesenteroides	20193^T	103316^T	4027^T
Oenococcus kitaharae	17330^T	109430^T	
Oenococcus oeni	10519		
Oenococcus oeni	20255		4028
Oenococcus oeni	20257		4029
Oenococcus oeni	20252^T	106144^T	217^T
Weissella cibaria	14295	108011	
Weissella cibaria	15878^T	107376^T	7032^T
Weissella confusa	20196^T	103172^T	4707^T
Weissella halotolerans	20190^T	103005^T	573^T
Weissella hellenica	7378^T	109879^T	7033^T
Weissella kandleri	20593^T	102809^T	4307^T
Weissella minor	20014^T	102978^T	572^T
Weissella paramesenteroides	5623		
Weissella paramesenteroides	20288^T	102421^T	4268^T
Weissella soli	14420^T	107584^T	7031^T
Weissella thailandensis	15832^T	106751^T	7052^T
Weissella viridescens	20248		
Weissella viridescens	20410^T	102810^T	283^T

Table 3. List of equivalent *Pseudomonas* type strains held in the DSMZ and UVEG-CECT culture collections.

Species and subspecies for which the spectrum of the type strain was not available or was available from only one collection were not considered.

Name	DSM	CECT
Pseudomonas agarici	11810^T	4467^T
Pseudomonas alcaligenes	50342^T	929^T
Pseudomonas anguilliseptica	12111^T	899^T
Pseudomonas argentinensis	17259^T	7010^T
Pseudomonas asplenii	17133^T	4468^T
Pseudomonas chlororaphis* subsp. *chlororaphis	50083^T	4470^T
Pseudomonas cichorii	50259^T	4471^T
Pseudomonas corrugata	7228^T	124^T
Pseudomonas fluorescens	50090^T	378^T
Pseudomonas fragi	3456^T	446^T
Pseudomonas lutea	17257^T	5822^T
Pseudomonas marginalis	13124^T	229^T
Pseudomonas mendocina	50017^T	320^T
Pseudomonas oleovorans	1045^T	4079^T
Pseudomonas pseudoalcaligenes* subsp. *pseudoalcaligenes	50188^T	318^T
Pseudomonas putida	50202^T	324^T
Pseudomonas rhizosphaerae	16299^T	5726^T
Pseudomonas simiae	18861^T	7078^T
Pseudomonas stutzeri	5190^T	930^T
Pseudomonas syringae	10604^T	4429^T
Pseudomonas tolaasii	19342^T	4472^T
Pseudomonas viridiflava	11124^T	458^T

Table 4. Distance levels of branching points in dendrograms correlated to Log Score Values of identification results of the BioTyper Version 2.0

Strains	**Distance level in dendrogram**	**Log Score Value to database entry of BioTyper 2.0**
Lactobacillus murinus		
DSM 20452^{T} /		2.201 (DSM 20452^{T})
CECT 4135^{T}	80 (Fig. 4)	2.168 (DSM 20452^{T})
CIP 104818^{T}	140 (Fig. 4)	2.221 (DSM 20452^{T})
Lactobacillus diolivorans		
DSM 14421^{T} /		2.337 (DSM 14421^{T})
CIP 109567^{T}	170 (Fig. 3)	2.096 (DSM 14421^{T})
Lactobacillus lindneri		
DSM 20690^{T} /		2.019 (DSM 20690^{T})
CECT 5957^{T}	180 (Fig. 4)	1.790 (DSM 20690^{T})
Lactobacillus perolens		
DSM 12744^{T} /		2.441 (DSM 12744^{T})
CECT 5955^{T}	240 (Fig. 5)	2.395 (DSM 12744^{T})
Lactobacillus suebicus		
DSM 5007^{T} /		2.228 (DSM 5007^{T})
CIP 103411^{T}	280 (Fig. 6)	2.139 (DSM 5007^{T})
CECT 5917^{T} 230610	600 (Fig. 6)	2.313 (DSM 5007^{T})
Lactobacillus manihotivorans		
CIP 105851^{T} /		1.775 (CIP 105851^{T})
DSM 13343^{T}	330 (Fig. 4)	2.368 (CIP 105851^{T})
Pseudomonas agarici		
DSM 11810^{T} /		2.540 (DSM 11810^{T})
CECT 4467^{T}	330 (Fig. 9)	2.466 (DSM 11810^{T})
Pseudomonas alcaligenes		
DSM 50342^{T} /		2.564 (DSM 50342^{T})
CECT 929^{T}	990 (Fig. 9)	2.417 (*Stenotrophomonas maltophila* DSM 50170^{T})

Fig. 1. Dendrogram *Lactobacillus* A

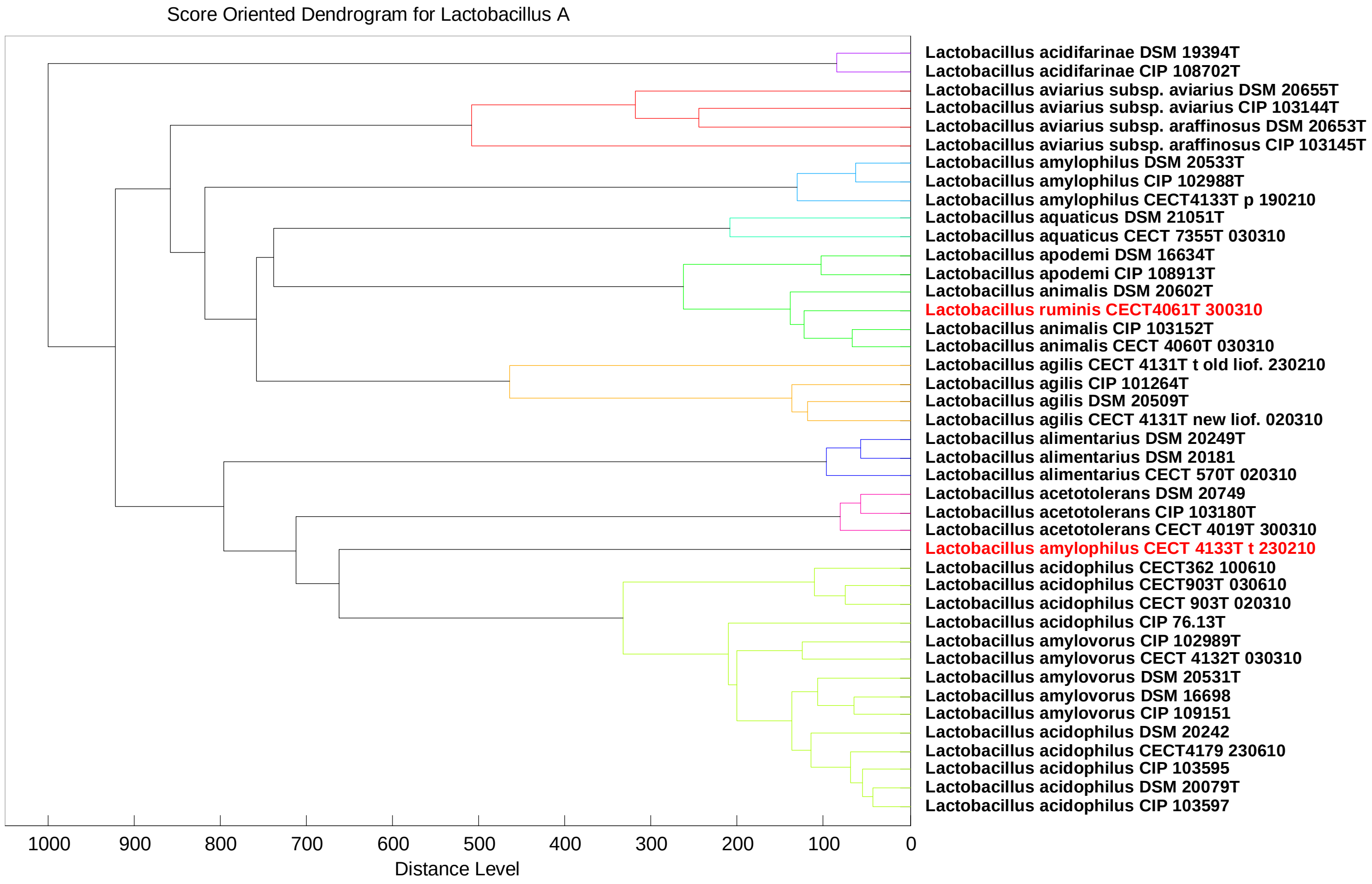


Fig. 2. Dendrogram *Lactobacillus* B-C

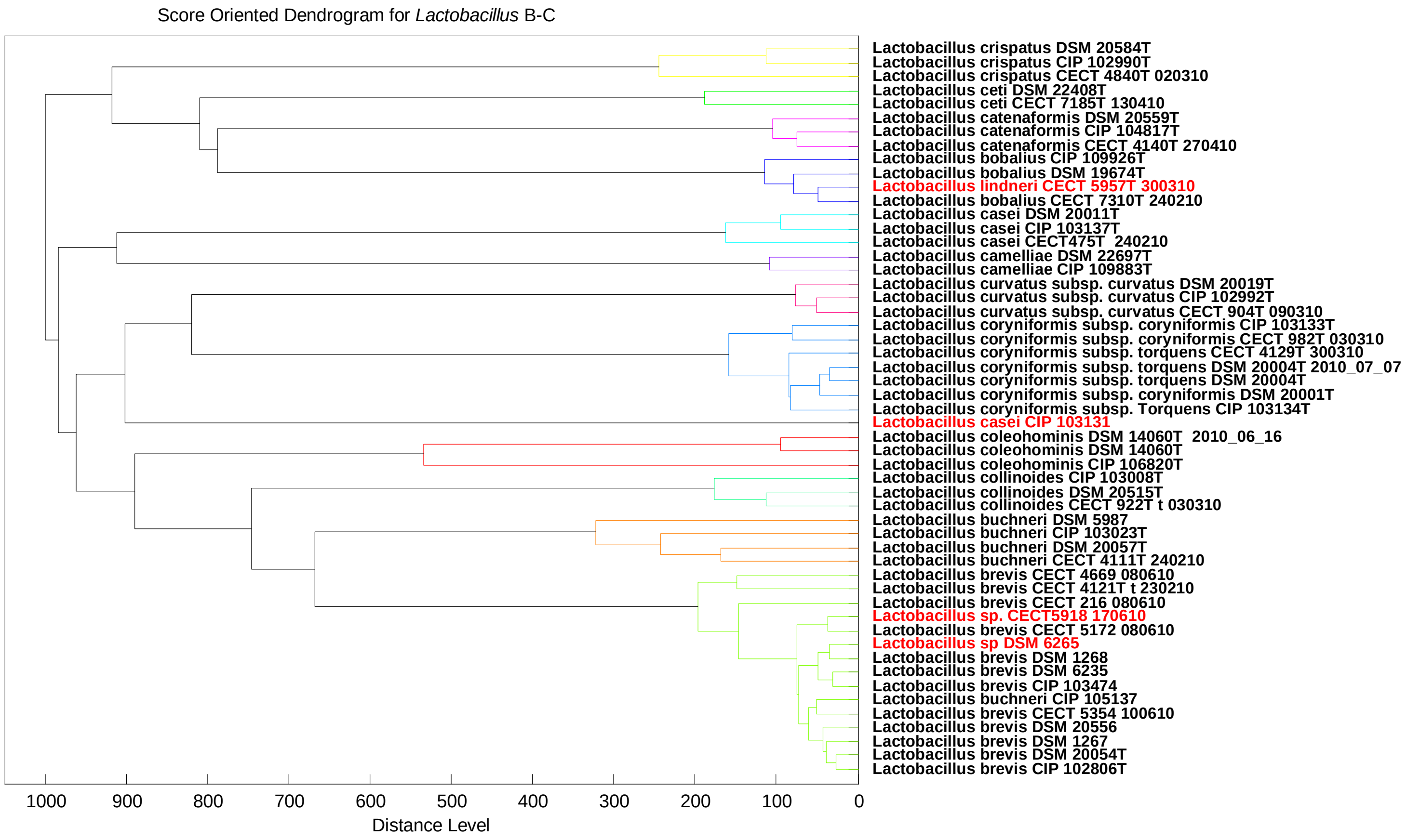


Fig. 3. Dendrogram *Lactobacillus* D-G

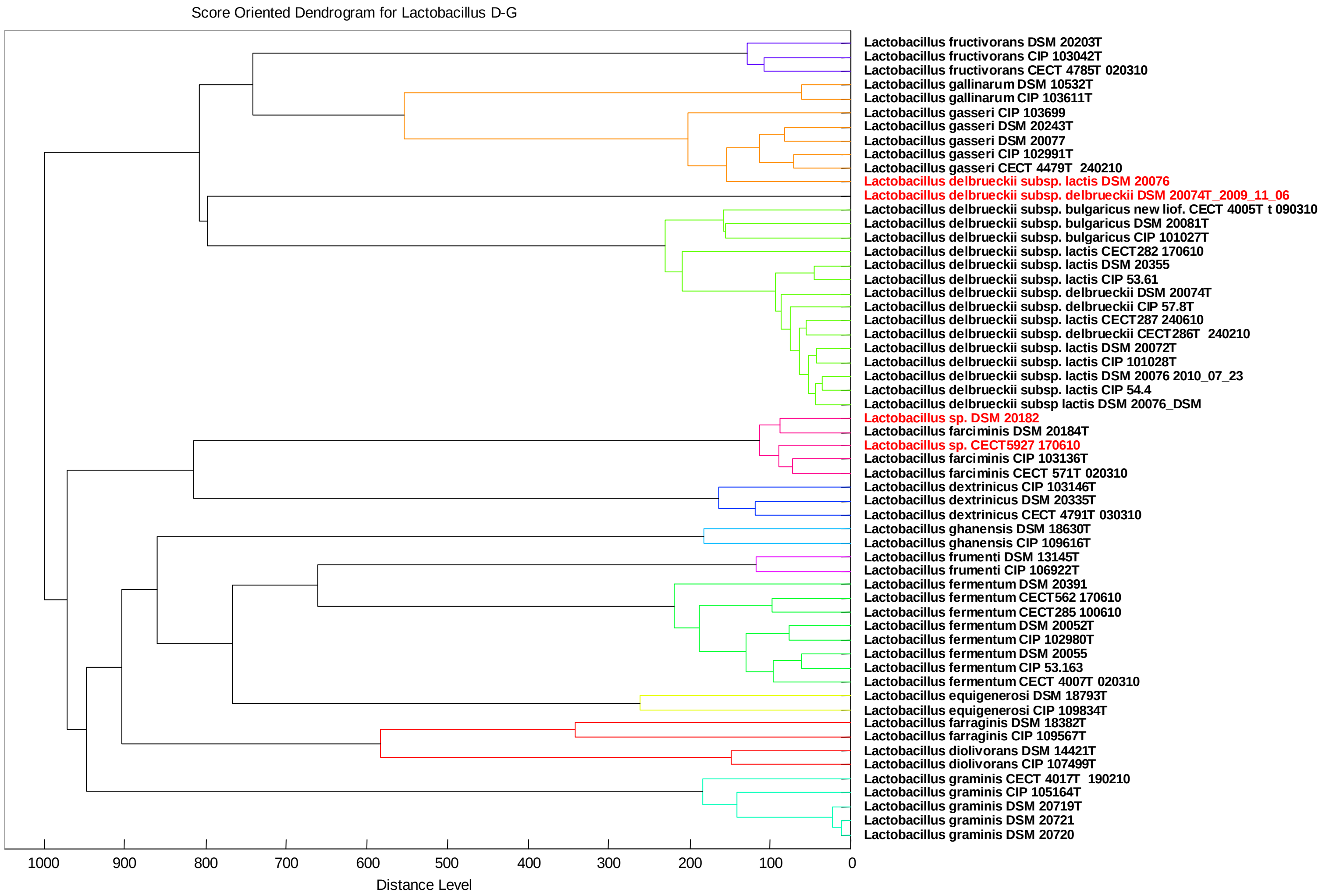


Fig. 4. Dendrogram *Lactobacillus* H–M

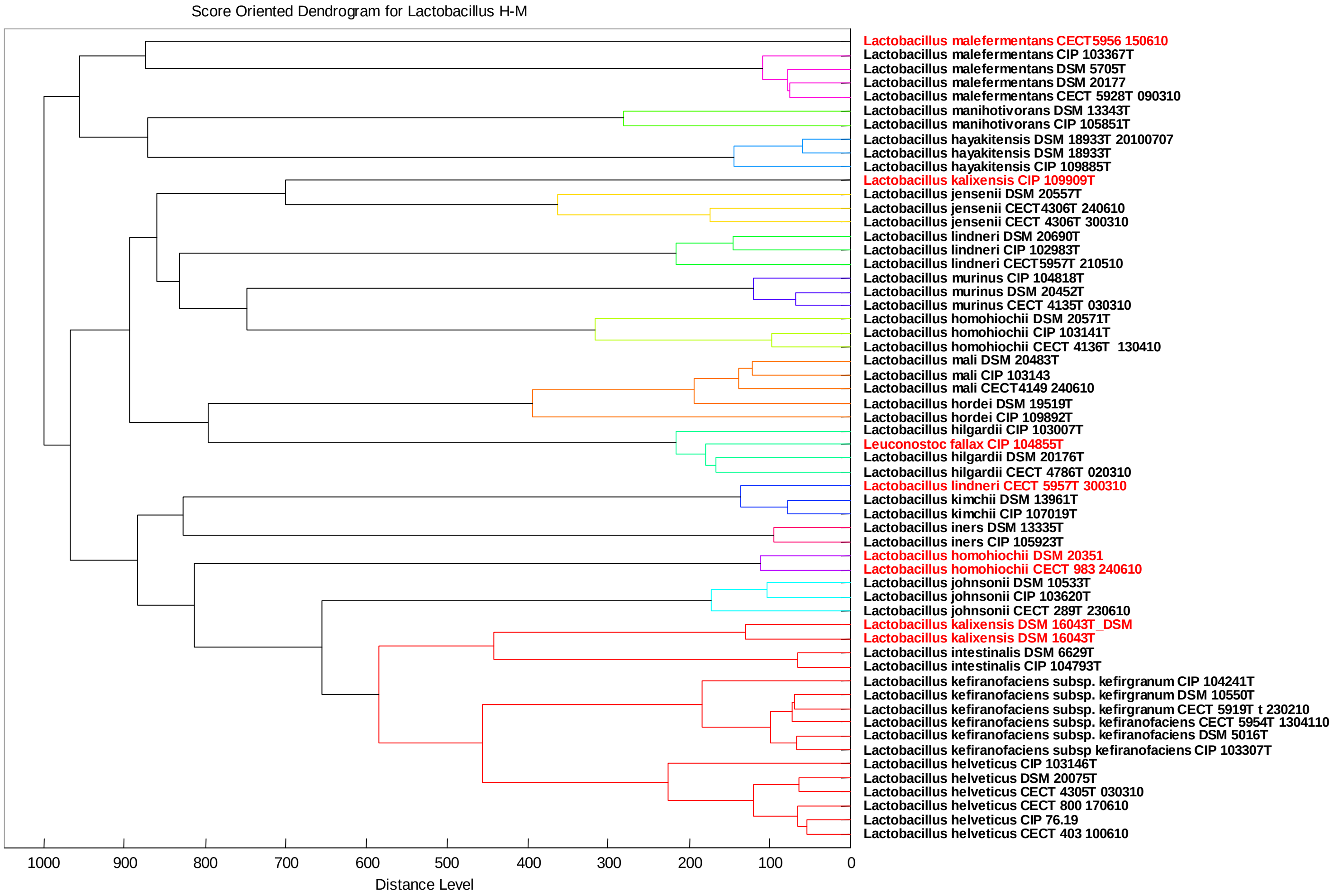


Fig. 5. Dendrogram *Lactobacillus* N-R

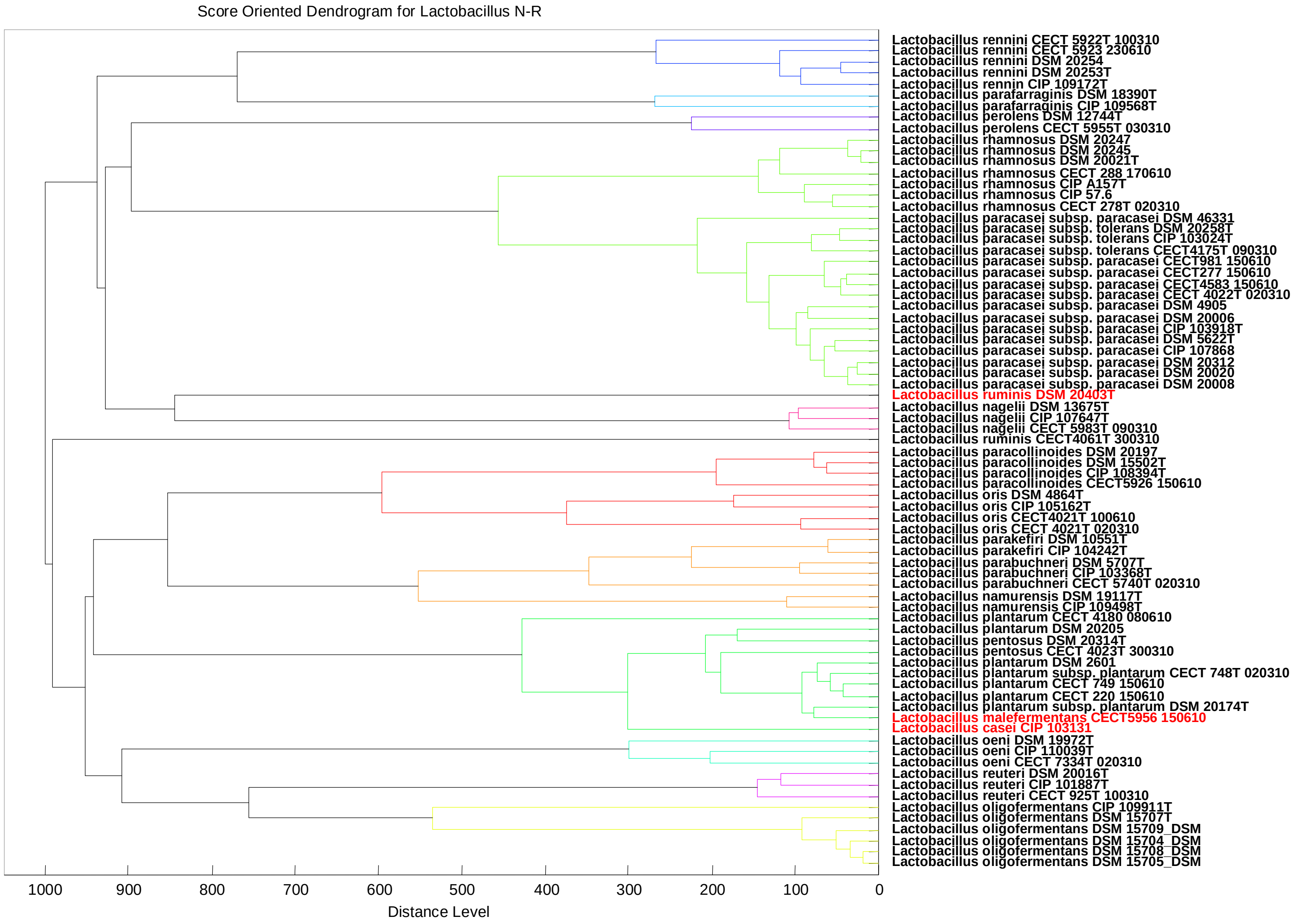


Fig. 6. Dendrogram *Lactobacillus* S-Z

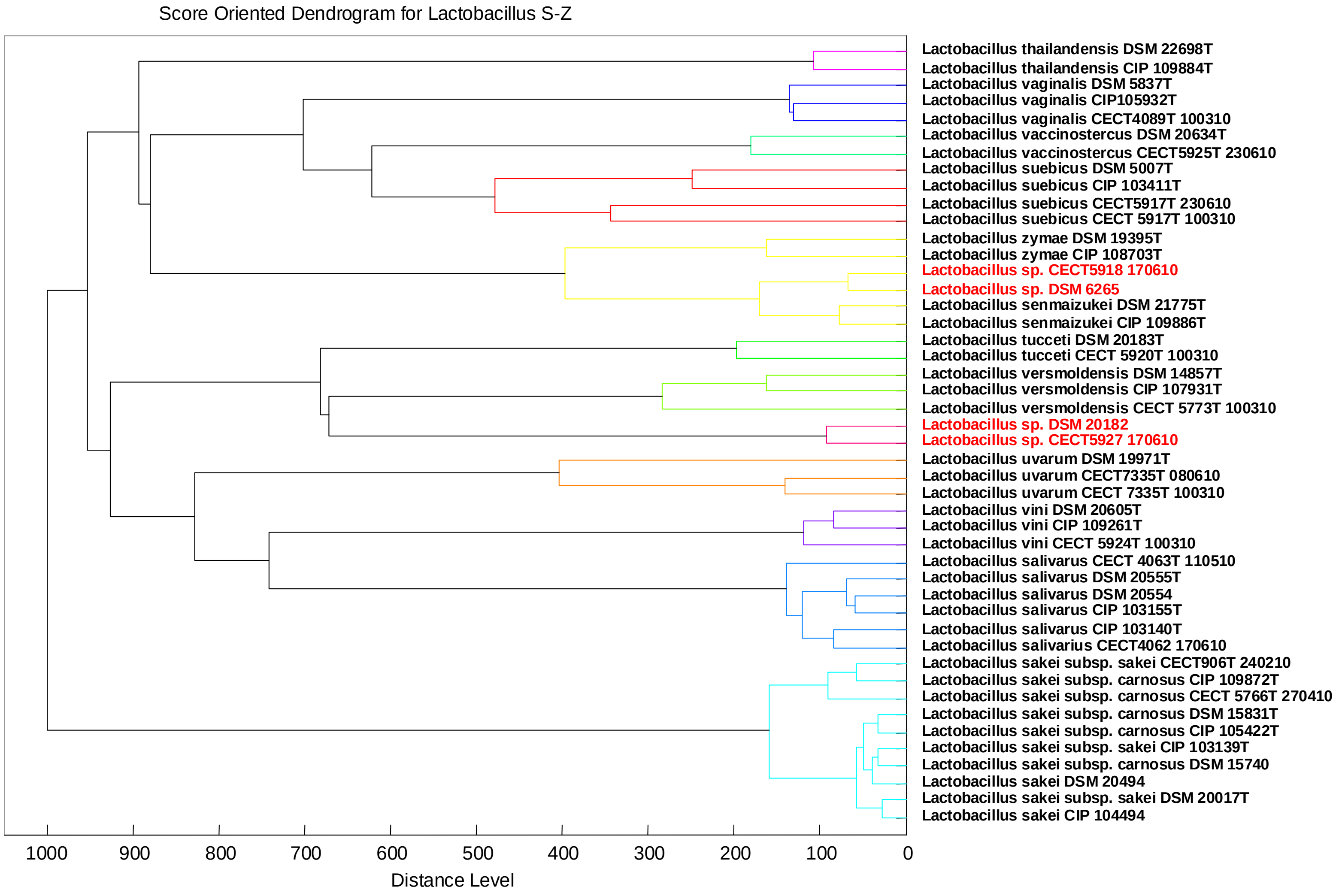


Fig. 7. Dendrogram *Leuconostoc*

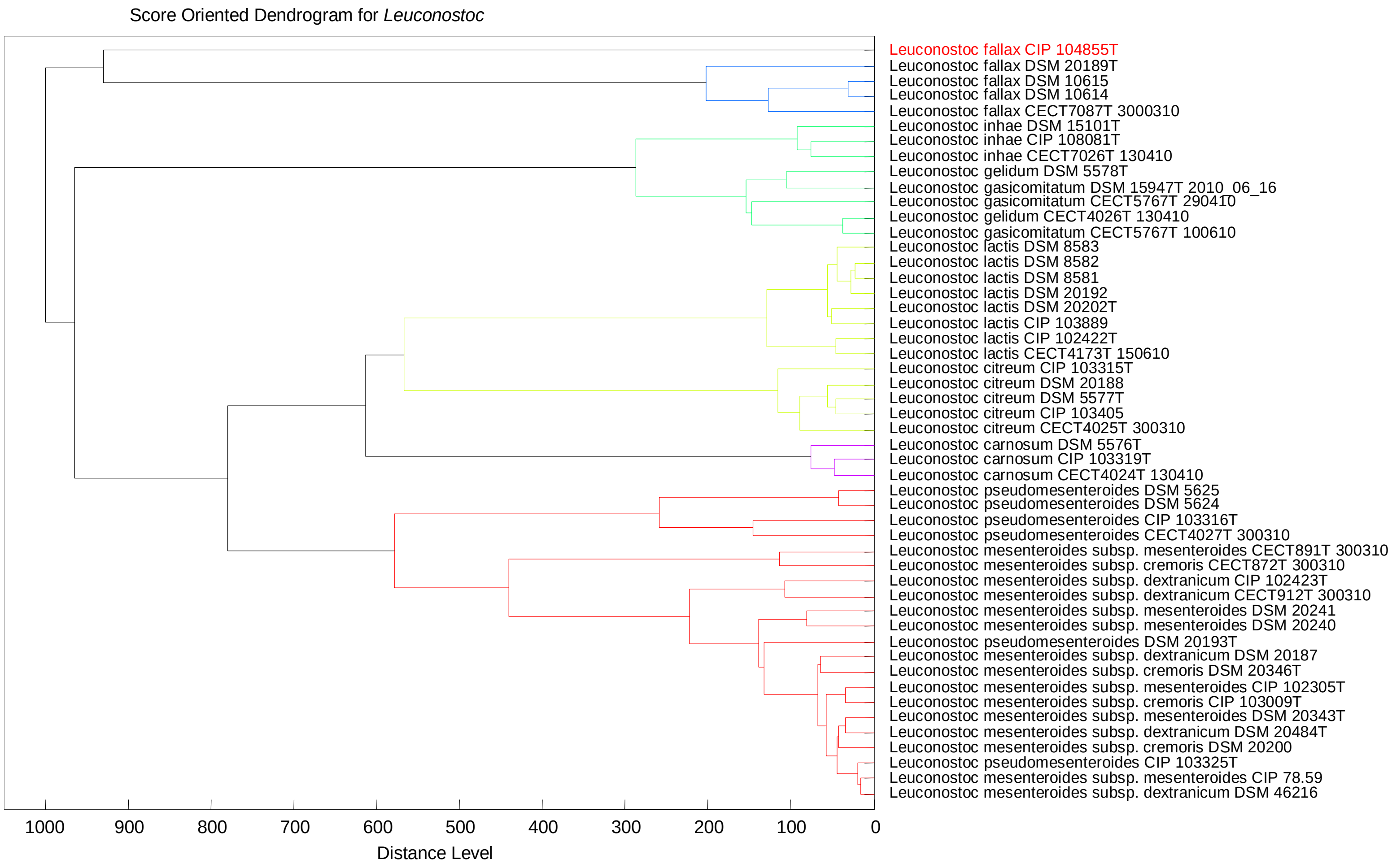
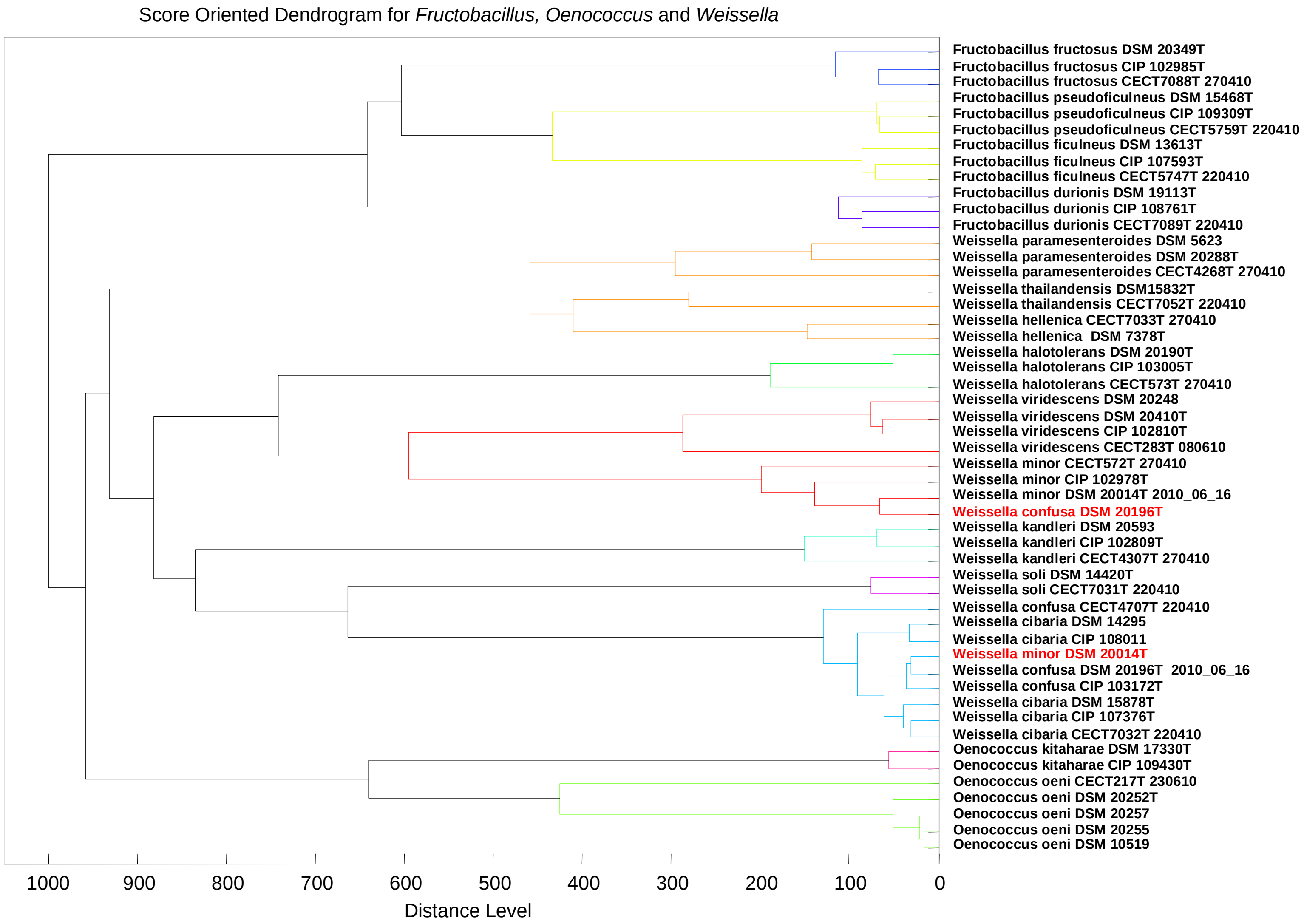
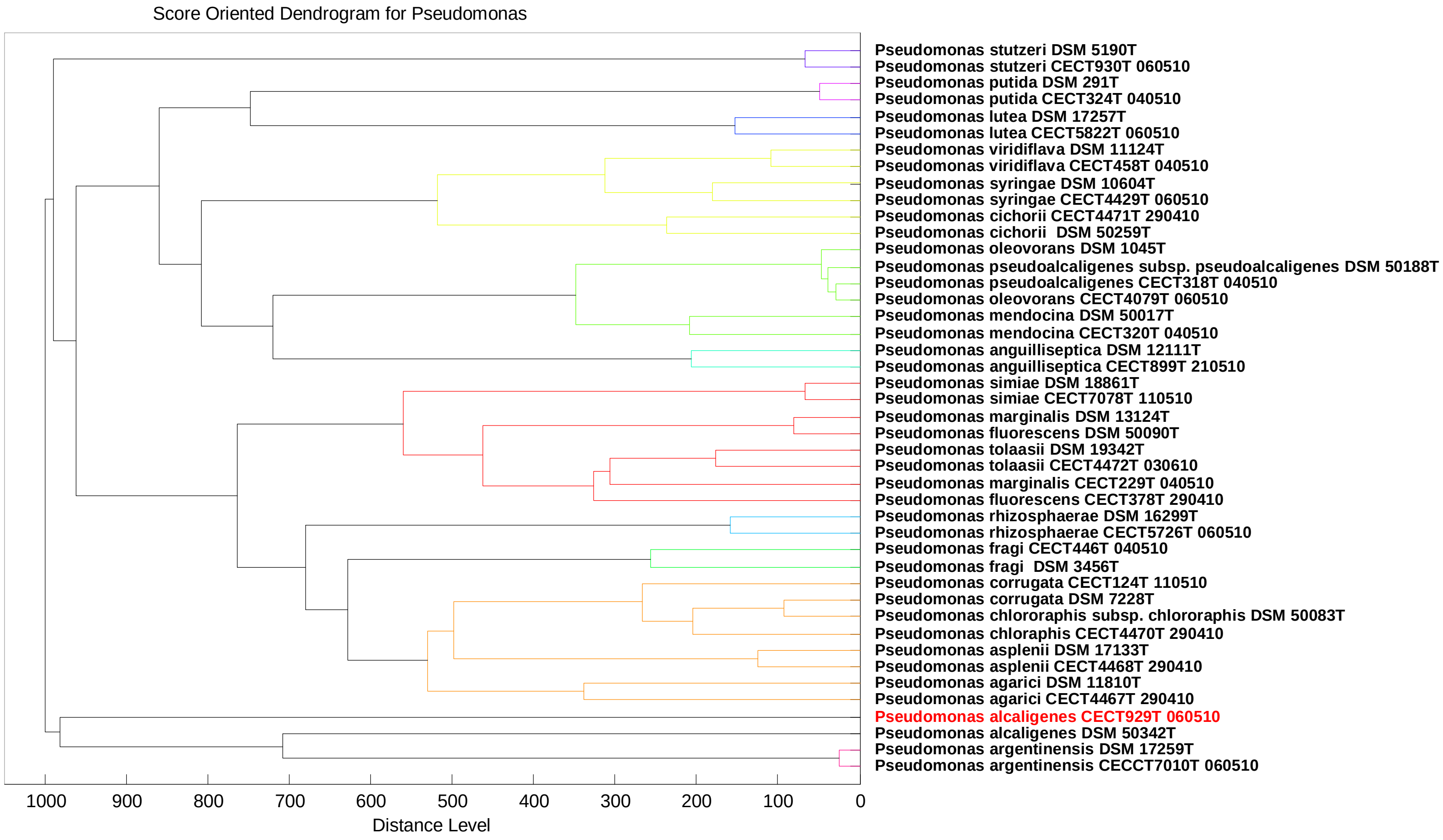


Fig. 8. Dendrogram for *Fructobacillus, Oenococcus* and *Weissella*



Score Oriented Dendrogram for *Fructobacillus, Oenococcus* and *Weissella*

Fig. 9. Dendrogram *Pseudomonas*



Significance of this deliverable

MALDI-TOF MS is a quick, appropriate and efficient tool to verify strain identify between collections and to detect misclassified strains.

Harmonization of the quality of spectra generated by different partners has been performed (quality control of existing databases of spectra); however, for easy spectra comparison the type of equipment has to be the same.