



HAL
open science

MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles

Menouar Nacef, Mickaël Chevalier, Sylvie Chollet, Djamel Drider, Christophe Flahaut

► **To cite this version:**

Menouar Nacef, Mickaël Chevalier, Sylvie Chollet, Djamel Drider, Christophe Flahaut. MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. *International Journal of Food Microbiology*, 2017, 247, pp.2-8. <10.1016/j.ijfoodmicro.2016.07.005>. <hal-04538577>

HAL Id: hal-04538577

<https://hal.science/hal-04538577v1>

Submitted on 9 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Copyright - All rights reserved



MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles



Menouar Nacef^{a,b}, Mickaël Chevalier^b, Sylvie Chollet^a, Djamel Drider^b, Christophe Flahaut^{c,*}

^a Institut Charles VIOLLETTE (ICV) EA 7394, ISA, F-59000 Lille, France

^b Univ Lille, F-59000 Lille, France

^c Univ Artois, F-62307 Lens, France

ARTICLE INFO

Article history:

Received 9 November 2015

Received in revised form 11 June 2016

Accepted 7 July 2016

Available online 8 July 2016

Keywords:

Mass spectrometry

MALDI-TOF MS

Bacteria identification

Lactic acid bacteria

Cow-milk cheese

ABSTRACT

In this study we identified the culturable population of mesophilic lactic acid bacteria (LAB) from a French cheese Maroilles made either with raw or pasteurized milk using MALDI-TOF mass spectrometry (MS). Samples from rind and heart of Maroilles cheese were used, the LAB were selected on MRS agar at 30 °C and 197 Gram-positive and catalase-negative strains were subjected to identification by MALDI-TOF MS profiling. All strains were unambiguously identified: 105 strains from Maroilles made with raw milk (38 on the rind and 67 in the heart) and 92 strains from Maroilles made with pasteurized milk (39 on the rind and 53 in the heart). MALDI-TOF MS identification allowed identification of three genera belonging to LAB including *Lactobacillus*, *Enterococcus* and *Leuconostoc*. *Lactobacillus* was the most represented genus with seven species: *Lactobacillus plantarum* (*L. plantarum*), *L. paracasei*, *L. curvatus*, *L. rhamnosus*, *L. fructivorans*, *L. parabuchneri*, *L. brevis* found in Maroilles made with both kind of milk. The correlation between the 16S rDNA-based identification performed on selected strains and those obtained by MALDI-TOF-MS demonstrates that this fast, economically affordable, robust and reliable method for bacteria characterisation stands as an attractive alternative to the commonly-used methods and its application in food industry is discussed.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The Maroilles is a soft French cheese with a washed rind, produced in the Nord and Aisne departments, more accurately in a region named Thiérache and benefits from a protected designation of origin in Europe. This label is granted to traditional agricultural products and describes foodstuffs that are produced, processed and prepared in a given geographical area using recognized know-how. The Maroilles has the protected designation of origin status since 1996. The earliest traces of the Maroilles cheese date back to the 7th century, afterwards the making method was improved by monks from the Maroilles abbey at the end of the 10th century. Traditionally, the Maroilles is a craft cheese elaborated from raw milk. However, the use of pasteurized milk was developed with the emergence of industrial processes and the increase of health risks due to the potential presence of pathogens.

Four ingredients are required to produce the Maroilles cheese: milk, rennet, salts and microorganisms (including a consortium of yeasts and bacterial strains, provided by Danisco®, inoculated during the first stage of production process and eventually in subsequent washing steps during ripening). Classically, the milk protein coagulation is carried out by rennet addition. The curd is cut, broken up to obtain desired particle sizes and molded into squares. The molds are then turned back every few hours for 2 days for draining the whey excess. During the ripening period, each cheese is individually and periodically washed (4 to 6 times) with brine or pure salts to trigger the formation of the crust. The Maroilles cheese is then moved to the maturing cellars (temperature between 9 and 16 °C and humidity close to 90%), where the cheese ripening is extended for up to four months.

Microbiological studies aiming at highlighting the microbial content of the Maroilles cheese are seldom undertaken although a recent report unveiled the predominance of Gram-positive bacteria in the microbiota of red-smear cheeses such as Maroilles, Munster, Epoisse, Livarot and Pont-l'Évêque (Dalcenserie et al., 2014). In 2013, Gori et al. showed that the surface bacterial microbiota on the farmhouse cheeses were dominated by *Corynebacterium* spp. and/or *Brachybacterium* spp. *Brevibacterium* spp. was found to be subdominant compared to other bacteria, and no *Brevibacterium* spp. was found on the cheese from the

Abbreviations: CFU, colony forming unit; LAB, lactic acid bacteria; MALDI-TOF MS, matrix-assisted laser desorption/ionisation-time of flight mass spectrometry; (*m/z*), mass over charge.

* Corresponding author.

E-mail address: christophe.flahaut@univ-artois.fr (C. Flahaut).

industrial dairy, even though *Brevibacterium linens* (*B. linens*) was used as surface-ripening culture (Gori et al., 2013). The orange-red crust of these cheeses is obtained by the action of *B. linens* which is commonly called 'the red ferment'. This bacterium was considered for a long time as the lone major microorganism on the surface of this kind of cheese. However recent studies showed that a mixture of two species, *B. linens* and the new species *B. aurantiacum*, which forms pale yellow to deep orange or brown colonies, are responsible for the orange to red colours of many smear-ripened cheeses. Moreover other species of bacteria were found, including *Arthrobacter arilaitensis* (*A. arilaitensis*) and *A. bergerei*, originally isolated from Reblochon and Camembert cheeses (Cogan, 2011). This study aimed at portraying the LAB content of Maroilles cheese from various industrial and craft manufactures to assess the impact of the production process on the LAB population.

The large diversity of microorganisms and the successive discoveries in several science fields (physics, biochemistry, (micro)biology, medicine) were the main drivers of development and progress of identification methods dedicated to microorganisms. The phenotypic characterization of microorganisms was originally performed using macroscopic/microscopy methods, culture-based methods and physiological/biochemical methods. Even if some of these methods are still used today for economical or regulatory reasons, the recent identification methods are nowadays based on cellular, immunological and molecular techniques. Among these latter, the tools dedicated to nucleic acid analysis are the most developed (e.g. G/C ratio, enzyme restriction use, PCR-based techniques, hybridization-based techniques, isothermal amplification, sequencing-based techniques...) and the more widely used in the world (Scheler et al., 2014; Yu et al., 2015). Main criteria for the choice of bacterial identification method are often (i) the sensitivity and specificity of identification tests used, (ii) their robustness and (iii) the cost per analysis. In this context, bacterial identification based on matrix-assisted laser desorption/ionisation (MALDI)-time of flight (TOF) mass spectrometry (MS) is becoming a method of choice for determining the genus, species and even subspecies of bacterial isolates (Carbonnelle et al., 2012; Dušková et al., 2012). Seemingly, this technology is also achievable for other microorganisms (e.g. yeasts, fungi,...) from various sources (Chalupová et al., 2014). Initially developed for the academic research area, the use of the MALDI-TOF-MS to obtain the molecular fingerprint of a given microorganism is now widely spread in activity sectors such as the human and animal health, the environment, the pharmaceutical but still remains underused in food industries.

MS-specialists have developed many kinds of ion sources, of mass analysers and of detectors to measure the molecular masses of various molecules. Even if several kinds of mass spectrometers are able to analyse complex mixtures of microorganism proteins (Hynek and Hochel, 2013), the MALDI-TOF mass spectrometer has emerged as the tool of choice for (i) the adequacy between the solid nature of bacterial samples and the MALDI ion source, (ii) the absence of complex, critical sample preparation steps (even if numerous standard operating protocols are now available), (iii) a rapid acquisition time of directly-usable MS-data and (iv) a low financial cost per analysis (see (Freiwald and Sauer, 2009; Welker, 2011) for details). A MALDI-TOF mass spectrometer is composed of an ion source for solid samples coupled to a mass analyser that sorts the ions according to their time-of-flight to travel a given distance in a free-field environment.

This work aimed at reporting the mesophilic LAB diversity for four types of samples: heart and rind of both raw and pasteurized milk based Maroilles cheese. We compared the LAB content in the heart and the rind of French Maroilles made with raw and pasteurized milk using the MALDI-TOF MS method. To strengthen the potency of this technology, we conducted a correlation analysis between the MALDI-TOF-MS data and the 16S rDNA identification results performed from selected strains.

2. Materials and methods

2.1. Cheese samples and sampling

Two commercially available Maroilles cheeses of large size (720 g), made with raw- and pasteurized-milk, were purchased and sampled as described below. The cheeses were, on average, 65-day old postproduction which is slightly before the average "consumption age" of 70 days for an expiry date of 120 days. The hearts and rinds of cheeses were analyzed separately. Rind sampling was performed on the first 5 mm deep of each cheese side. Heart sampling was carried out on the remaining part of the cheese at a depth of >5 mm. The 25 g samples were homogenized with a stomacher (BagMixer^R 400, Interscience, ST Nom, France) and used for the microbiological analysis.

2.2. Growth conditions and lactic acid bacteria isolation

Twenty five grams (25 g) of samples were homogenized in 225 mL of 0.9% sterile saline solution, then serial dilutions (10^{-1} to 10^{-7}) were performed and 1 mL of each dilution was plated onto MRS medium (VWR international, Fontenay-sous-Bois, France) agar plates. Afterwards, plates were incubated for 72 h at 30 °C and then inspected. The selection of mesophilic colonies were based on the characteristics shape and colour of *Lactobacilli* and *Leuconostoc* genera from countable plates (30 to 300 CFU) and were purified by re-streaking on the MRS medium.

2.3. MALDI-TOF MS profile acquisition

Solid samples of a same isolated bacterial strain were loaded three times onto ground steel MALDI target according to the manufacturer's instructions (Bruker Daltonics, Bremen, Germany). Briefly, 3 CFU from a bacterial strain were taken off from agar plates with a 10 µL pipet tip, smeared onto the target, dried at room temperature and overlaid with 2 µL of matrix solution (10 mg/mL of α -cyano-4-hydroxycinnamic acid in acetonitrile:water:trifluoroacetic acid, 50:47.5:2.5 v/v/v).

Samples were automatically analyzed using a MALDI-TOF mass spectrometer (AutoflexSpeed from Bruker Daltonics) running Flexcontrol 3.4 software. Calibration of mass spectrometer was achieved with the Bruker's bacterial test standard (Bruker Daltonics). This calibration kit contains a typical protein extract of *E. coli* DH5 alpha spiked with two additional pure proteins (RNase A and myoglobin) to cover an overall mass range from 4 to 17 kDa. For each loaded sample, the MS-signals were acquired in positive linear mode in the 2000–20,000 Da *m/z* range by summing 480 laser-shot spectra, according to the manufacturer's automatic method MBT_FC.par (voltage values of ion sources #1 and #2 set as 19.5 and 18.30 keV, respectively; lens tension = 6.9 keV; pulsed extraction = 280 nsec; laser intensity between 40 and 60%).

2.4. Bacteria identification

2.4.1. MALDI-TOF-MS bacterial identification

Mass spectra were processed using BioTyper software (version 3.0; Bruker Daltonics) running with the BioTyper database version DB-5989, containing 5989 reference MALDI-TOF MS profiles (5298 of bacteria, 626 of yeasts and 65 of filamentous fungi). Matching between experimental MALDI-TOF MS profiles obtained from bacteria isolates and the reference MALDI-TOF MS profiles is expressed by BioTyper according to a Log(Score) and an associated-colour code (green, yellow and red). Briefly, a BioTyper Log(score) exceeding 2.3 (green colour) indicates a highly probable identification at the species level. A Log(score) between 2.0 and 2.3 means highly probable identification at the genus level (green colour) and probable identification at the species level. A Log(score) between 1.7 and 2.0 (yellow colour) implies only probable

genus identification, while score value under 1.7 (red colour) means no significant similarity between the unknown profile and any of those of the database. Dendrogram of identified strains was performed with the BioTyper MSP Dendrogram Creation Standard Method (version 1.4) using the default settings. The mass spectra triplicates of 197 strains were combined in 197 mass spectrum profiles (MSP) and used to generate the dendrogram. The default settings were as follow: the distance measure was set as correlation; the linkage was set as average; the score oriented dendrogram was selected, the score threshold value for single organism was 300 and the score threshold value for related organisms was 0.

2.4.2. DNA extraction and 16S rDNA PCR

One isolate of each cluster identified by MALDI-TOF-MS was selected for 16S rDNA gene sequencing. DNA was extracted from O/N culture obtained in MRS at 30 °C with the Promega Wizard® Genomic DNA purification kit according to the manufacturer recommendations. The 16S rDNA was amplified using two universal primers S1 (5'-AGAGTTG ATCMTGGCTCAG-3') and S2 (5'-GGTACCTGTACGAYTTC-3') yielding 1200 to 1500 bp PCR products. The PCR mixture was performed according to the manufacturer's instructions (DreamTaq, PCR Master Mix, Thermo Scientific, Waltham, MA, USA). The following PCR conditions were used: initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min and a final elongation step at 72 °C for 10 min. The PCR products were analyzed by agarose (1%) gel electrophoresis and purified with the GeneJET PCR purification kit (Thermo Scientific). PCR product sequencing was performed by Eurofins Genomics (Germany). The partial 16S rDNA gene sequences were aligned to the NCBI database in order to identify the genus. Phylogenetic tree was built using MEGA7 program package (Kumar et al., 2016).

2.5. Statistical analysis

All experiments were carried out in triplicate and LAB data were expressed as a mean with standard error. Differences between raw and pasteurized milk and heart and rind samples were considered significant for a p -value < 0.05 calculated using two-way ANOVA (milk and sampling point) (XLSTAT, Version 2015.4.01.21575).

3. Results

3.1. LAB counts in the Maroilles cheeses revealed slight differences

The mesophilic LAB population of Maroilles made with raw or pasteurized milk was estimated by plate counting on MRS agar medium. LAB colonies appeared with characteristics shape and colour of *Lactobacilli* and *Leuconostoc* genera which are generally round, with translucent or white colour and various aspects. Although the Maroilles cheese made with raw milk contained a slightly more important amount of LAB (rind: $1.33 \cdot 10^7 \pm 0.57$ CFU/g and heart: $7.33 \cdot 10^6 \pm 3.79$) compared to the cheese made with pasteurized milk (rind: $9.23 \cdot 10^6 \pm 0.6$ CFU/g and heart: $6.2 \cdot 10^6 \pm 2.08$ CFU/g), this difference was not statistically significant ($p > 0.05$). However, the LAB population in the rind tends to be higher than in the heart ($p = 0.053$).

3.2. MALDI-TOF MS identification led to typical signatures for LAB strains

Different LAB colonies were identified by MALDI-TOF MS. All LAB strains yielded MALDI-TOF mass spectra with protein/peptide mass signals within the 2–12 kDa mass range. Fig. 1 shows the raw MALDI-TOF MS profiles (black & white panels) and the bacteria identification obtained from 3 CFU of distinct strains. As illustrated in Fig. 1A, B, C and D, the raw MALDI-TOF MS profiles clearly differ in terms of number and intensity of mass signals. The obtained MALDI-TOF MS profiles were then compared to the reference spectra of the BioTyper database

and their similarity was expressed by a BioTyper Log(score). The colour panels (Fig. 1E, F, G and H) illustrate, as stick spectra, the matching of the experimental MALDI-TOF MS profile of strain CFU (top half coloured panels) and the matching reference MALDI-TOF MS profiles of the BioTyper database (bottom half coloured panels). The green, yellow and red sticks in the top half coloured panels indicate that the peak matching (intensity and m/z value) between experimental and database reference MALDI-TOF MS profiles is excellent, medium and low, respectively. The relevance of the matching is evaluated by the Log(score) as mentioned in the legend of the Fig. 1. The Log(score) takes into account the number of matching peaks, the total number of peaks, the peak weight representing species specificity and a correlation factor related to the matching peak intensity (Cherkaoui et al., 2010). In fine, three different genera of LAB including *Leuconostoc pseudomesenteroides*, *Enterococcus devriesei* and 7 species belonging to the *Lactobacillus* genus; e.g. *L. plantarum*, *L. paracasei*, *L. curvatus*, *L. rhamnosus*, *L. fructivorans*, *L. parabuchneri*, *L. brevis* were identified.

The 197 LAB colonies (105 and 92 from Maroilles made with raw and pasteurized milk, respectively) isolated on MRS agar plates were identified by MALDI-TOF MS (158 isolates (80.20%) with Log(score) ≥ 2.3 ; 34 isolates (17.26%) with Log(score) ≤ 2.3 and ≥ 2.0 ; 5 isolates (2.54%) with Log(score) ≤ 2.0 and ≥ 1.7 (Supplemental data Table 1). Seventy seven isolates of the rind of the two types of Maroilles were identified (38 and 39 in the cheese made with raw milk and pasteurized milk, respectively) as 4 species of *Lactobacillus* genus (*L. paracasei*, *L. curvatus*, *L. fructivorans*, *L. brevis*). However *L. brevis* was identified only on the rind of cheese made with raw milk. The 120 isolates of cheese heart were identified (67 and 53 in the cheese made with raw milk and pasteurized milk, respectively) as 3 different genera of LAB, including *Leuconostoc*, *Enterococcus* and 5 species belonging to the *Lactobacillus* genus (*L. plantarum*, *L. paracasei*, *L. rhamnosus*, *L. parabuchneri*, *L. brevis*). Notably, the species *E. devriesei* and *L. rhamnosus* were isolated only from the heart of cheese made with raw milk. As depicted in Fig. 2, *L. parabuchneri* was isolated only from the heart of cheese made with pasteurized milk. To strengthen these MALDI-TOF-MS data, we performed the identification of 10 selected strains by 16S rDNA sequencing and concluded to a perfect correlation with the MALDI-TOF MS based identifications performed on the same strains (Supplemental data Table 2). Finally, the MSP-based dendrogram (Fig. 3) of the 197 LAB strains constructed using the MALDI BioTyper explorer module illustrates the hierarchical clustering of strains from the Maroilles cheeses and provides the relationships of similarity among each of clusters detected. The dendrogram based on the bacterial identification obtained following 16S rDNA sequencing is given as Supplemental data 3.

4. Discussion

4.1. Lab content of raw versus pasteurized milk made Maroilles reveals usual and unusual trends

Pasteurization of milk does not affect the total mesophilic LAB number in comparison to the value obtained from the cheese made with the raw milk since no significant difference in the number of CFU/g between both kinds of cheese ($p < 0.05$) was observed. Nevertheless, many LAB species were found in both kinds of Maroilles cheeses, e.g. *L. plantarum*, *L. paracasei*, *L. curvatus*, *L. rhamnosus*, *L. fructivorans*, *L. parabuchneri*, *L. brevis*. As previously reported, some *Lactobacilli* are present in the natural microflora of dairy products and arise from animals, farms and dairies: *L. casei* ssp. *casei*/*L. paracasei* ssp. *paracasei*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. brevis*, *L. buchneri*, *L. curvatus*, *L. acidophilus* and *L. pentosus* (Corbo et al., 2001; Demarigny et al., 1997; Gobetti et al., 2002; Jordan and Cogan, 1993; López and Mayo, 1997; Medina et al., 2001). *Lactobacilli*, especially *L. curvatus*, represents a type of milk microbiota that is resistant to pasteurization (Martley and Crow, 1993). Moreover the presence of LAB

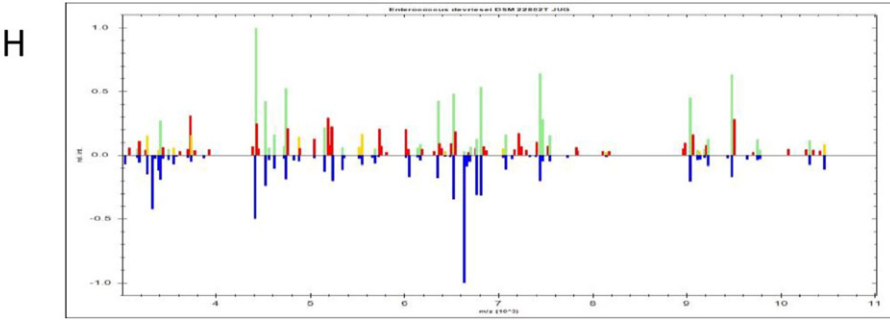
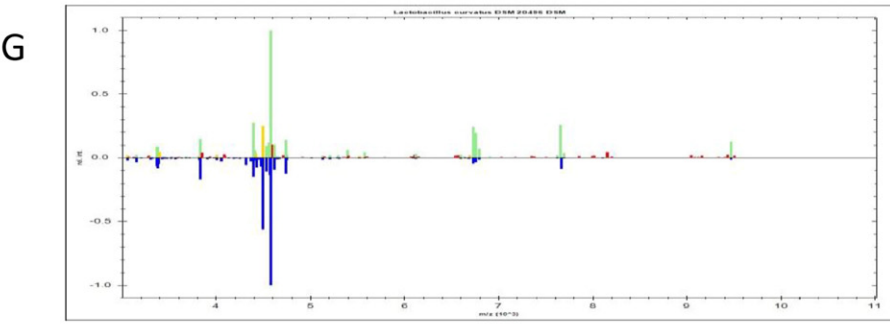
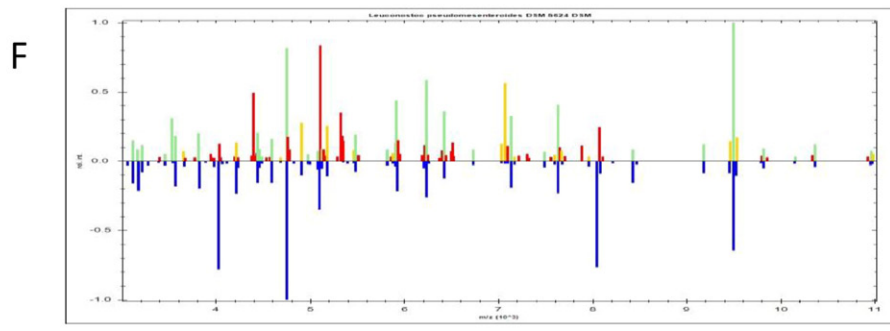
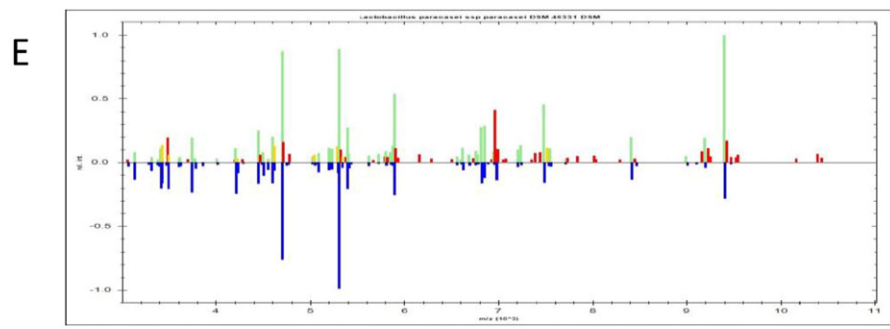
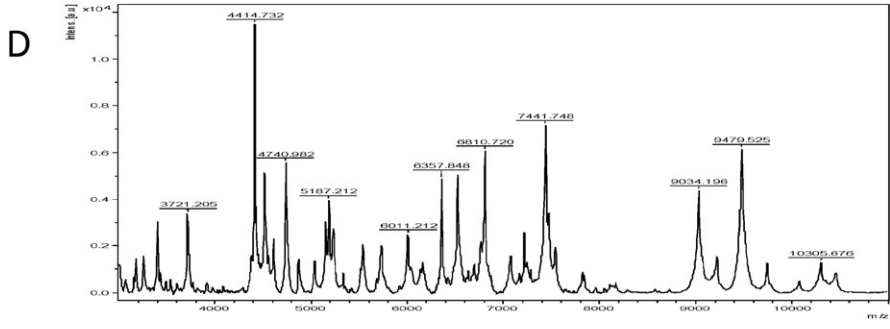
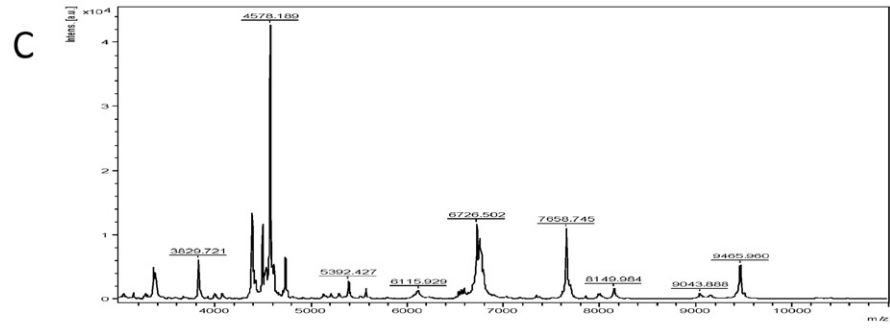
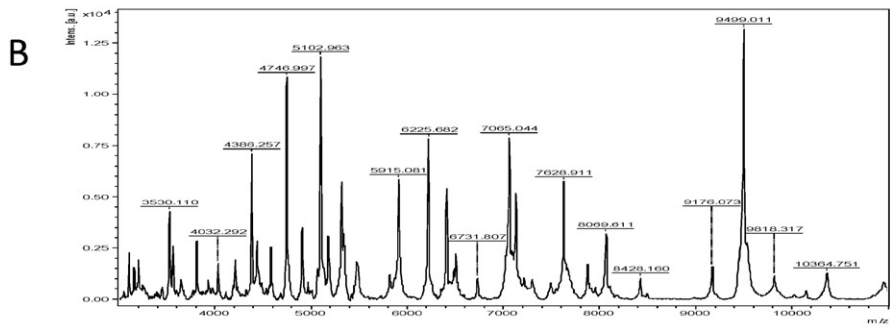
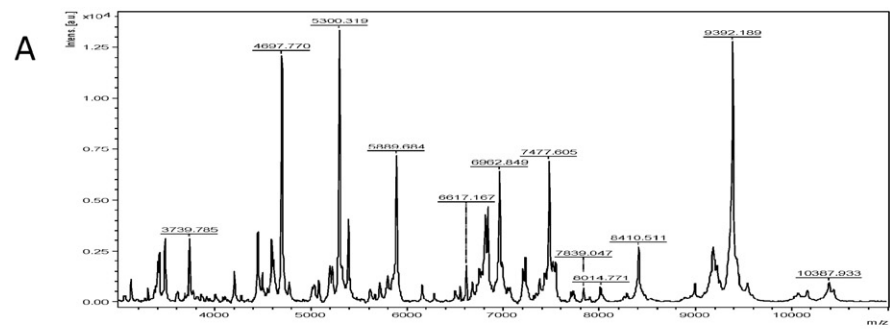


Fig. 1. Raw MALDI-TOF MS profiles (A, B, C and D panels) and the bacteria identifications obtained from 3 CFU of distinct strains (E, F, G and H panels). The identification log(scores) were 2.55 (*Lactobacillus paracasei* ssp. *paracasei*; panel E), 2.32 (*Leuconostoc pseudomesenteroides*; panel F), 2.47 (*Lactobacillus curvatus*; panel G) and 2.24 (*Enterococcus devriesei*; panel H).

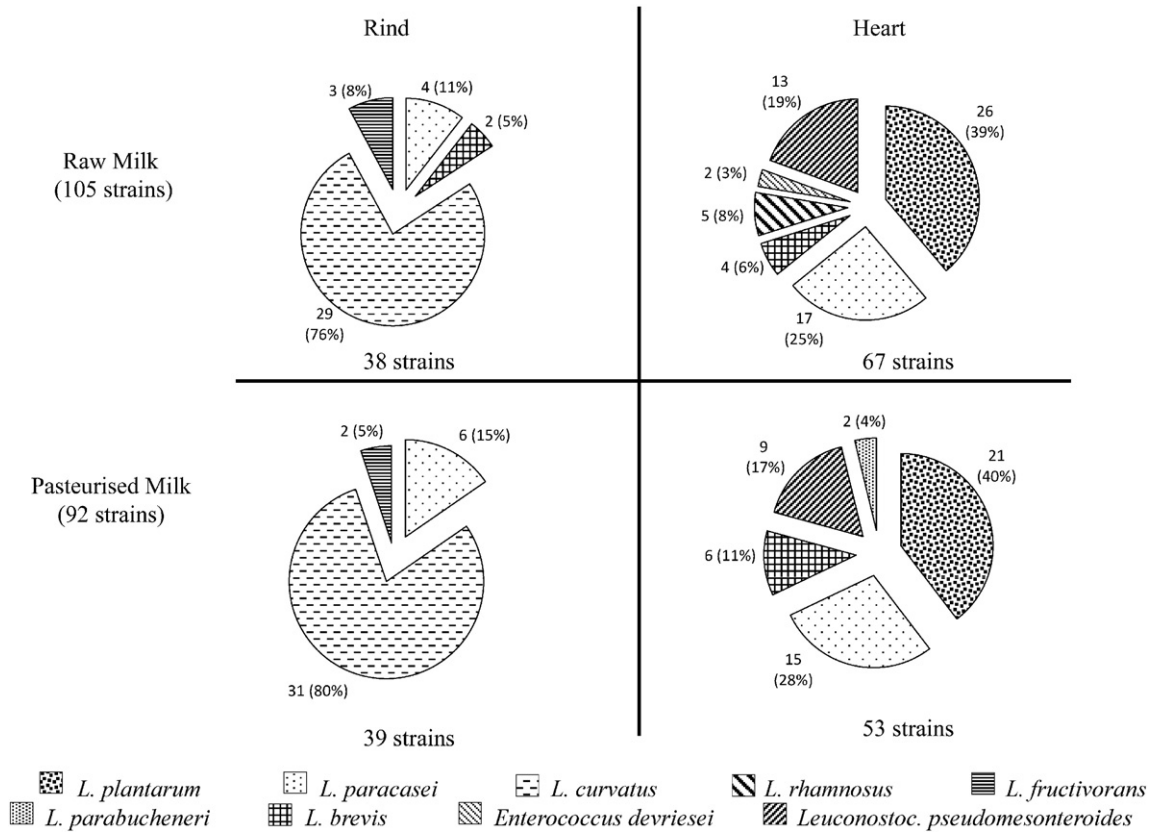


Fig. 2. Repartition of LAB strains on the rind and in the heart of cheeses made with raw and pasteurized milk.

could be also attributed to contamination occurring after pasteurization (Martley and Crow, 1993).

However, pasteurization of milk could affect only specific species as some species were only identified in raw milk cheese or pasteurized

milk cheese. Notably, the *E. devriesei* species was identified by MALDI-TOF MS profiling only in the Maroilles cheese made from raw milk likely due to its thermal sensitivity to the pasteurization conditions (temperature and time) (Svec et al., 2005). Moreover, LAB diversity is higher in

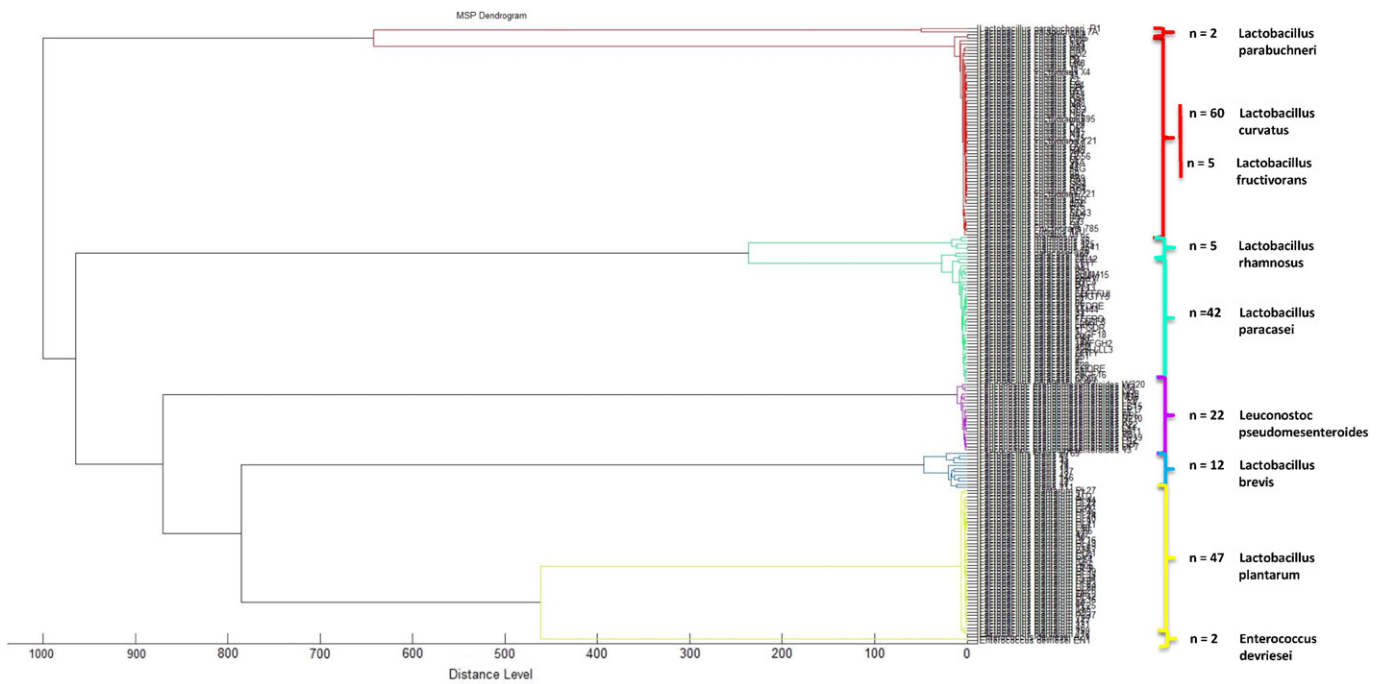


Fig. 3. Dendrogram illustrating the hierarchical clustering of the 197 strains identified by MALDI-TOF-MS. The dendrogram was created using the BioTyper MSP Dendrogram Creation Standard Method, version 1.4 with default settings as follow: Distance measure: correlation; Linkage: average, Score oriented dendrogram: selected; Score threshold single: organism–300, related–0.

the heart of the cheese because LAB are well adapted to growth conditions of low pH, high NaCl concentration, anaerobiosis and lack of fermentable carbohydrates (Montel et al., 2014). Interestingly, we found *L. paracasei* and *L. plantarum* in the heart of both cheeses, species which were described as the most frequently encountered species in the camembert cheese (Henri-Dubernet et al., 2008). Elsewhere *L. plantarum* could become the dominant LAB species in several types of cheese during ripening process (Hynes et al., 2003; Rantsiou et al., 2008; Sánchez et al., 2005). In view of the manufacturing process, the salt-tolerance of *L. curvatus* (Belfiore et al., 2013) can explain its presence only on the rind of cheeses studied in this work but also demonstrates that all other species isolated from the rind are tolerant to salt-washing process.

4.2. Analysis methods

In a general manner, identification of *Lactobacilli* from cheeses relies on methods based on phenotypic features and/or on physiological and biochemical criteria. However these complex and time-consuming methods can over- or underestimate the microbiological diversity of a food ecosystem (Ercolini et al., 2001). In contrast the nucleic acid-based molecular approaches have proven to be powerful tools to inventory the microbial diversity in food samples in spite of limitations, including the possibility of drawbacks related to DNA or RNA isolation, amplification and cloning (Coeuret et al., 2003).

The recent soaring of bacteria identification based on MALDI-TOF MS profiling relies on several advantages ranging from high-throughput, robustness, recognizing-based method, ultrafast tendency test, ease of use to low cost per test. MALDI-TOF MS profiling can be easily implemented as routine analysis (Pavlovic et al., 2013) and supplements the phenotypic methods for fast identification in the first step of characterization of the culturable microbial community in dairy products. This fast, cheap, robust and reliable method of bacteria identification stands as an attractive alternative to biochemical and even molecular biology-based identification approaches requiring no extraction of nucleic acids and PCR or sequencing steps (Dec et al., 2014; Vithanage et al., 2014). Regarding the identification of *Lactobacillus* species, Dušková et al. (2012) demonstrated the higher success rates of species level assignment using MALDI-TOF MS approach (93%) than PCR (77%) (Dušková et al., 2012). In some cases, MALDI-TOF MS profiling of bacteria allows identification of bacteria to subspecies level (Carbonnelle et al., 2011). Therein, the MALDI-TOF-MS method presented as affordable, sustainable, robust method could warrant a better management of microorganism identification.

However, the MALDI-TOF-MS based bacterial identification is not exempt of drawbacks: (i) restricted application fields without prior culture and fresh culture (<24 h) of isolated microorganisms are required (because the commercially available database has been constructed with fresh cultures); (ii) a method of low analytical sensitivity ($\pm 10^5$ – 10^6 bacteria/deposit) combined to a lack of susceptibility information; (iii) a sample preparation method essential and crucial; (iv) a difficult but not impossible discrimination of microorganisms phylogenetically very close (Paauw et al., 2015) and finally (v) a too restricted content of the MS-profile database. Besides, the need of a global quality control program of the method and that the principle of identification relies on databases distinct from one manufacturer to another act as both strengths and limitations. The set-up of a single, worldwide database of bacterial MALDI-TOF-MS reference spectra of a larger set of microbial strains and/or of a dedicated database for food industry correlated with genomic data will be a key progress in the spreading of this bacterial identification method.

5. Conclusions

The LAB diversity in Maroilles cheese is directly related to the treatment steps of the milk, although the number of CFU/g of LAB is not

directly affected by this treatment. Here, the use of MALDI-TOF MS profiling method for the LAB identification allowed to identify, with high match values, different LAB strains belonging to different genera and even discriminate, with statistically significant identification scores, phylogenetically close species into the same genus. Remarkably, the genera diversity of LAB is more important in the heart of the cheese made with raw milk with the presence of *E. devriesei*. Interestingly, *Lactobacillus* is the dominant LAB genus on the rind and in the heart of both kinds of cheeses. However, the species found are slightly different in the heart and the rind: *L. rhammosus* was only found in the heart of raw milk Maroilles cheese while *L. curvatus* was found only on the rind of both cheeses.

Fast microbial identification is becoming increasingly necessary in industry to improve microbial control, reduce biocide consumption, to avoid cost-intensive recall of contaminated products and damage to brand reputation. While MALDI-TOF-MS has revolutionized speed and precision of microbial identification for clinical isolates, in contrast few performance studies have been published so far focusing on suitability for particularly industrial applications. The MALDI-TOF MS based microbial identification is a molecular approach that bridges the gap between the reliability of test results produced from a biochemical-based phenotypic system and a genotypic identification system. The MALDI-TOF-MS-based bacteria identification appears as a method approximately 24 h shorter than other methods, cost-effective, robust, reliable, evolutive and well-suited for data management. Concomitantly, certain aspects, depending directly on the method principle, are both strengths and weaknesses (e.g. the dependence to a database, the need of a global quality control program, the inability to identify non-viable microorganisms having their DNA in the sample). In all cases, the real limitations (e.g. the need of fresh cultures, no susceptibility information, a challenging discrimination between phylogenetically closely-related strains) of this bacterial identification method should be considered and addressed.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.005>.

Acknowledgements

We are indebted to Dr. Florie DESRIAC for her helpful assistance, and Dr. Delphine Caly for the critical reading of the manuscript. The MALDI-TOF MS profiling experiments were performed on the REALCAT platform issued from French governmental subvention administrated by the French National Research Agency (ANR) within the frame of the 'Future Investments' program (ANR-11-EQPX-0037). The Nord-Pas-de-Calais Region and the FEDER are also acknowledged for their financial contributions to the acquisition of equipments.

References

- Belfiore, C., Raya, R.R., Vignolo, G.M., 2013. Identification, technological and safety characterization of *Lactobacillus sakei* and *Lactobacillus curvatus* isolated from Argentinean anchovies (*Engraulis anchoita*). SpringerPlus 2, 257. <http://dx.doi.org/10.1186/2193-1801-2-257>.
- Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J., Ferroni, A., Gutmann, L., Nassif, X., 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin. Biochem. 44, 104–109. <http://dx.doi.org/10.1016/j.clinbiochem.2010.06.017>.
- Carbonnelle, E., Grohs, P., Jacquier, H., Day, N., Tenza, S., Dewailly, A., Vissouarn, O., Rottman, M., Herrmann, J.-L., Podglajen, I., Raskine, L., 2012. Robustness of two MALDI-TOF mass spectrometry systems for bacterial identification. J. Microbiol. Methods 89, 133–136. <http://dx.doi.org/10.1016/j.mimet.2012.03.003>.
- Chalupová, J., Raus, M., Sedlářová, M., Šebela, M., 2014. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnol. Adv. 32, 230–241. <http://dx.doi.org/10.1016/j.biotechadv.2013.11.002>.
- Cherkaoui, A., Hibbs, J., Emonet, S., Tangomo, M., Girard, M., Francois, P., Schrenzel, J., 2010. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J. Clin. Microbiol. 48, 1169–1175. <http://dx.doi.org/10.1128/JCM.01881-09>.

- Coeuret, V., Dubernet, S., Bernardeau, M., Gueguen, M., Vernoux, J., 2003. Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. *Lait* 83, 269–306. <http://dx.doi.org/10.1051/lait:2003019>.
- Cogan, T.M., 2011. Bacteria, beneficial *Brevibacterium linens*, *Brevibacterium aurantiacum* and other smear microorganisms. In: Fuquay, J.W., Fox, P.F., McSweeney, P. (Eds.), *Encyclopedia of Dairy Sciences*, pp. 395–400.
- Corbo, M.R., Albenzio, M., De Angelis, M., Sevi, A., Gobbetti, M., 2001. Microbiological and biochemical properties of canestrato pugliese hard cheese supplemented with bifidobacteria. *J. Dairy Sci.* 84, 551–561. [http://dx.doi.org/10.3168/jds.S0022-0302\(01\)74507-9](http://dx.doi.org/10.3168/jds.S0022-0302(01)74507-9).
- Dec, M., Urban-Chmiel, R., Gnat, S., Puchalski, A., Wernicki, A., 2014. Identification of *Lactobacillus* strains of goose origin using MALDI-TOF mass spectrometry and 16S-23S rDNA intergenic spacer PCR analysis. *Res. Microbiol.* 165, 190–201. <http://dx.doi.org/10.1016/j.resmic.2014.02.003>.
- Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey, D., Korsak, N., Daube, G., 2014. Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. *J. Dairy Sci.* 97, 6046–6056. <http://dx.doi.org/10.3168/jds.2014-8225>.
- Demarigny, Y., Beuvier, E., Buchin, S., Pochet, S., Grappin, R., 1997. Influence of raw milk microflora on the characteristics of Swiss-type cheeses: II. Biochemical and sensory characteristics. *Lait* 151–167.
- Dušková, M., Šedo, O., Kšicová, K., Zdráhal, Z., Karpíšková, R., 2012. Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. *Int. J. Food Microbiol.* 159, 107–114. <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.07.029>.
- Ercolini, D., Moschetti, G., Blaiotta, G., Coppola, S., 2001. The potential of a polyphasic PCR-dGGE approach in evaluating microbial diversity of natural whey cultures for water-buffalo Mozzarella cheese production: bias of culture-dependent and culture-independent analyses. *Syst. Appl. Microbiol.* 24, 610–617. <http://dx.doi.org/10.1078/0723-2020-00076>.
- Freiwald, A., Sauer, S., 2009. Phylogenetic classification and identification of bacteria by mass spectrometry. *Nat. Protoc.* 4, 732–742. <http://dx.doi.org/10.1038/nprot.2009.37>.
- Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A., Di Cagno, R., 2002. Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. *Crit. Rev. Food Sci. Nutr.* 42, 223–239. <http://dx.doi.org/10.1080/10408690290825538>.
- Gori, K., Ryssel, M., Arneborg, N., Jespersen, L., 2013. Isolation and identification of the microbiota of Danish farmhouse and industrially produced surface-ripened cheeses. *Microb. Ecol.* 65, 602–615. <http://dx.doi.org/10.1007/s00248-012-0138-3>.
- Henri-Dubernet, S., Desmasures, N., Guéguen, M., 2008. Diversity and dynamics of lactobacilli populations during ripening of RDO Camembert cheese. *Can. J. Microbiol.* 54, 218–228. <http://dx.doi.org/10.1139/W07-137>.
- Hynek, R., Hochel, I., 2013. Identification of bacteria using mass spectrometry techniques. *Int. J. Mass Spectrom.* 353, 67–79. <http://dx.doi.org/10.1016/j.ijms.2013.04.016>.
- Hynes, E., Bach, C., Lamberet, G., Ogier, J.-C., Son, O., Delacroix-Buchet, A., 2003. Contribution of starter lactococci and adjunct lactobacilli to proteolysis, volatile profiles and sensory characteristics of washed-curd cheese. *Lait* 83, 31–43. <http://dx.doi.org/10.1051/lait:2002048>.
- Jordan, K.N., Cogan, T.M., 1993. Identification and growth of non starter lactic acid bacteria in Irish cheddar cheese. *Ir. J. Agric. Food Res.* 32, 47–55.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* <http://dx.doi.org/10.1093/molbev/msw054> (msw054).
- López, S., Mayo, B., 1997. Identification and characterization of homofermentative mesophilic *Lactobacillus* strains isolated from artisan starter-free cheeses. *Lett. Appl. Microbiol.* 25, 233–238.
- Martley, F.G., Crow, V.L., 1993. Interactions between non-starter microorganisms during cheese manufacture and ripening. *Int. Dairy J.* 3, 461–483. [http://dx.doi.org/10.1016/0958-6946\(93\)90027-W](http://dx.doi.org/10.1016/0958-6946(93)90027-W).
- Medina, R., Katz, M., Gonzalez, S., Oliver, G., 2001. Characterization of the lactic acid bacteria in ewe's milk and cheese from northwest Argentina. *J. Food Prot.* 64, 559–563.
- Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, N., Berthier, F., 2014. Traditional cheeses: rich and diverse microbiota with associated benefits. *Int. J. Food Microbiol.* 177, 136–154. <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.02.019>.
- Paaauw, A., Jonker, D., Roeselers, G., Heng, J.M.E., Mars-Groenendijk, R.H., Trip, H., Molhoek, E.M., Jansen, H.-J., van der Plas, J., de Jong, A.L., Majchrzykiewicz-Koehorst, J.A., Speksnijder, A.G.C.L., 2015. Rapid and reliable discrimination between *Shigella* species and *Escherichia coli* using MALDI-TOF mass spectrometry. *Int. J. Med. Microbiol.* 305, 446–452. <http://dx.doi.org/10.1016/j.ijmm.2015.04.001>.
- Pavlovic, M., Huber, I., Konrad, R., Busch, U., 2013. Application of MALDI-TOF MS for the identification of food borne bacteria. *Open Microbiol. J.* 7, 135–141. <http://dx.doi.org/10.2174/1874285801307010135>.
- Rantsiou, K., Urso, R., Dolci, P., Comi, G., Cocolin, L., 2008. Microflora of Feta cheese from four Greek manufacturers. *Int. J. Food Microbiol.* 126, 36–42. <http://dx.doi.org/10.1016/j.ijfoodmicro.2008.04.031>.
- Sánchez, I., Seseña, S., Poveda, J.M., Cabezas, L., Palop, L., 2005. Phenotypic and genotypic characterization of lactobacilli isolated from Spanish goat cheeses. *Int. J. Food Microbiol.* 102, 355–362. <http://dx.doi.org/10.1016/j.ijfoodmicro.2004.11.041>.
- Scheler, O., Glynn, B., Kurg, A., 2014. Nucleic acid detection technologies and marker molecules in bacterial diagnostics. *Expert. Rev. Mol. Diagn.* 14, 489–500. <http://dx.doi.org/10.1586/14737159.2014.908710>.
- Svec, P., Vancanneyt, M., Koort, J., Naser, S.M., Hoste, B., Vihavainen, E., Vandamme, P., Swings, J., Björkroth, J., 2005. *Enterococcus devriesei* sp. nov., associated with animal sources. *Int. J. Syst. Evol. Microbiol.* 55, 2479–2484. <http://dx.doi.org/10.1099/ijs.0.63851-0>.
- Vithanage, N.R., Yeager, T.R., Jadhav, S.R., Palombo, E.A., Datta, N., 2014. Comparison of identification systems for psychrotrophic bacteria isolated from raw bovine milk. *Int. J. Food Microbiol.* 189, 26–38. <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.07.023>.
- Welker, M., 2011. Proteomics for routine identification of microorganisms. *Proteomics* 11, 3143–3153. <http://dx.doi.org/10.1002/pmic.201100049>.
- Yu, J., Wang, H.M., Zha, M.S., Qing, Y.T., Bai, N., Ren, Y., Xi, X.X., Liu, W.J., Menghe, B.L.G., Zhang, H.P., 2015. Molecular identification and quantification of lactic acid bacteria in traditional fermented dairy foods of Russia. *J. Dairy Sci.* 98, 5143–5154. <http://dx.doi.org/10.3168/jds.2015-9460>.