University of Stavanger

Faculty of Science and Technology

MASTER'S THESIS

Study program/ Specialization: Biological Chemistry	**Spring semester, 2015** Open /Restricted access
Writer: CHEUZEVILLE Lisa	…… (Writer's signature)
Faculty supervisor: LILLO Cathrin	
Thesis title: Rapid detection of microorganisms in the dairy value chain by MALDI-TOF MS	
Credits (ECTS): 60	
Key words: MALDI-TOF MS Identification Species Yeasts Bacteria Milk Cheese Mastitis Resistance B-lactamase	**Pages: 80** **+ enclosure: 77** **Stavanger, June, 12th 2015**

University of Stavanger

Master thesis in Biological Chemistry

Rapid detection of microorganisms in the dairy value chain by MALDI-TOF MS

CHEUZEVILLE Lisa

2014-2015

Supervisors:

FLIEGEL Daniel, PhD
Tine FoU, Fagleder hurtiganalyser
Tine R&D, Division leader Analytical Instrumentation

LILLO Cathrin
University of Stavanger, TN-IMN
Centre for Organelle Research

List of contents

List of Figures

List of Tables

Acknowledgements

First of all, I would like to thank TINE Company for giving me the opportunity to do this master thesis with an interesting subject.

More particularly, thank you to Daniel Fliegel for this interesting subject and his encouragement, to Inger Ødegård for her tips during my lab experiments and to Liv Sølverød and her team for their help, support.

I wish to thank the various professionals who were able to convey to me their passion for their job and knowledge. Thank you for your support.

I also want to thank my trainers for their guidance during this master degree. Also, thank you to my supervisor for her availability and advice.

Finally, I want to thank my boyfriend, Romain Grime, my parents, brother and sister for their support throughout this training.

Also, I would like to thank my friends for their encouragement and particularly to Tim Rice for his reading and comments about my thesis.

Abstract

Recently, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has developed as vigorous method for the identification of microorganisms in the clinical sector. MALDI-TOF MS is able to detect different chemical components such as peptides, proteins, and other biomolecules. Identification of microorganisms by this tool has been demonstrated to be accurate, rapid and lower cost than conventional methods.

Safety and quality are keywords for food industry, and in particular dairy industry; consequently there is a requirement for rapid identification of the pathogenic and spoilage microorganisms.

From a variety of raw milk and milk products, strains have been collected from different culture media and identified by MALDI-TOF MS (Biotyper, Bruker Daltoniks). Identification was made by comparison with the Bruker database, or to a reference library created. Detection of β-lactamase resistance has been studied using mastitis samples collected from the TINE Mastitis.

The results demonstrate that MALDI-TOF MS is an effective tool for microorganisms identification. Collection strains (such as ATCC strains) or strains identified by other methods can be incorporated into the database which was made for the identification of clinical strains. Detection of β-lactamase resistance is achieved faster than using antibiotic diffusion disk methods even if some of the parameters used in the method have potential for further improvement.

Abbreviations

2,5-DHB: 2,5-Dihydroxybenzoic acid

16S: component of small subunit ribosomal (30S) for Prokaryotes.

ACN: Acetonitrile

ATCC: American Type Culture Collection

CCP: Critical Control Point

CFU: Colony-forming unit

CNS: Coagulase negative Staphylococcus strains

Da: Dalton

DRBCA: Dichloran-Rose Bengal Chloramphenicol Agar

DT: Direct Transfer

eDT: Extended Direct Transfer

Eth: Ethanol

EX: Formic acid Extraction

HACCP: Hazard Analysis Critical Control Point

HCCA: α-Cyano-4-hydroxycinnamic acid

HIB: Heart Infusion Broth

ISO: Internal Organization for Standardization

LAB: Lactic Acid Bacteria

MALDI: Matrix Assisted Laser Desorption Ionization

MALDI-TOF MS: Matrix Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry

McF: Mac Farland (unit)

min: minutes

mPCA: skimmed milked Plate Count Agar

MRS: Man, Rogosa, Sharpe agar

MS: Mass Spectrometry

MSP: Main Spectra Profile

MW: Molecular Weight

MYP: Mannitol Egg Yolk Polymyxin agar

m/z: Mass/charge

nm: Nanometer (unit)

NMKL: Nordisk metodikkomité for næringsmidler

PCR: Polymerase Chain Reaction

PPC: Post-pasteurization contamination

RCM: Reinforced Clostridial Medium

R&D: Research and Development

RNA: Ribonucleic acid

RODAC: Replicate Organism Direct Agar Contact

rpm: Revolutions per minutes (speed of revolution). Define the speed of rotation of the centrifugation.

RTC: Real Time Classification

TFA: Trifluoro acetic acid

TOF: Time of Flight

TSA: Tryptic Soy Agar

TGEA: Tryptone Glucose Extract Agar

VRBD: Violet Red Bile Dextrose agar

Introduction

1. Scope of the study

For the food industry, quality of product is very important. Food authorities as European Commission described numerous microbiological criteria based on norms such as International Organization for Standardization (ISO).

For industry, these norms are mandatory in order to ensure control of the quality of products. Microbiological criteria allow:

- The security of consumers by identifying hazards (presence/absence of pathogens).
- Control of the natural flora and contamination. Microorganisms can be pathogens or spoilage microorganisms. Spoilage microorganisms are not dangerous for consumers but can modify the flavor and odor of products.
- Decrease the cost of wastage and provide benefits for the company.

Identification of microorganisms allows control of which kind of microorganisms are present and where in the dairy value chain (raw milk, heat-treated milk, cheeses). Hazard Analysis Critical Control Point (HACCP) method is used by the food industry to avoid unsafe products and control them during the production process.

Objectives of this study were to identify microorganisms from the dairy value chain by using a rapid method: Matrix Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry (MALDI-TOF MS).

My goal was to collect and isolate microorganisms from raw milk samples and milk products as cheese and consumer milk. These microorganisms were conserved (collections) until their identification by MALDI-TOF MS.

Different microorganisms have been isolated:

- Spores and thermoduric bacteria from the raw milk,
- Psychrotrophic bacteria from consumer milk at the end of the shelf life,
- Yeasts from surfaces of hard white type cheeses.

Moreover, samples which are responsible of mastitis have been collected by the Tine Mastitis Laboratory in Molde, Norway. These samples have also been analyzed and identified by MALDI-TOF MS. The detection of antibiotic resistance mechanisms have been tested for in some of these strains.

My internship was conducted in the Research and Development department in TINE firm (Måltidet hus, Stavanger, Norway) to filful my Biological Chemistry master degree at the University of Stavanger.

In the first time, evolution of the identification methods will be described. Then, the different microorganism groups relative to the dairy products analyzed in this thesis will be explained.
Then, an observation of different analysis will be described.
Finally, the results will be discussed in order to evaluate the MALDI-TOF MS system in the dairy value chain.
The conclusion will put into perspective all the results and provide a general discussion on the subject of this report.

2. Theoretical background

2.1. History of the identification of microorganisms: from GRAM staining to MALDI-TOF Mass Spectrometry

In microbiology, identification of microorganisms is one of the main interests and the technology used to identify microorganisms is under constant evolution.

Taxonomy is the study and classification of microorganism's diversity and the relationships which can exist between them.

Microorganism identification compares characteristics of each microorganism to those of microorganisms available in the classification.

Many species (e.g.: *Escherichia coli*) constitute a genus (e.g.: *Escherichia*), many genus constitute a family (e.g.: Enterobacteriaceae), group of families constitute a class (e.g.: Gamma proteobacteria), then many classes constitute a phylum (e.g.: Proteobacteria) and then group of phylums constitute a kingdom (e.g.: Bacteria).

There are different characteristics for bacteria used to classify them:

- Phenotypic characteristics
- Genetic characteristics
- Immunologic characteristics
- Chemical characteristics

Previously, identification was based on conventional tests (Marcadé, 2013) such as:

- Macroscopic aspects: morphology of colonies.
- Microscopic examination: hanging drop, Gram staining, culturing tests (aerobes, anaerobes, facultative aerobes, and microaerophilic), and biochemical tests (e.g.: metabolism of sugar degradation).

Chromogenic media have also been introduced later. This is culture medium whose principle is based on the ability of microorganisms "target" hydrolyzing, through specific enzymes, specific substrates: chromogenic substrates. In this fact, coloration of colonies appears (Perry et al., 1999).

In 1970's years, API (Analytical Profile Index) systems were developed. This miniaturized system commercialized by bioMérieux is composed of 20 tests and allow a quick identification (Smith et al., 1972).

Then, automatic systems such as Vitek 2® (bioMérieux) or Phoenix® (Becton Dickinson) were developed. These systems use a short incubation time (at least 8h) and allow a very quick identification which is completely autonomic.

Immunologic tests have been developed in order to identify some species of bacteria.

It used specific antibodies which bind to the antigen of the bacteria (Chattopadhyay et al., 2013;Wieckowska-Szakiel et al., 2002).

Later, a new molecular method appeared in order to identify bacteria. The first step consists of amplifying the 16S RNA of bacteria by PCR. Each cycle allows the duplication of the 16S RNA sequence, this is an exponential amplification. Nucleic acid molecules are then run into electrophoresis gel to be separated in function of their size. A profile is obtained which allows bacterial identification (Avaniss-Aghajani et al., 1994;Chiang et al., 2006).

Mass spectrometry and particularly MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry) have been introduced for bacterial identification. This method is known to detect and identify specific molecules. Charge molecules migrate to a detector and are separated by their molecular weight. The mass spectrum is then given and compared against a database of mass spectrum profiles.
All microorganisms from the same species or from the same genus have the same mass spectrum signature (fingerprint).
MALDI-TOF MS identifies microorganisms at strain level or at genus level and is able to obtain reproducible mass spectra.

2.2. Milk

Milk is a complex fluid secreted by the mammary glands of mammals and that is essential for all mammals at the beginning of their life. This is an emulsion of fatty mater which is in globular form in a liquid that is a matter proteic suspension in a serum. It is composed mainly of water but also consist of lipids, proteins and carbohydrates.
Milk is a good medium for bacterial growth. Fresh cow milk contains up to 5.10^3-5.10^4 CFU/g (Hayes, 1992). For the food industry, the presence of bacterial spoilage is responsible of economic loses (Dogan and Boor, 2003;He et al., 2009;Sørhaug and Stepaniak, 1997).
The goal of the pasteurization is to decrease the level of spoilage bacteria in raw milk and to eliminate pathogens (McAuley et al., 2012). The microflora found in the pasteurized milk is often the same as in the raw milk. The microorganisms found in the pasteurized milk come from different sources (He et al., 2009;Robinson, 2005):

- Teat surface,
- Contamination from teats surface can contaminate the milking machine,
- Hygiene of the environment,
- Hygiene of the milking machines.

In raw milk, different kind of microorganisms can be found:

- Thermophiles thermoduric bacteria,
- Mesophiles thermoduric bacteria,
- Psychrophiles thermoduric bacteria.

2.3. Definitions

Thermoduric bacteria resist heat treatment, and they contribute to the bacterial spoilage of the product (Dogan and Boor, 2003;He et al., 2009;Sørhaug and Stepaniak, 1997).

Thermoduric bacteria commonly found in the raw milk are *Microbacterium, Micrococcus, Enterococci, Lactobacilli and Corynebacteria (Gleeson et al., 2013).*

Thermoduric bacteria are enumerated using a laboratory method which consists to the heat-treatment of the milk for 30 min at 62.8 °C and then enumerate them by using standard plate count methods (Frank et al., 1992).

In the dairy industry, spore-formers such as *Bacillus cereus* group and *Clostridium* but also thermoduric bacteria such as *Streptococcus* (*S. thermophilus*), *Micrococcus* (*M. luteus*), *Corynebacterium* (*C. lacticum*) are the main microorganisms found in pasteurized milk (Sørhaug and Stepaniak, 1997).

Members of *Bacillus cereus* group are responsible in the pasteurized milk of “sweet curdling” (Daley and Hayes, 1992).

Psychrophilic *Bacillus spp.* produces heat-resistant enzymes similar to heat-resistant enzymes of *Pseudomonas* but its generation time is longer than those of *Pseudomonas*.

Thermophiles bacteria grow at 45 and above. Their optimal growth conditions occur between 55-65°C (Jay et al., 2005). Problematic of these kinds of microorganisms is that they can grow during the pre-heating step and also multiply during the cooling-down step (Murphy et al., 1999).

Mesophiles bacteria grow at between 20-45°C. Their optimal growth conditions are between 30-40°C (Jay et al., 2005). Microorganisms classified in this group can be spore-formers or non spore-formers.
Strains commonly found in the milk are *Bacillus (*spore-formers*), Microbacterium, Micrococcus, Enterococcus, Streptococcus* and *Arthrobacter.*

Spores are dormant, dehydrated and it is the resistant that some bacteria produced by spore-forming bacteria when environmental conditions become unfavorable. For example, bacteria produce spores when nutrients are not sufficient to survive, or when environmental conditions change (high temperatures, and extreme pH values) (Setlow, 2006).
Cycle of spore production by bacteria is described in Figure 1 below.

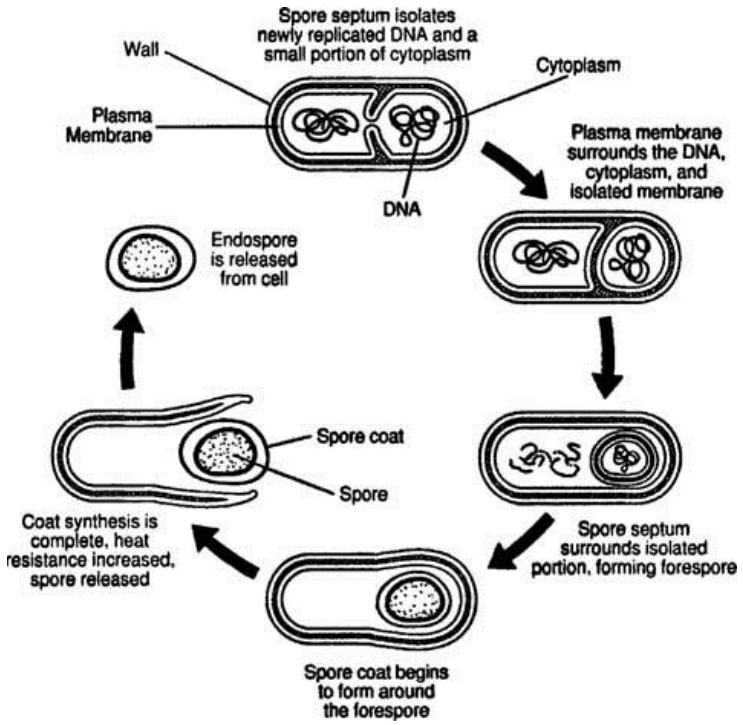


Figure 1: Cycle of production of spores by spore-forming bacteria as *Bacillus* and *Clostridium* (**Source:** http://www.cliffsnotes.com/sciences/biology/microbiology/prokaryotes-and-eukaryotes/prokaryotic-cells)

Spores stay dormant until that the environmental conditions become favorable. When good conditions are achieved, the spore germinates and produces a vegetative cell.
In the dairy industry, spore-forming bacteria and their spores have to be highly controlled since the spores survive to the pasteurization process.
Bacillus cereus group is restrained in dairy industry. The bacilli spore-forming bacterium, Gram positive, facultative anaerobes is known as a human pathogen due its ability to produce enterotoxins: emetic and diarrheal (Borge et al., 2001;Granum and Lund, 1997;Pácová et al., 2003;Rowan and Anderson, 1998).

Strains of *Bacillus* in raw milk are also found in the pasteurized milk (Pácová et al., 2003;Robinson, 2005). Strains commonly found are: *B. licheniformis, B. cereus and B. cereus var. mycoïdes, B. circulans, B. weihenstephanensis, B. subtilis, B. brevis, B. carotarum, B. firmus, B.lateropsorus, B.lentus, B. megatarium, B. polymyxa, B. pumilus, B. sphaericus, B. stearothermophilus and B. thuringiensis.*

Psychrotroph bacteria are able to grow at 0-20°C. Their optimal growth conditions are between of 10-20°C (Jay et al., 2005). In the milk, the psychrotrophic bacteria commonly found are:

- Gram negative bacteria: *Pseudomonas, Achromobacter, Aeromonas, Serratia, Alcaligenes, Chromobacterium* and *Flavobacterium*.
- Gram positive bacteria: *Bacillus, Clostridium, Corynebacterium, Streptococcus, Lactobacillus* and *Micrococcus* (Sørhaug and Stepaniak, 1997).

Some mesophilic bacteria are able to grow at refrigeration temperatures (0-7°C) these are also called psychrotrophs.

Enzymatic activities produced by these bacteria are important for Lactic Acid Bacteria (LAB) because they boost their growth. LAB consume peptides, amino acids and ammonia synthetized by these psychrotrophic bacteria. However, production of free fatty acids by *Pseudomonas* on the milk is unfavorable for LAB (Sørhaug and Stepaniak, 1997).

A lot of psychrotrophic bacteria are not heat-resistant and do not survive after heat-treatment. However, their presences after heat-treatment (pasteurization) indicate a post-pasteurization contamination.

Pseudomonas is the predominant microorganism (more than 10% of the microflora) implicated in the limitation of the shelf-life of processed milk at 4°C.

Moreover, a lot of these strains are heat resistant due to their heat-stable extracellular lipases, proteases, phosphatases and lecithinases. These enzymes are also responsible of the milk spoilage and their enzymatic activity can decrease the shelf life of the milk as they can decrease the organoleptic quality (Dogan and Boor, 2003;He et al., 2009;Sørhaug and Stepaniak, 1997). *Pseudomonas* is also often implied as post-pasteurization contamination (PPC). Species of *Pseudomonas* that are commonly identified in the milk are: *P. fluorescens, P. putida, P. fragi, P. maltophilia, P. migulae* (He et al., 2009;Robinson, 2005). Due to its metabolic activity, *Pseudomonas* is known as the “single most detrimental factor”. In fact, parameters of pasteurization (72°C-15s) and storage condition 4-7°C have been established to keep quality of final product. PPC of Pseudomonas is often at 0.001-1 CFU/mL. Generation time *P. fluorescens is* 9.4h at 4°C. Then after 10 days of storage, there are 3.10^7 CFU (colony-forming unit) /mL. To maintain the quality of pasteurized milk at 4-8°C, the dairy industry prefers to have *Pseudomonas* at low amount levels (Martin et al., 2011;Sørhaug and Stepaniak, 1997). *Paenibacillus spp.,* spore-forming bacteria can also be a factor which limits the shelf life of the pasteurized milk at 21 days (Martin et al., 2011).

The presence of Enterobacteriaceae in the pasteurized milk is often linked to inadequate heat-treatments, and post-pasteurization contamination from materials, the environment. This indicates poor food processing hygiene (Cirolini et al., 2013).

In the dairy industry, psychrotrophic thermoduric bacteria are able to grow at refrigeration temperatures (in the tank after milking and in the final products) and to survive the pasteurization process. These bacteria have an impact on the shelf life due to their grown at refrigerate temperatures and thus limit it (Te Giffel et al. 1997).

The quality of the raw milk from cows, goats, ewes are important. Milk is subject to control by authorities in order to have a good milk quality. They use certain criteria for the detection of microorganisms responsible of mastitis and determine the physical content (water content) to insure the quality of products.

2.4. Mastitis

Mastitis is a disease which corresponds to bacterial infection of cows, goat, and ewe udder and other mammals. *Staphylococcus aureus* is known as the more significant pathogen responsible for mastitis (Deshpande, 2007;Ruegg, 2012;Whist et al., 2009).

This infection of mammary glands is problem for farmers because quality of milk is modified (modification of taste, less nutritional, shelf life reduced) and quantity of milk produced by the cow is highly reduced. The poor quality and quantity of milk results in important economic loses (Nielsen and Emanuelson, 2013;Ruegg, 2012).

Different pathogens are able to cause mastitis. Environmental pathogens live in the cow's environment (for example: bedding materials, moisture, mud and manure). These bacteria are opportunistic: they infect host (e.g.: cow / goat) when they are immunosuppressed. Microorganisms invade teats and then bacteria are multiplied inside the gland (Ruegg, 2012;Whist et al., 2009).

Pathogens implied in the mastitis infection can be Gram negative as *Escherichia coli* and *Klebsiella spp.* or Gram positive as *Streptococcus dysagalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae and Staphylococcus aureus*.

Some of these pathogens are contagious and can be spread from an infected animal to others during the milking process. Hygiene of milking and cleaning milking machines is very important to avoid this contamination and transmission between animals.

In order to limit infection, the "5-points plan" has been done. Treatment of infected animals with dry cow therapy (antibiotic treatment) after bacteriological examination of cow milk is done. After treatment, if the infection remains, an appropriate treatment is given to them. In case of chronically infected animals, culling of them is done. Finally, maintenance of the milking machine has to be done frequently to avoid further contaminations from cow to cow and mechanical stress for the teats (Ruegg, 2012;Whist et al., 2009).

In Norway, only treatment by penicillin G is used by veterinary to treat infected cows.

In the laboratory, detection of mastitis is done by analysis of milk of infected quarter. Mastitis infection can be recognized by increase of the somatic cell count in the quarter milk infected (Ruegg, 2012;Whist et al., 2009).

In farms, when cows are infected and treat by antibiotics, they have to be identified. The presence of antibiotic residues in their milk is controlled before milking process and addition in the tank.

In some countries, antibiotics are often added to the cattle's food. This antibiotic ingestion is used in order to avoid infections and to have better performance of cows.

2.5. Cheeses: hard white type

Cheeses are produced by the coagulation of milk accompanied by draining process.
It is mainly composed caseins gel which keeps the butterfat globules and a part from the water-based fluid of milk.
Fabrication process of cheese (Hayes, 1981) is given in Figure 2 below.

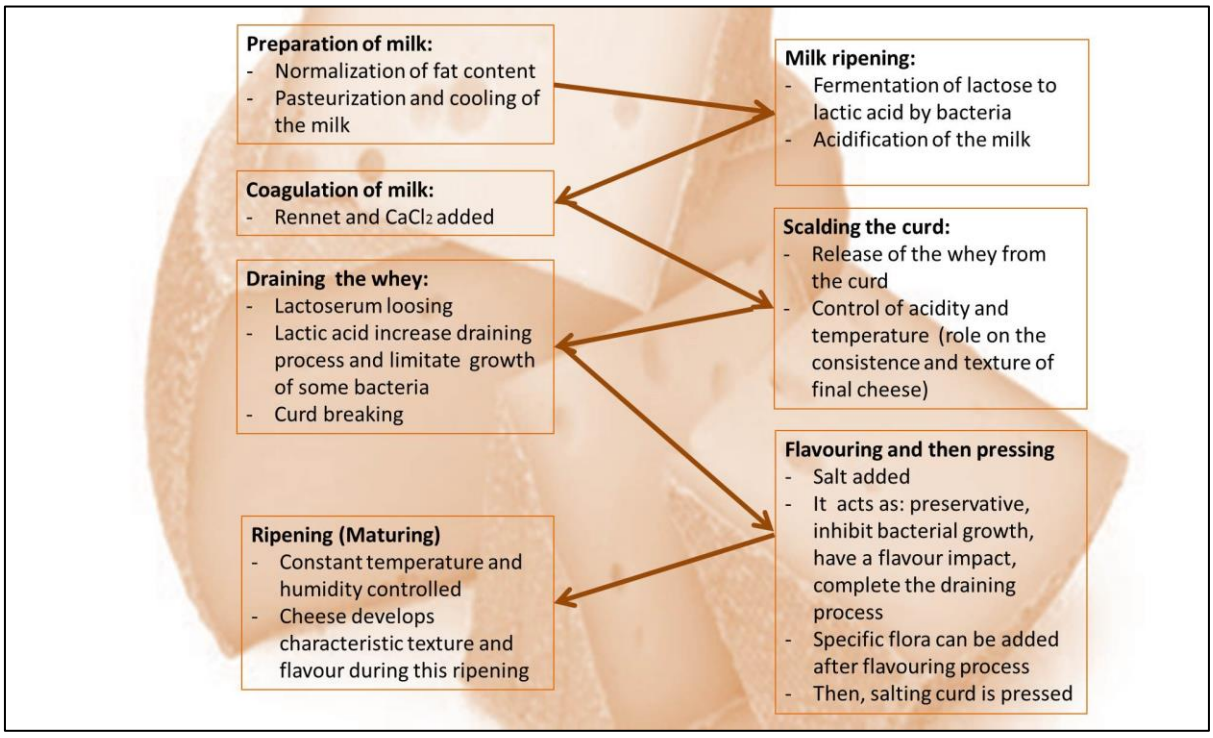


Figure 2: The stages of cheese production.

Hard white type cheeses are making with pasteurized milk.
Microbiota of cheese is complex and a wide range of microorganisms can be involved in cheese production. Two groups of microorganisms found in the milk process are described (Beresford et al., 2001;Bockelmann and Hoppe-Seyler, 2001;Smit, 2003):

- Starter flora: bacteria are added to the milk or they are naturally present in the milk (milk ripening step) which ferment the lactose of milk to lactic acid. As the pH of the milk decreases and other microorganisms in the milk cannot survive.
  The most common bacteria used as starter are: *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus helviticus* and *Lactobacillus delbrueckii*.
- Secondary flora: microorganisms derived from the ingredients or from the environment (during ripening process, cheese surfaces are exposed to non-sterile environment). These microorganisms included bacteria, yeast and fungi. All of these microorganisms have an interest in developing flavor and a casual effect on the texture during the ripening process (Beresford et al., 2001;Smit, 2003).

Yeasts on the cheese surface:

Yeasts are commonly found in the secondary flora of a wide variety of cheeses. However, their action in the ripening process has still not been identified.

Some of them are used to neutralize acidification due to their growth in presence of high salt concentrations.

A. Corsetti, J.Rossi and M. Gobbetti have studied yeast on the surface of cheeses. In their article, they described the different microorganisms identified.

From smear ripened cheeses (as Tilsit, Gruyère, Beaufort), yeasts described below were identified (Corsetti et al., 2001):

- *Candida* species*: Candida spp, C.zeylanoides, C. intermedia*
- *Debaryomyces* species*: Debaryomyces spp, D. hansenii*
- *Geotrichum* species*: Geotrichum candidum*
- *Kluveyromyces* species*: Kluveyromyces spp.*
- *Rhodoturola* species*: Rhodoturola spp, R. minuta, R. incospicua*
- *Yarrowia* species*: Yarrowia lipolytica*

In this smear ripened cheeses, yeasts can be added to the cheese surface. In the hard white type cheese, no yeasts or other microorganisms are voluntary spreading on the cheese surfaces.

2.6. HACCP (Hazard Analysis Critical Control Point)

The quality of milk and other products is important in the dairy industries to avoid economic lost and insure safety of consumers. Hazard Analysis Critical Control Point (HACCP) is a system which exists to prevent, suppress or reduce to an acceptable level every hazard (biologic, chemical or physical).

Currently, the HACCP preventive system is used in the food industry as a Standard.

HACCP system is based on seven steps (Mortimore and Wallace, 2013):

- **1:** Conduct a hazard analysis.
- **2:** Determine the critical control points (CCP: Critical Control Point).
  These are key points of the manufacturing process in which hazard control can be applied.
- **3:** Set the critical limits.
- **4:** Establish a monitoring system of hazard control measures to CCP.
  The goal of this monitoring system is to ensure that everything is under control during the manufacturing process at each CCP. Their goals ensure products are safe for human consumption.
- **5:** Determine the corrective actions. When the monitoring system indicates that a control measure at a given CCP has failed, these corrective actions are applied.
- **6:** Conduct verification procedures. These procedures ensure ensuring that the HACCP system is working efficiently.
- **7:** Create record keeping procedures (traceability). This folder contains all the procedures and records relating to these principles and their implementation in the manufacturing process.

Materials and Methods

The goal of this section is to describe the various methods used to collect and identify the microorganisms from different products in the dairy value chain. Moreover, the method used for some mastitis samples from the TINE SA Mastitis laboratory in Molde to detect the β-lactamase resistance mechanism will be described.

1. Sampling and cryoconservation

1.1. Thermoduric bacteria and spore-formers bacteria from the raw milk

Milk samples:

In order to collect microorganisms in the raw milk, 30 milk samples have been collected from different farms.

Aerobes collection:

1/ Sampling of *Bacillus cereus* (spore-formers) (Christiansson et al., 1995)

For all raw milk tested, 4mL of it was transferred in 3 sterile tubes. These tubes were previously sterilized using dry heat sterilization process (160°C-4h).
Then tubes were heat treated at 72 ± 1°C for 5 minutes in order to kill the vegetative cells (Christiansson et al., 1995;Xu et al., 2006). Another tube filled with 4mL of water was prepared in order to control and start the timer when 72°C ± 1°C was reached.
After the heat treatment, these tubes were deposited in a cold water bath over maximum of 5 minutes. The tubes were then incubated at 20 ± 1°C for 24 ± 3 hours(Christiansson et al., 1995).
After incubation, the presence of *Bacillus cereus* was confirmed by withdrawing one drop (approximately 30µL) from each tube, which was previously mixed by using a vortex, on MYP agar (Christiansson et al., 1992).
Recipe of the MYP agar is given in Appendix 1.
Plates were kept for 1-2 hours at room temperature before their incubation in order to dry the drop of milk, and then they were incubated at 30 ± 1°C for 24-48 hours (Christiansson et al., 1995).
The number of positive tubes indicates roughly a high, medium or low level of spores of *Bacillus cereus*.
Bacillus cereus is able to germinate rapidly if the temperature of the sample rises from 20 to 30°C. Spores are able to germinate at temperatures between 4 and 12°. At 10 to 12°C, it may happen that a few sprouts.

Keeping the temperature of the samples as low as possible was very important. *Bacillus cereus* cannot rise uncontrollably to an elevated temperature over a long period of time.

2/ Sampling of thermoduric microorganisms

A volume of 6 mL of each milk sample was transferred to a sterile tube and heated at 63°C for 30 minutes (Frank et al., 2004). These tubes were previously sterilized using a dry heat sterilization process (160°C-4h).
Another tube filled of 6 mL of water was prepared in order to control and start the timer when 63°C ± 1°C was reached.
After 30 minutes at 63°C, tubes were cooled down in a cold water bath.
From these tubes, serial dilutions were prepared in Dilucup which were mixed by using a Dilushaker (Dilushaker II, variosensor, Lab robot.Dilucup) as described in the part 1.2, Figure 3.
Pour-plate method has been used to inoculate all the media listed in the Table 1 below. Recipes of all culture media used are given in Appendix 1.
When plate was filled with 1 mL of milk and the culture medium, plates was mixed to homogenize the milk into the culture medium.

Table 1: Inoculation using pour plate method of the milk samples on different culture media. Inoculation of medium, incubation parameters and target of each culture media used (VRBD, MRS and mPCA) are described.

		Volume of heat-treated milk tested		
	Dilutions of each raw milk tested	**10^0**	**10^{-1}**	**10^{-2}**
Culture medium (Pour 10-12 mL of melted culture medium(45°C)) — *Incubation time*	**mPCA :** *30 ± 1°C for 72 ± 2 hours* (Target: Total viable count) (Frank et al., 2004;ISO 4833, 2003)	**1 mL**	**1 mL**	**1 mL**
	VRBD: *37 ± 1°C for 24 ± 2 hours* (Target: *E. coli*) (Jay et al., 2005;NMKL no.192, 2011)	**1 mL**		
	MRS: *30± 1°C for 48h* (Target: mesophile *Lactobacillus* species) (Curk et al., 1996)	**1 mL**		
	MRS: *42°C± 1°C for 48h* (Target: thermophile *Lactobacillus* species) (Di Cagno et al., 2006)	**1 mL**		

A deviation concerning inoculation of VRBG medium has been done. The 10 first milk tested (RM1 to RM10) have been prepared belong the method described previously.

However, the method used concerning the detection of *E.coli* at 37°C has been modified in order to add a reparation step of damaged and stressed bacteria.
In fact, 1 mL of milk sample (dilution 10^0) and 5 mL of autoclaved TSA temperate at 45°C (non-selective agar medium) are mixed in a Petri plate. Then, a pre-incubation at 20°C for 1-2 hours is done. After this pre-incubation step, VRBD medium is poured on the surface of the pre-incubated medium. VRBD plate is then incubated at 37°C for 24 hours (NMKL no.125, 2005).

Anaerobes collection

1/ Sampling of anaerobes spore-formers bacteria (Brendehaug, 2008)

In sterile culture tubes added growth medium for anaerobic spores and paraffin/vaseline mixture, a certain amount of milk is added. The recipe of the RCM broth and the preparation of these tubes are given in Appendix 1.

For each milk sample studied, 3 tubes were filled with 10 mL of the milk.

The method is normally described to use 9 tubes (Brendehaug, 2008):

- 3 tubes filled with 10 mL of milk
- 3 tubes filled with 1 mL of milk
- 3 tubes filled with 0.1 mL of milk.

In normal milk, number of spores expected is < 1000/liter (Everitt and Christiansson, 1996). Then, number of spore in the raw milk is very few. In order to isolate *Clostridium*, only 3 tubes containing 10 mL of milk were used.
Then, sample were heat-treated at 80 ± 1°C for ten minutes and then cooled to room temperature. The cooling was done at room temperature to allow to the Paraffin/Vaseline mixture to solidify correctly on the top, and avoiding the presence of gas.
Another tube filled of water (same level than a tube of RCM broth with 10mL of milk) was prepared in order to control and start the timer when 80°C ± 1°C was reached.(Demeter, 1952)
This step kills the vegetative cells but allows the spores to survive.
Paraffin/Vaseline mixture formed a tight lid on the top after cooling, providing anaerobic conditions during incubation.
Any spores germinated and gas production move on the Paraffin/Vaseline mixture.
Tubes were incubated at 37 ± 1°C for 72h (Demeter, 1952).
After incubation, the presence of gas in the tubes was verified. From each tube, the presence of gas indicated the presence of Clostridium. They were called “positive” (Demeter, 1952).

1.2. Psychrotrophic bacteria in consumer's milk (end of the shelf-life)

Milk samples

In order to collect microorganisms in the milk at the end of the shelf-life, 16 milk available in the market have been selected. They were made by different producers (A, B and C). The kind of milks selected were Hel (whole milk), Lett (semi-skimmed milk), Ekstra lett (semi-skimmed milk, low content of fat), økologisk lett (ecologic milk). Volumes of consumer package chosen were 0.5L, 1L, 1.5L.
All of these milks were kept in the fridge and analyzed on the day of their expiry.

Sampling of bacteria from milk at the end of the shelf-life

To collect microorganisms, different culture media have been used and prepared:

- Mannitol Egg Yolk Polymixine agar (MYP),
- Violet Red Bile Dextrose agar (VRBD),
- Tryptone Glucose Extract agar (TGEA).

Recipes of these media are given in Appendix 1.

For a batch of milk tested, serial dilutions from 10^0 to 10^{-3} have been prepared, see Figure 3 below. Milk from the bottle was shaken and then transferred in a sterile flask in order to homogenize and take more easily the 1000µL to transfer to the Dilucup.
All the preparation of dilution done for this study followed this method.
These dilutions were made by using Dilucups which were automatically homogenized with a Dilushaker (Dilushaker II, variosensor, Lab robot.Dilucup).

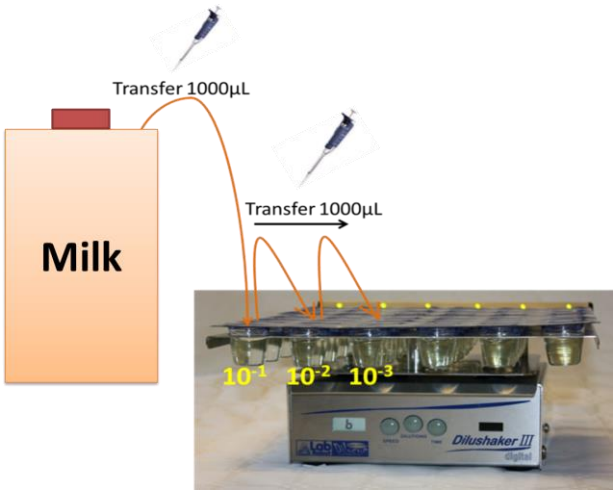


Figure 3: Preparation of serial dilutions by using of Dilucups shook on a Dilushaker.

Spread-plate method has been used to inoculate all the media listed in the Table 2 below. Actually, 100µL of the selected dilution were inoculated on the surface of the medium by using a sterile spreader.

Table 2: Inoculation by using spread plate method of the milk samples on different culture media. Inoculation of medium, incubation parameters and target of each culture media used (TGEA, VRBD and MYP) are described.

		Volume of heat-treated milk tested			
	Dilutions of each raw milk tested	10^0	10^{-1}	10^{-2}	10^{-3}
Culture medium (Pour 10-12 mL of melted culture medium(45°C)) / *Incubation time*	**2 plates of TGEA** *30 ± 1°C for 3 days* (Target: Total viable count)	N/A	100µL	100µL	100µL (milk A to I)
	2 plates of VRBD *30 ± 1°C for 24 ± 2 hours* (Target: Enterobacteriaceae)	100µL	100µL		
	2 plates of VRBD *25± 1°C for 3 days* (Target: *Pseudomonas)*	100µL	100µL		
	2 plates of MYP *30°C± 1°C for 24-48 hours* (Target: Bacillus species)	100µL	100µL	100µL	100µL (milk A to I)

Selection of the colonies

After incubation, plates were enumerated. For TGEA plates, when the enumeration was between 30-300 colonies, plates were kept and from 5 to 10 different colonies were randomly selected. However, for VRBD and MYP plates, they were kept when enumeration was between 15-150 colonies. On these plates, 5 colonies were randomly selected.

1.3. Yeasts on the cheese surfaces

Cheeses tested

Four different hard type white cheeses available in the shop were selected. These cheeses are called G, H, I, J. Each cheese was tested in duplicate, in a first time in September 2014 and in a second time in January 2015.
Only the surfaces have been tested. Surfaces of cheese cut from the final matured cheeses were out of scope. From each cheese, two surfaces have been analyzed: top and bottom.
Consumer package of cheese are squares about 1kg have been selected, in order to have more than 6 cm of thickness. This thickness was important because sampling of yeast has been done by using of RODAC (Replicate Organism Direct Agar Contact) dishes, also called contact plate. So, all the surface of these Petri dishes should be in a contact to the cheese surface analyzed.

Sampling of yeasts on the cheeses surfaces:

- Dichloran-Rose Bengal Chloramphenicol Agar (DRBCA)

Sampling of yeasts on the cheeses surfaces has been done by using Dichloran-Rose Bengal Chloramphenicol Agar (DRBCA). Recipe of the DRBCA is given in Appendix 1.

After sterilization and brief cooling, the media were maintained at 45°C until pouring.

Under aseptic condition using a laminar flow cabinet, the RODAC dishes were poured with approximately 13mL of DRBCA medium. The contact plates were completely filled forming a convex surface and then solidified.

Then, the each contact plate was deposited on the pressed surface of the cheese and firmly pressed for 10 seconds against the cheese.

Plates were incubated upside down for 3-5 days at 25°C (ISO 7954:1987, 2008).

1.4. Purification and collection

Purification on Tryptic Soy Agar (TSA)

Tryptic Soy Agar (TSA) has been used for the purification of:

- Yeasts collected on DRBCA from the cheese surfaces,

- Microorganisms collected on VRBD, MYP, TGEA media from the milks at the end of the shelf-life,

- Microorganisms collected on VRBD, MYP and mPCA media from the raw milks heat treated.

Between 5 to 10 colonies were randomly selected from countable plate. Countable plates are defined by criteria which are listed in Table 3 below.

Table 3: Criteria to define if colonies from plates enumerated can be selected. Under or upper this criteria, no enumeration results (UFC/mL, UFC/g or UFC/cm^2) can be done.

Culture Media	Countable plate if number of colonies is…	References
Non selective medium as TGEA, mPCA	30-300	(ISO 7218:2007)
Selective medium as MYP, VRBD	15-150	(ISO 7218:2007)
RODAC plates	<100	(Guillet et al., 2002)

With a sterile loop, an isolated colony was taken and streaked on TSA. The recipe of the TSA is given in Appendix 1. This step was repeated (1-2 times) in order to obtain pure growth.

Plates were incubated in aerobes conditions by following their growth conditions, see Table 4 below.

Table 4: Incubation conditions of the TSA plates in function of their origins

Colony isolated from…	Temperature (°C)	Time
DRBCA (yeasts from cheeses surfaces)	25	2-3 days
VRBD at 25°C (from milk at the end of shelf-life)	25	3 days
VRBD at 30°C (from milk at the end of shelf-life)	30	24h
VRBD at 37°C (from heat-treated raw milk)	37	24h
MYP(from milk at the end of shelf-life, from heat-treated raw milk)	30	1-2 days
TGEA (from milk at the end of shelf-life)	30	1-3days
mPCA (from raw milk after heat treatment)	30	1-3 days

Purification on MRS agar

MRS agar has been used for the purification of *Lactobacillus* species from the heat-treated raw milk.

From plates which contain between 30-300 colonies, 5 to 10 colonies were randomly selected.

With a sterile loop, isolated colony was picked up and streaked on MRS agar in order to purify the sample. This step was repeated two times.

Recipe of the MRS agar is given in Appendix 1.

Plates were incubated by following their growth condition, see Table 5 below.

Table 5: Incubation conditions of the MRS agar plates following origins of samples

Colony isolated from…	Temperature (°C)	Time
MRS agar at 30°C	30	48 hours
MRS agar at 42°C	42	48 hours

Purification on RCM agar

Under aseptic condition, one loop of positive tubes was streaked on the RCM agar.

Recipe of the RCM agar is given in Appendix 1.

RCM agar plates were incubated in anaerobic jar at 37± 1°C for 2-3 days.

In each jar, one bag (like Anaerocult® A, Merck) and one strip were added. The bag was used to create an anaerobic atmosphere. The strip was used to control the anaerobia's in the jar were used.

Make a collection in Microbank™

Microbank made by Pro Lab Diagnostics was used to store the microorganisms selected and purified as described previously.

Each Microbank box contains 80 vials which are sterile and composed of porous beads in a cryopreservative fluid.

One tube per strain has been inoculated under aseptic condition. Colonies from fresh pure culture (1-3 days) were taken by using sterile loop or swab and transferred to the Microbank vials. This was done to achieve 3-4 Mac Farland (McF). The turbidity of the vials was compared to the Mac Farland Standards.

Vials were mixed by inverting 4-5 times. In order to allow the fixation of the microorganisms to the beads, vials were let sit for 2 minutes. Then, the cryopreservative solution was removed by a using sterile Pasteur pipette and the vials were store in the freezer at -80°C.

59 strains from cheese samples were collected in September 2014, 359 strains from the milks at the end of the shelf-life and 557 strains from the heat-treated raw milks have been collected.

Moreover, 329 *Staphylococcus* coagulase negative and 133 *Streptococcus* strains have been collected by the Mastitis Laboratory in Molde.

About cheese collected in January 2015, 73 samples were purified on TSA and kept in the fridge at 4°C until to be streak and identification by MALDI-TOF MS.

2. Mastitis samples

Sampling of microorganisms, preparation of culture media and cryoconservation step have been done by the Tine Mastitis Laboratory in Molde, Norway.

2.1. Sampling and registration of the raw milk from the cow

This step was done for the goat and cow's milk tested. Farmers were responsible of this sampling using the procedure given in Appendix 2.

After sampling, samples were sent to the Tine Mastitis laboratory in Molde (4 tubes for cows/2 tubes for goats). Samples were registered and a unique number was given for each cow/goat tested. The quarter milk samples are given extending journal numbers when they were registered.

A roadmap allows to follow analysis to do and done on each sample. This roadmap is given in Appendix 3.

2.2. Microbial testing and collection

From the sample of cow composed of 4 tubes, 0.01 mL of milk is streaked using a sterile loop on Heart Infusion agar with esculin and blood. This plate is dived in 4 quarters, one for each teat which corresponds to each teat tested. Plates were incubated at 37°C for 24/48h.

The recipe of the Heart Infusion agar with esculin and blood is given in Appendix 1.

A confirmation of *Staphylococcus* coagulase negative and *Streptococcus* was done visually (morphology, characteristic colonies). Some biochemical confirmation tests like catalase, camp reaction and hippurat were also conducted.

Then, confirmed colonies of *Staphylococcus* coagulase negative or *Streptococcus* species were purified once or twice on the Heart Infusion agar with esculin and blood plates.

After purification, strains were freezing in Heart Infusion Broth (HIB) with Glycerol (18%) vials. Recipe of these vials is given in Appendix 4.

Under aseptic condition, purified strains were frozen in HIB with glycerol 18% to obtain approximately 3 McF by using sterile loop (10µL).

Vials were store at -20°C.

3. Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) identification

3.1. Principle of the MALDI-TOF MS

MALDI is an ion source technology which allows to protonation of ions. An ion is defined as atom or atoms group (molecule) which have lost or gained electrons. These molecules or atoms are charged negatively or positively. TOF-MS is known as a method of Mass Spectrometry which measures the mass-over-charge ratio of ions.

Mass Spectrometry has been used for chemistry applications for several years. However, in 1975, Anhalt and Fenselau identified microorganisms by using pyrolysis Mass Spectrometry (Anhalt and Fenselau, 1975). In 1980's, this new technology allowed the analysis of a large spectrum of proteins. These proteins allowed the discrimination and classification of closely related species. Identification was done at species level (Demeter, 1952;Fox, 2006;Murray, 2010).

In 2002, Koichi Tanaka and John B. Fenn received a Nobel Prize in Chemistry. K. Tanaka was nominated as the first person to discover ionization of biomolecules as proteins using soft laser desorption. As for John B. Fenn, he discovered the principle to protonate ions by using an electric field [1].

In microbiology, MALDI-TOF MS allows the identification of microorganisms such as yeasts and bacteria. This identification is based on the analysis of the peptidic spectra (also called protein fingerprint signature) which is specific of each species, family (Fenselau and Demirev, 2001;Lavigne et al., 2013;Seng et al., 2009;Sogawa et al., 2011;Van Veen et al., 2010). MALDI-TOF MS is also used in clinical fields to determine antibiotic resistance as β-lactamase activity, the detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) or the detection of vancomycin-resistant (Hrabak et al., 2013).

This method has a lot of benefits compared to phenotypical identification (Gram staining and biochemical characteristics) and to molecular identification as Polymerase Chain Reaction (PCR). It is a rapid detection, simple preparation, inexpensive and accurate method (Holland et al., 1996;Seng et al., 2009;Van Veen et al., 2010)

MALDI-TOF MS involves an ionization of the sample covered of an excess of matrix by using a laser which form protonated molecules, an acceleration of molecules by an electric field until a detector trough a vacuum flight tube, and a mass spectrum obtained from data analysis, as shown in Figure 4 (Marvin et al., 2003).

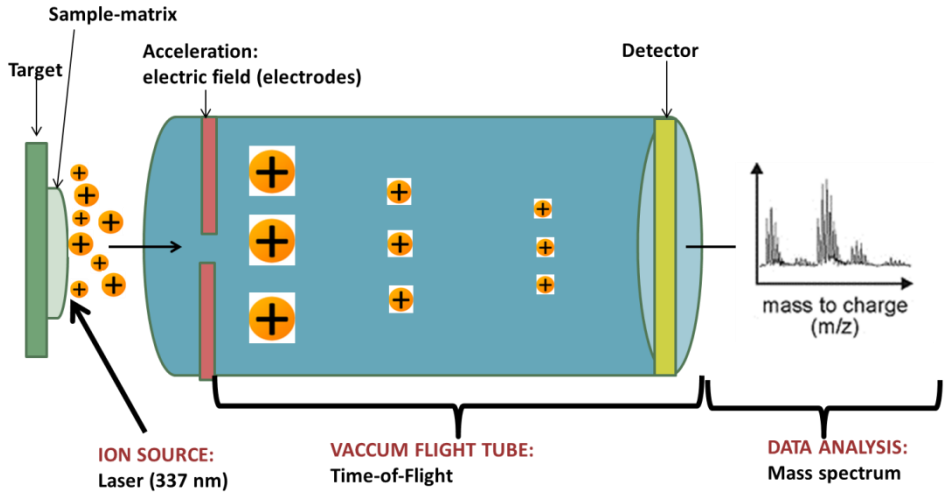


Figure 4: Principle of the MALDI-TOF MS.
Co-crystallized matrix-sample ionized by a laser, followed by desorption and transfer of proton from the matrix to the sample which formed ions. An electric field is applied which accelerated ions. Then, ions fly though a vacuum flight tube until a detector. Heavy ions fly slowly to compare to light ions.

Mass spectrometer is composed of three compounds:

- An ion source,
- A mass analyzer
- A detector.

MALDI is used in mass spectrometry as an ionization method. The sample is covered by a matrix onto a target. A co-crystallization of the matrix-sample mixture is done.

This matrix is generally a strong acid (e.g.: α-Cyano-4-hydroxycinnamic Acid (HCCA)). In the case of the HCCA matrix, the strong organic acid is suspended in water and organic solvent (s). Organic solvents and strong acids allow the lysis of the cell wall. For some microorganisms (such as yeasts or some Gram positive bacteria), an extraction method is used in order to lyse them using strong organic acids and/or alcohols (Fenselau and Demirev, 2001;Lavigne et al., 2013).

First, the co-crystalized mixture irradiated is by a laser, at the wavelength used (337 nm), matrix molecules have a strong absorption. The laser allows the ionization of co-crystallized samples (composed of proteins). This ionization results of desorption of ions created by proton (H^+) transfer between photo-excited matrix (M) and molecule (A). This reaction (1) is given below.

$$MH^+ + A \rightarrow M + AH^+ \qquad (1)$$

Then, ionized proteins are accelerated through a linear vacuum flight tube which is a TOF analyzer by using an electric field. The TOF is a method which measures the m/z ratio of ions. The ions fly to a detector in function of m/z ratio. Ions with small m/z ratio fly faster than ions with a big m/z ratio (Lavigne et al., 2013).

3.1.1. Identification of microorganisms

MALDI-TOF MS is used to identify microorganisms, mainly in the clinical sector. However, it has become increasingly interesting to the food industry due to its advantages (Bruker Daltonics, 2008;Pavlovic et al., 2013;Quintela-Baluja et al., 2013):

- Accuracy: more than 92% of species identification are correct,
- Inexpensive method because it is 2-3 less expensive than standard identification methods,
- Simple and minimal preparation of samples,
- Rapid analysis (few minutes per sample) which makes fingerprints based on the highly abundant proteins (intrinsic proteins as ribosomal proteins).
- Results given rapidly, where spectra are specific and selective given a classification at genus, species (see Figure 5), strains level (see Figures 6). These spectra from unknown microorganisms can be compared to a database or to each other.

The proteome of the microorganisms is identified by MALDI-TOF MS (low mass range 2000-20000 Da). Proteins are synthetized as a function of the growth conditions (time, temperature, culture medium).
In fact, identification of microorganisms have to be done using pure and fresh strain cultivated on culture medium (Bruker Daltonics, 2008;Pavlovic et al., 2013).

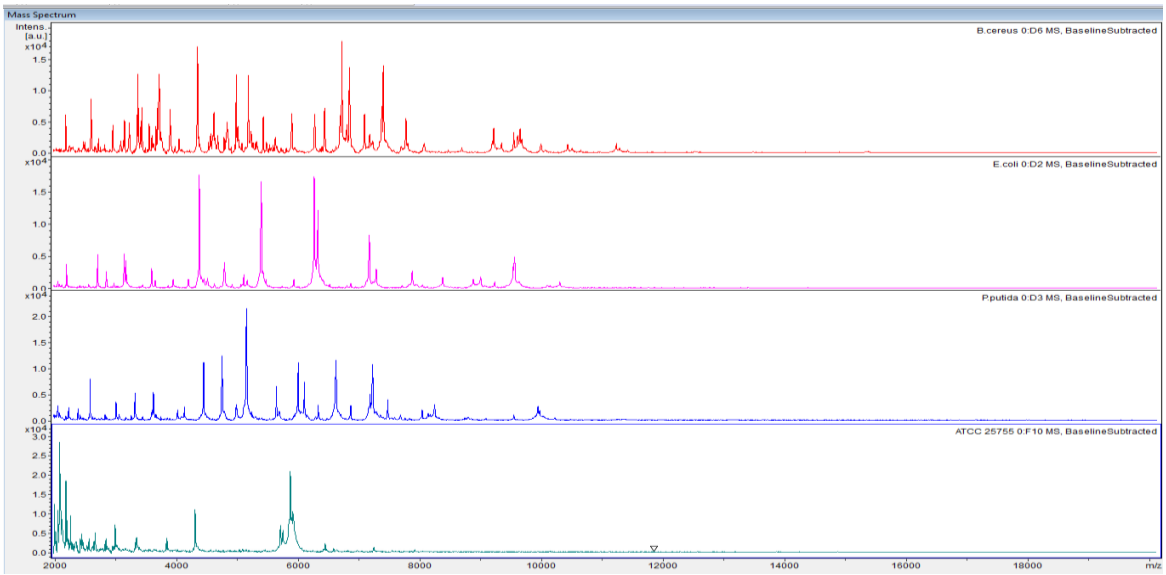


Figure 5: Comparison at strains level of spectra of 4 different microorganisms.
Spectra have been obtained using MALDI Biotyper system, with the FlexAnalysis software. Red spectrum corresponding to *Bacillus cereus*, pink spectrum corresponding to *Escherichia coli;* blue spectrum corresponding to *Pseudomonas putida* and green spectrum corresponding to *Clostridium tyrobutyricum* ATCC® 25755.

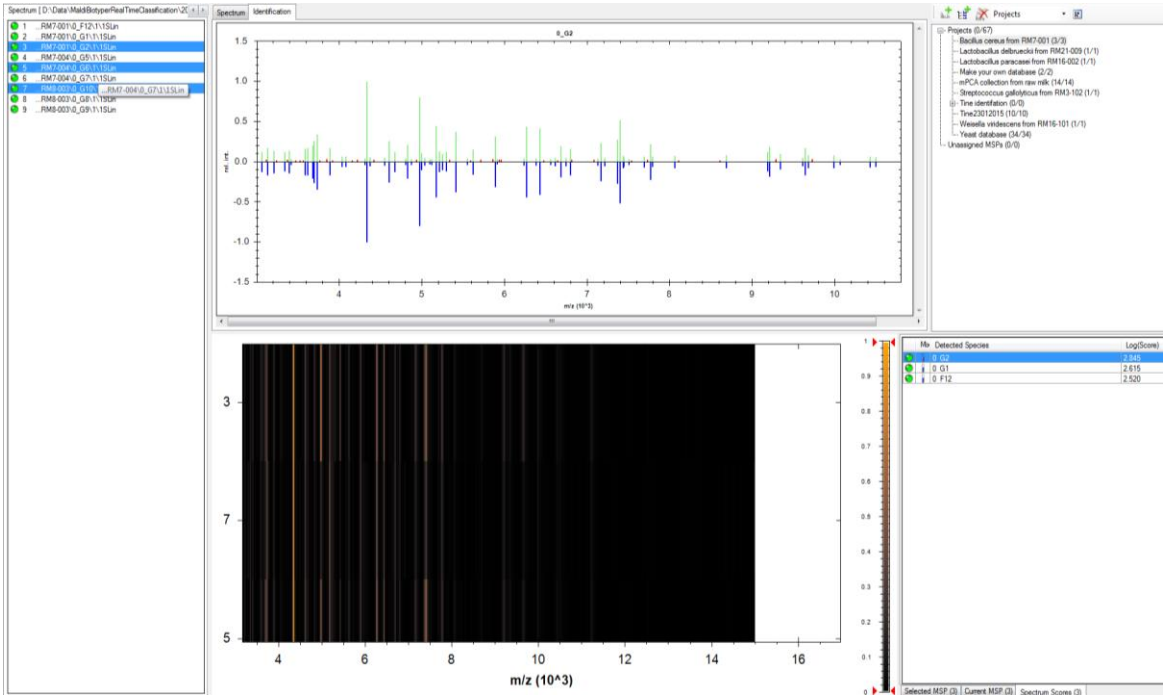


Figure 6: Comparison of strains of *Bacillus cereus*, using MALDI Biotyper V3.0 software.
Green peaks indicate a perfect matching. Red peaks represent peaks which are not aligned with the reference spectrum of the database (*Bacillus cereus*, strain RM7-001). Yellow peaks correspond to a partial matching (peak close to the reference peak). The black board gives the matching of 3 strains selected (on the list on the left side), it is a comparison at the strains level. The samples are the same at the strain level if bands are exactly at the same position. The table given on the right side indicates the score of the alignment between database and sample analyzed.

3.1.2. Detection of resistance mechanisms: B-lactamase

In the mastitis laboratory, Clover Leaf method is used for detection of β-lactamase positive *Staphylococcus* coagulase.
Penicillin G is a natural antibiotic from the β-lactam antibiotic family.

Antibiotics, generality

Antibiotics are medicine drugs used for the treatment of bacterial infections. They are natural or synthetic and can inhibit the bacterial growth (bacteriostatic effect) or destroy population of bacteria (bactericidal effect).

13 families of antibiotics exist (bioMerieux, 2004) which are classified according their chemical formulation. One of these families is β-lactams act on the cell wall of the bacteria.

Antibiotics resistance, generality

Antibiotic resistance of bacteria is its capability to fight against antibiotics and to grow in their presence.

This resistance can come from:

- Natural resistance inside the chromosome,
- Chromosome mutation,
- Acquisition of mobile genetic elements as plasmids.

B-lactam family

β-lactams are composed of a β-lactam core (Figure 7). They inhibit the peptidoglycan synthesis in the cell wall (bioMerieux, 2004).

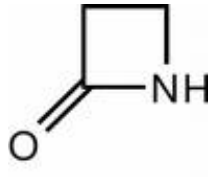


Figure 7: β-lactam core

This huge family is divided in four groups (bioMerieux, 1998):

- Penicillins,
- Cephalosporins
- Carbapenems
- Monobactams.

Mechanisms of resistance

They are 4 mechanisms of resistance against β-lactams (Courvalin et al., 2006).

- Modification of penicillin-binding proteins (PBPs) which induces an affinity default,
- Efflux phenomenon: antibiotic is actively and immediately discharged
- Impermeability resistance: the size and number of porins change, then β-lactams cannot go into the bacterium. This mechanism concerns only Gram-negative bacteria.
- Enzymatic destruction by β-lactamases.

β-lactamases are enzymes produced by bacteria to fight off β-lactams (Courvalin et al., 2006). They open the β-lactam ring. Their synthesis is done in the periplasmic space.

The β-lactamase classification which has been created by Amblers (1980) is based on genetic similarities and on the enzymes functionalities. Four different groups of β-lactamase (A-D) are described on this classification (Hrabák et al., 2013;Yang et al., 1999).

Penicillinases are classified as Class A serine enzyme.

Two mechanisms of resistance are used by β-lactamases(Kostrzewa et al., 2013):

- Hydrolysis reaction which break an amide bond by addition of a water molecule (H_2O),
- Decarboxylation reaction which remove one molecule of carbon dioxide (CO_2).

MALDI-TOF MS, an instrument able to detect the β-lactamase activity

MALDI-TOF MS is a rapid method used in the clinical sector. Using this instrument, β-lactamase resistance is not directly determined. However, the chemical reaction of hydrolyse of the β-lactam ring by β-lactamase is determined.
If the β-lactam antibiotic is hydrolyzed by β-lactamase, then the molecular weight of the antibiotic will change (+18 Da). After this hydrolysis, a carboxylation will happen (- 44Da). The scheme of the β-lactam antibiotic hydrolyzed by β-lactamase is given in Figure 8 below. Characteristic peaks are determined by MALDI-TOF MS. This method is used for some β-lactam antibiotics.

However, this method has not been validated for Penicillin G antibiotic.

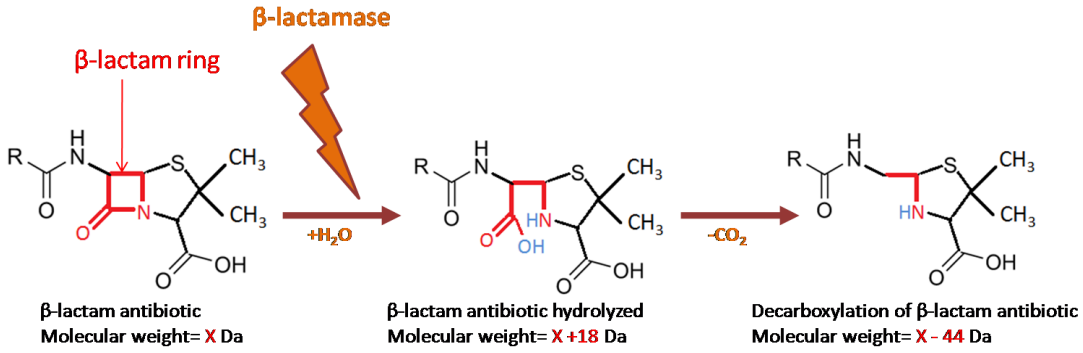


Figure 8: β-lactam antibiotic hydrolyzed by β-lactamase.
This hydrolysis of β-lactam ring induce a modification of the molecular weight (-18Da). After that, decarboxylation is identified by loosing of -44Da.

3.2. Methods for microorganisms identification by MALDI-TOF MS

3.2.1. Bacterial and yeasts isolates from the freezer or fridge

Microorganisms collected as described previously have been streaked on their respective culture medium. In order to have a good identification, strains have to be fresh (Bruker Daltonics, 2012;ISO 7954:1987, 2008). The culture media used were the same than culture media used for purification of strains before their freezing. The culture medium used and the growth condition are given in Table 6 below.

Table 6: Culture media used to streak microorganisms from the freezer or fridge

Colony isolated from…	Culture media	Temperature (°C)	Time
DRBCA (yeasts from cheeses surfaces)	TSA	25	2-3 days
VRBD at 25°C (from milk at the end of shelf-life)	TSA	25	3 days
VRBD at 30°C (from milk at the end of shelf-life)	TSA	30	24h
VRBD at 37°C (from raw milk)	TSA	37	24h
MYP(from milk at the end of shelf-life, from raw milk)	TSA	30	1-2 days
TGEA (from milk at the end of shelf-life)	TSA	30	1-3days
mPCA (from raw milk)	TSA	30	1-3 days
MRS at 30°C (from raw milk)	MRS	30	48h
MRS at 42°C (from raw milk)	MRS	42	48h
RCM at 37°C (from raw milk)	RCM	37	2-3 days
Heart Infusion agar with esculin and blood (strains from Molde)	Heart Infusion agar with esculin and blood **and** Heart Infusion agar	37	1-2 days

3.2.2. Identification of microorganisms

Direct transfer

One single colony from sample was deposit on one spot of the target plate (MSP 96 target polished steel BC, Bruker Daltonik GmbH).

This method has been used for all bacteria identification. For yeasts, the extended direct transfer method has been used. Moreover, in order to increase the identification score obtained for some bacterium/yeast samples, the Formic Acid extraction method have been used.

Extended direct transfer method

One single from sample to identify was deposit on one spot of the target plate (MSP 96 target polished steel BC, Bruker Daltonik GmbH). Then, 1µL of 70% Formic acid was added on the spot and dried at room temperature for few minutes.

Formic acid extraction method

In an Eppendorf vial, 300µL of deionized water are transferred. Then, one sterile loop (1µL) is filled of fresh and pure strain to identify. The vial is mixed with vortex.

900µL of Ethanol (absolute) are added and mixed thoroughly.

The Eppendorf vials from different samples are centrifuged at 13 000 rpm (revolutions per minute) for 2 minutes.
Supernatant is removed. Vials are centrifuged again for a few seconds (10) and pipette all the Ethanol residues. The volume of pellet was estimated.
The pellet is dried at room temperature for 3-5 minutes.

In function of the volume estimation of the pellet, formic acid (70%) is added as described in Table 7 below. After that, the Eppendorf vial is mixed to suspend the pellet.

Table 7: Volume of reactants added according to the volume estimation of the pellet. Reactant used are Formic acid (FA) 70% and Acetonitrile (ACN).

Estimated volume of the pellet	FA 70% (µL)	Absolute ACN (µL)
Small colony	5	5
Large colony	10	10
1µL loop	20	20

As described previously, the equivalent volume of Acetonitrile is added and then mixed carefully. Vials are centrifuged 2 minutes at 13 000 rpm.
Finally, 1µL of the supernatant is deposited on the target to be analyzed.

Matrix used

All samples deposited on the target were overlaid with 1µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix. Samples deposited using direct transfer method or extended direct transfer method were duplicated. However, extracted samples were not tested as duplicate. Preparation HCCA matrix is described in Appendix 5.

Air-drying of the matrix-sample mixture at room temperature allowed the crystallization.

MALDI-TOF MS identification

Every week, a calibration was done to validate runs. It was done by using a Bacterial test standard (Bruker Daltonik). This calibration consists to deposit 1µL of BTS on the target and overlaid with 1µL of HCCA matrix. Then, after drying, calibration was done on the FlexControl software (click on Calibration and calibration was done automatically).

After drying of samples, measurements were performed by using MALDI Biotyper CA system which is equipped with microflex™ LT instrument (Bruker Daltonik) and FlexControl software. The microflex instrument has a nitrogen laser (337nm) and allows exciting ions. Ions are recorded in a positive linear mode. The mass analyzer is configured in a mass range of 2,000 to 18,000 Da. Spectra were acquired by a succession of 240 shots (60 shots /second by using automatic mode) with variable laser intensity.

Then, data were collected and compared to the reference library by using the Biotyper Real Time Classification 3.1 software (RTC). Bruker reference library is a database of over than 5000 spectra. These spectra have been collected from approximately 2,000 different species. For E.coli species, 40 spectra are present in the database. However, the user needs only one spectrum to characterize his microorganisms.

For identifications performed, a score of identification was given as explained in the Table 8 below.

Table 8: Criteria for identification of isolates, color code associated and meaning of the score value

Score	Meaning	Color code
≥ 2	Species identification	
Between 1.7 and 1.9	Genus identification	
<1.7	Not reliable identification	
≤0	No peaks found	

Creation of my own Main Spectra Profile (MSP)

From some unreliable identification results, own database has been compiled. Own databases were made by using the MALDI Biotyper 3.0 software.

The procedure to create own MSP is described below:

- Click on File/ Add Spectra
- The Spectrum Browser opens and loads the spectrum of interest from the results store in the “RealTime Classification project” folder.
- Select the spectrum of interest, then right click and select “Create MSP series”.

Then, spectrum is load in “unassigned MSPs” folder. Select “project” and then click on the “Tree” icon (Figure 9). Taxonomy Editor Window appears and creation of a new node can be done. Then, spectrum from strains of interest can be download and rename (e.g.: *Clostridium tyrobutyricum* ATCC 25755).

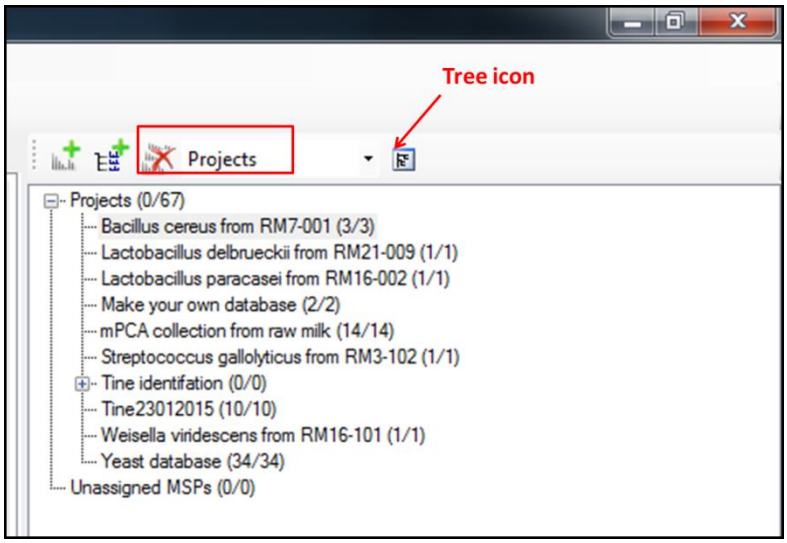


Figure 9: Creation of own MSP.
To create own MSP, a project have to be selected selection of a project and a new folder can be insert by using the Tree icon (Taxonomy Editor) by using MALDI Biotyper 3.0 software.

Some unidentified microorganisms have been collected to be an own MSP and compared to the results obtained by RealTime Classification projects.

Milk analysis (deposit of milk on the target), target: *Bacillus cereus*:

Raw milk samples have been collected randomly from different farms. 18 milk samples have been studied.

Method used was the same than method described part 1.1 (Sampling of *Bacillus cereus* (spore-formers).
After heat-treatment (72°C-5 min), tubes were incubated at 20°C for 24 hours.
From tube, one drop has been deposit on the surface of the MYP agar.
At the same time, incubated milk was deposit using a toothpick on to the MALDI-TOF MS target. Then, the milk was overlaid with 1µL of HCCA matrix.
Concerning the MYP plate, *Bacillus cereus* group are recognized on this medium by colony with pink lecithinase halo. Identification by MALDI-TOF directly from the colonies which grow on the MYP were done (*Bacillus cereus* group typically and others which did not have any halo).

Colonies which grew on MYP were deposit on the target and then overlaid with 1µL of HCCA matrix.

3.2.3. Detection of the resistance mechanisms by MALDI-TOF MS

Bacterial strains

From samples isolated as *Staphylococcus* coagulase negative in the mastitis laboratory of Molde, the detection of the penicillin G resistance was done by using diffusion test method for some samples.

From the samples list given in the Appendix 6, 40 strains were randomly selected:

- 20 strains which were resistant to the Penicillin G,
- 20 strains which were sensible to the Penicillin G.

These strains have been cultivated on Heart infusion agar and incubated overnight at 37°C.

Procedure

To start with, Penicillin G solution at 1 mg/mL has been prepared. Then, 3 Eppendorf vials were filled:

- 1 vial contained 30µl of 1mg/mL Penicillin G (for strain tested)
- 1 vial contained 30µl of 1mg/mL Penicillin G (only antibiotic: control)

In the vial containing 30µl of 1mg/mL Penicillin G prepared for strain tested, one sterile loop (1µL) was filled with the fresh strains and re-suspended into the vial.
Then, all of the vials were incubated under agitation (400 rpm) at 37°C for 2 hours.
After incubation, vials were centrifuged for 2minutes at 13,000 rpm.
A scheme of this procedure is given in Appendix 7.
Then, 1µL of the supernatant was deposit on the target and let it dry for 3-5 minutes.
All samples were duplicated:

- Half of them were overlaid with 1µL of HCCA matrix
- The rest were overlaid with 1µL of 2.5-Dihydroxybenzoic acid (2.5-DHB) matrix. Preparation of 2.5-DHB matrix is described in Appendix 5.

MALDI-TOF measurements

The measurements were done by using the flexControl software.

A method called “antibiotic” has been created by Application Specialist from Bruker during the training. The characteristic of this method is that the mass range is localized between 100-1000 Daltons. This method has been calibrated using the Peptide Calibration Standard II in order to optimize the acquisition method.

Due to ionization, the molecular weight of the component is protonated and called adduct ions (Fuchs et al., 2010). An exchange of ions (commonly H^+ and Na^+) between the matrix and component appears.

Sensivity pattern is recognized because β-lactam ring is intact. It is not hydrolyzed. Then, peaks revealed on the mass spectra are:

- $[M + H]^+$
- $[M + Na]^+$
- $[M + 2Na]^+$

"M" corresponds to the molecular weight of the component. Here, it corresponds to the molecular weight of the Penicillin G (356.4 g/mol)

The resistant pattern represents the hydrolysis of the β-lactam ring. In this fact, hydrolysis of the β-lactam ring induced a mass shift of +18Da.

Peaks corresponding to the resistant pattern are:

- $[M_{hydrolyzed} + H]^+$
- $[M_{hydrolyzed} + Na]^+$
- $[M_{hydrolyzed} + 2Na]^+$

"$M_{hydrolyzed}$" corresponds to the molecular weight of the component added of 18Da. Here, it corresponds to the molecular weight of the Penicillin G (356.4 + 18 = 374.4 g/mol)

After acquisition of the spectra, they were analyzed by using FlexAnalysis software:

- Baseline subtraction was applied for all spectra
- Determination of peaks, see Table 9 below.

Table 9: Peaks expected in function of the resistance pattern of the strain: sensible or resistant. Molecular weight (g/mol) of the Penicillin G; if β-lactam ring is hydrolyzed, the strain has a resistance mechanisms, if β-lactam ring is not hydrolyzed, the strain is sensible to the antibiotic.

	MW	Sensitivity pattern			Resistance pattern		
	(g/mol)	$[M+H]^+$	$[M+Na]^+$	$[M+2\ Na]^+$	$[M_{hydr.}+H]^+$	$[M_{hydr.}+Na]^+$	$[M_{hydr.}+2\ Na]^+$
Penicillin G	356.4	357.4	379.4	402.4	375.4	397.4	419.4

Results

1. Identification of thermoduric bacteria and spore-from raw milk by MALDI-TOF MS

1.1. Identification of the spore-formers bacteria: target *Bacillus cereus* group

36 strains have been collected in Microbank™ and identified by MALDI-TOF MS. All of these samples were selected using the MPN method (MYP agar plate) from 13 different milks.

Characteristic spot with bacterial growth (pink halo and white precipitate around the colony) were found for the spot test of three milks: RM10, RM26 and RM27.

Non-characteristic colonies (white colonies or colonies with yellow halo) were also selected and purified from 5 milk samples. These strains are not considered to belong to the *Bacillus cereus* group. They may be other *Bacillus* species or Gram positive microorganisms.

All of these strains have been identified using the Direct Transfer (DT) method. Results of identification of 4 strains were lower than 1.7 and between 1.7 and 1.9 for 11 strains. In this fact, the Formic acid Extraction (EX) method has been used in order to improve their score and identification at species level.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 8.

The results of identification using the DT method were:

- 20 strains identified at species level (score higher than 1.9)
- 11 identified at genus level (score between 1.7 and 1.9)
- 5 strains were not reliably identified (score lower than 1.7).

Otherwise, all the results of identification obtained at a genus level by using the DT method have been identified again by using EX method. Identification results obtained were:

- 6 strains identified at species level,
- 4 identified at genus level,
- 1 strain was not reliably identified.

Different identification of microorganisms at species and genus found were summarized in Table 10 below.

Table 10: MALDI-TOF MS identification of microorganisms collected from raw milks on MYP agar plate at a strain or genus level.

		Raw milk samples heat-treated (72°C-5min)												
		RM1	RM2	RM3	RM5	RM7	RM8	RM9	RM10	RM19	RM24	RM26	RM27	RM28
At species level														
Bacillus cereus group	*Bacillus cereus*					x	x							
	Bacillus weihenstephanensis											x		
	Bacillus mycoides								x			x		
	Bacillus thuringiensis								x					
Others species	*Bacillus pumilus*					x				x	x		x	x
	Bacillus subtilis								x				x	
	Paenibacillus brasilensis	x												
	Paenibacillus polymyxa	x												
	Lysinibacillus fusiformis												x	
	Lysinibacillus sphaericus	x												
	Kocuria varians		x											
	Microbacterium lacticum			x										
At genus level														
	Lysinibacillus												x	
	Paenibacillus	x												

Comparison at the species level:

By using the software MALDI Biotyper V3.0, own database (MSP) has been created with from the strains RM7-001 (identified by MALDI Biotyper RTC 3.1 software as *B. cereus*, score=2.274) and RM8-003 (identified as *B. cereus*, score=2.322).
Strain RM10-002 (identified as *B. thuringiensis*) has been compared to these two strains. Results of these comparisons are given in Figure 10 below.

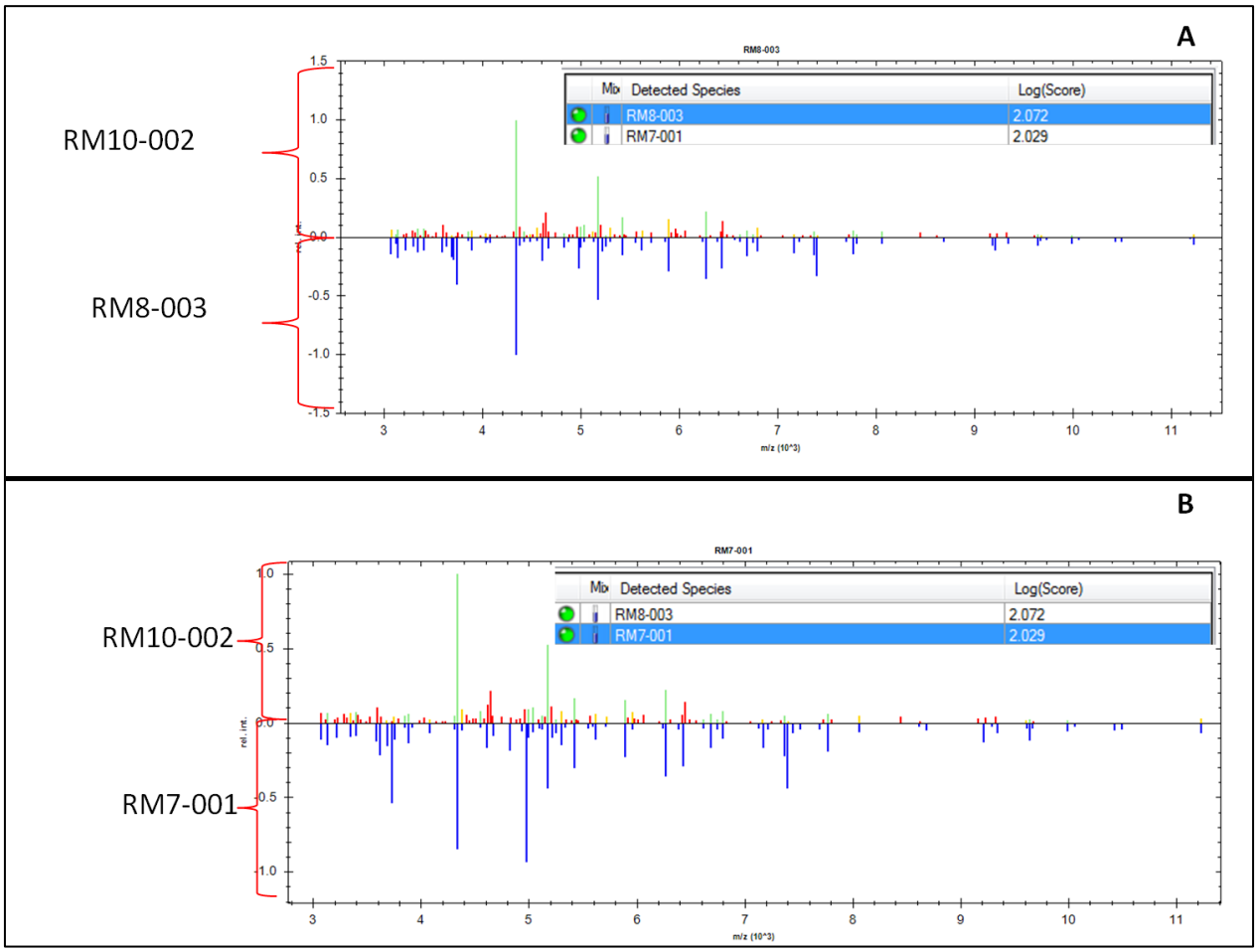


Figure 10: Comparison of MALDI-TOF MS spectra at strain level of 2 strains
Mass spectra of 2 strains from own MSP of *Bacillus cereus* (RM7-001 and RM8-003) were compare against one strain (identified as *B. thuringiensis*) using MALDI Biotyper V3.0. Peaks were identified between 2,000 and 15,000 Da. Similar peaks between two strains are color in green, peaks identified at the same size but different intensity are colored in yellow and finally peaks which are not similar to the reference strain are colored in red.
(A), comparison of the *B. cereus* strain (RM8-003) against a strain of *B. thuringiensis* (RM10-002), score found is 2.072 (identification at strain level);
(B), comparison of the *B. cereus* strain (RM7-001) against a strain of *B. thuringiensis* (RM10-002), score found is 2.029 (identification at strain level).
.

Comparison at strain level:

By using the software MALDI Biotyper V3.0, spectra of 3 strains of *Bacillus cereus* have been compared to each other.
Results of this comparison are given in the Figure 11 below.

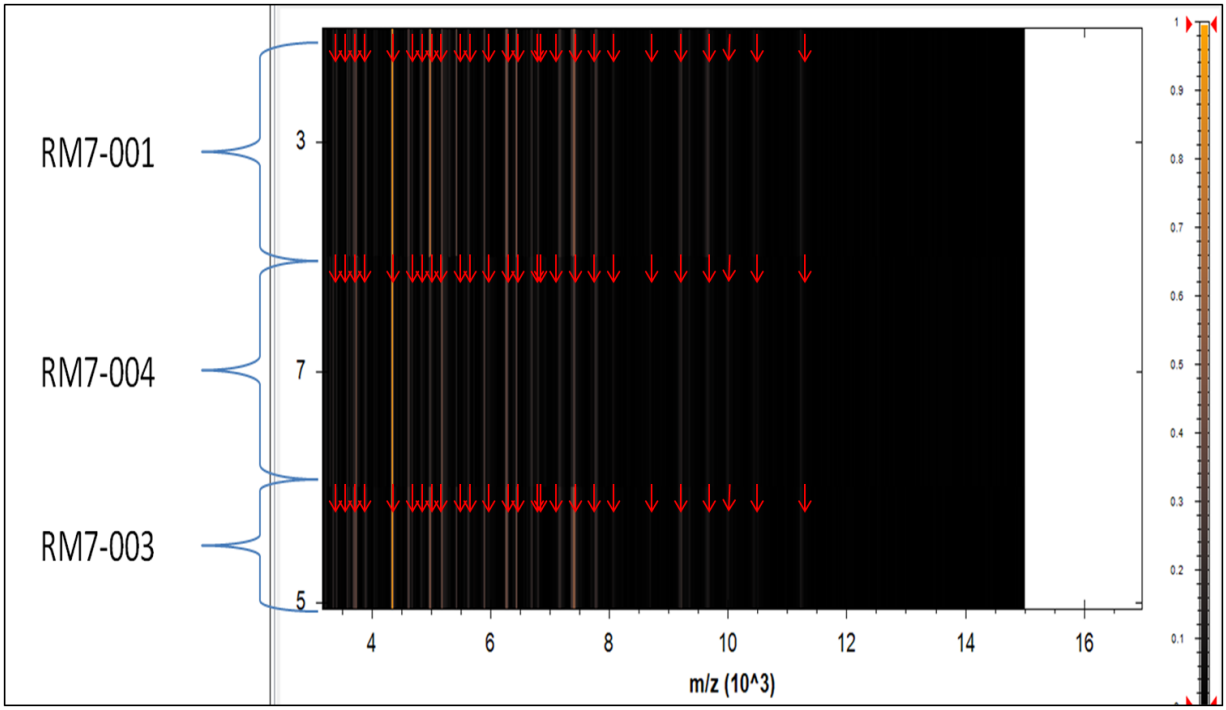


Figure 11: Comparison at species level of *Bacillus cereus* of 3 strains.
The three strains compared in this Figure are strains RM7-001, RM7-003 and RM7-004.Peaks identified (between 2,000 and 15,000 Da) are marked by red arrows.

Peaks found for these three strains are similar. However, peaks of strain RM7-004 are less strong than strains RM7-001 and RM7-003.

1.2. Milk analysis (deposit of milk on the target), target: *Bacillus cereus*

18 different raw milks were collected and analyzed (3 tubes heat-treated/milk sample) using DT method from the raw milk heat-treated and pre-incubated (milk deposit directly on the target and colonies from the drop of milk on the MYP agar).

Results from the milk deposited directly on the target were:

- “No peaks found” for 2 milk samples (milk 1 and 2),
- “Not reliable identification” for 9 milk samples (milks 3, 4, 5, 6, 7, 8, 9, 10 and 13),
- 7 other milks were not tested.

Concerning the drop of milk deposited on the surface of MYP agar, results were:

- For 9 milks:
  - *Bacillus pumilus* identified (**score > 2.0**) for milks 3, 4, 11 and 17. For these milks, species *B. pumilus* was confirmed,
  - *Micrococcus luteus* identified at species level (**score > 2.0**) for milks 2 and 8,
  - *Staphylococcus capitis identified* at species level (**score > 2.0**) for milk 8,
  - *Bacillus pumilus* identified (**score between 1.7 and 1.9**) for milks 3, 11, 13 and 17. For these milks, the genus Bacillus was confirmed,
  - *Kocuria varians* identified at genus level (**score between 1.7 and 1.9**) for the milk 8,
  - "Not reliable identification" (**score < 1.7**) for 6 milks (milks 2, 4, 8, 9, 13, 18),
  - "No peaks found" (**score <0**) for milks 11 and 18.
- For the others milks (1, 5, 6, 7, 10, 12, 14, 15, 16), no identifications were done. From the drop of milk, no colonies grew.

Results (detailed) are given in Appendix 9.

1.3. Identification of spore-formers bacteria: target *Clostridium* species

Using the Microbank vials, 47 strains have been collected. Concerning these samples, morphology of colonies was different (like 2 different microorganisms). Then, these colonies with the different morphologies were purified and called by their sample name with the annotation "a", "b", "c"… In this fact, 69 samples were identified by MALDI-TOF MS.

Samples were selected from the tubes (RCM broth with paraffin/vaseline mixture) with gas production from 14 different milks. When the paraffin/vaselin lid of the tubes moved to the top with presence of gas, strains were collected. Six other milks were not affected by this kind of microorganisms.

All of these strains have been identified by MALDI-TOF MS using the Direct Transfer (DT) method.

Results of identification using the DT method were:

- 33 strains identified at species level (score higher than 1.9)
- 15 identified at genus level (score between 1.7 and 1.9)
- 20 strains were not reliably identified (score lower than 1.7).

The Formic acid Extraction (EX) method has been used in order to improve the score and identification at species level of 8 strains. Moreover, 4 samples which have a green result have been identified again by using the EX method in order to confirm the identification results.

Identification results obtained with the EX method was:

- 10 strains identified at species level:
  - Similar identification of 4 strains (green score by DT method also)
  - Improvement of the score of the identification and same species found of 5 strains
  - Score higher than 2.0 for 1 strain which was not tested with DT method.
- 2 strains identified at genus level:
  - Improvement of the score with the same identification but this identification were still at a genus level for one of these strains
  - Decrease in the score with the same identification for one of these strains. Score lower than 2.0, then this identification is at a genus level.

The Bruker database is mainly used for the clinical purposed. *Clostridium tyrobutyricum* is species commonly found in milk. This species was not included in the Bruker database. The spectra from the *Clostridium tyrobutyricum* ATCC® 25755™ has been done. Then, a MSP was conducted using the MALDI Biotyper V3.0. Then, spectra from this strain were compared to all the anaerobes microorganisms identified by the MALDI Biotyper RTC V3.1. In fact, 11 strains have been matched at the strain *Clostridium tyrobutyricum* ATCC 25755. Examples of comparison at the strain level are given in Figure 12 and 13. Different identification of microorganisms at species and genus found were described Table 11 below.

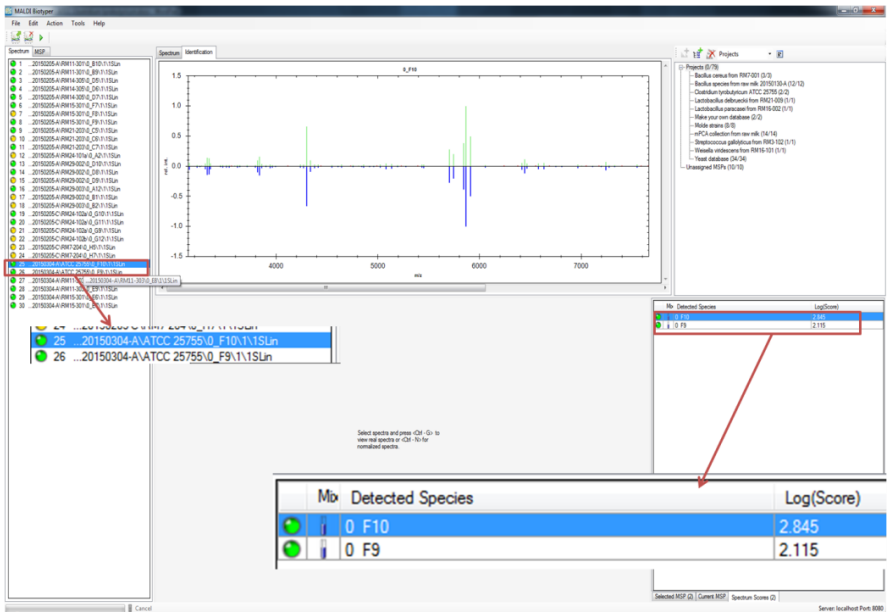


Figure 12: Comparison at a strain level of *Clostridium tyrobutyricum* ATCC 25755 strains.
Spectrum of *Clostridium tyrobutyricum* strain ATCC 25755 was performed by MALDI Biotyper and integrated in own MSP. This comparison shows that the spectrum of the strain ATCC 25755 is exactly the same than the spectrum of the strain registered in our own database (only green spectra).

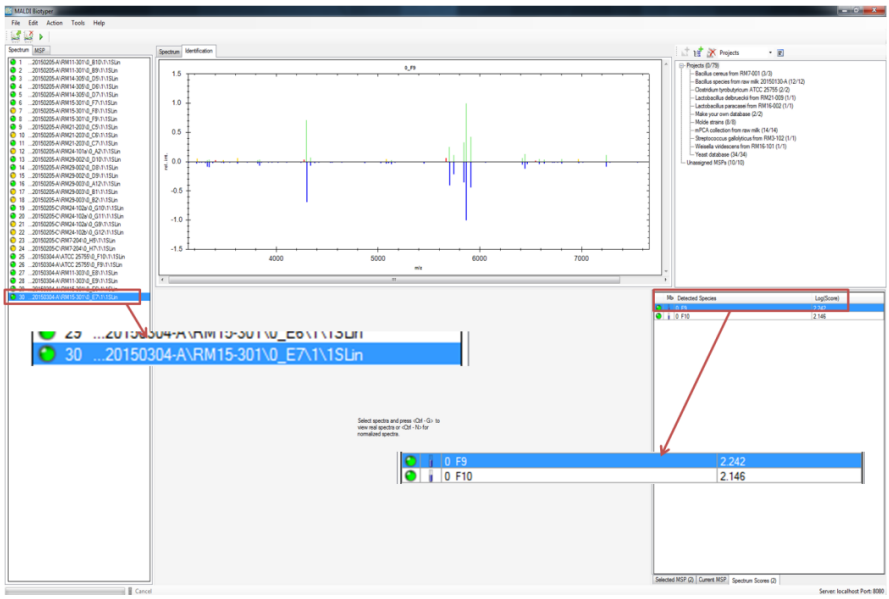


Figure 13 Comparison of *Clostridium* strain against a strains from own MSP.
Spectrum of *Clostridium tyrobutyricum* strain ATCC 25755 was performed by MALDI Biotyper and integrated in own MSP. This comparison shows the comparison of the strain RM15-301 and the strain *Clostridium tyrobutyricum* ATCC 25755. Peaks in green indicate a perfect match, and in red are spectra which did not exist in the MSP to compare to the reference from own MSP (blue peaks).

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 10.

Table 11: MALDI-TOF MS identification of microorganisms collected from raw milks samples and isolated in RCM broth a strain or genus level.

		Raw milk samples heat-treated (80°C-10min)												
		RM1	RM2	RM4	RM7	RM10	RM11	RM14	RM15	RM16	RM19	RM21	RM24	RM29
At species level														
Clostridium	*Clostridium perfringens*					x				x				
	Clostridium sporogenes	x	x		x	x		x				x		x
	Clostridium diolis				x									
	Clostridium tyrobutyricum						x	x	x			x	x	x
Others spore-formers species	*Bacillus thermoamylovarans*										x			
	Bacillus licheniformis				x		x	x			x			
	Bacillus cereus							x						
	Paenibacillus turicensis						x							
Others species	*Lactobacillus delbrueckii*													x
	Staphylococcus pasteuri				x									
	Propionobacterium acnes						x							
	Moraxella_sg_Moraxella osloensis			x										
At genus level														
	Clostridium				x								x	x
	Paenibacillus						x							
	Bacillus			x			x	x			x			

1.4. Identification of mesophiles and thermophiles bacteria: target *Lactobacillus* species

Using the Microbank vials, 21 mesophiles strains (growth at 30°C for 48h on MRS agar) and 27 thermophile strains (growth at 42°C for 48h on MRS agar) have been collected and identified by MALDI-TOF MS. Mesophiles strains were isolated from 5 milks (RM3, RM7, RM10, RM16 and RM23) and thermophile strains were isolated from 4 milks (RM3, RM13, RM17 and RM21). All of them were identified by using the Direct Transfer (DT) method.

Results of identification of mesophiles bacteria using the DT method were:

- 9 strains identified at species level (score higher than 1.9)
- 9 identified at genus level (score between 1.7 and 1.9)
- 3 strains were not reliably identified (score lower than 1.7).

The Formic acid Extraction (EX) method has been used in order to improve the score and identification at species level of 3 strains.

Identification results obtained with the EX method was:

- 2 strains identified at species level:
  - Improvement of the score for the identification and same species found.
- 1 strain identified at genus level:
  - Decrease of the score with the same identification. The score obtained with the EX method were lower than the score obtained with DT method.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 11 and 12. Different identification of microorganisms at species and genus found are summarized in Table 12 below.

Table 12: MALDI-TOF MS identification of microorganisms collected from raw milks samples and isolated on MRS agar at 30°C or 42°C at a strain or genus level.

		Raw milk samples heat-treated (63°C-30min)								
		Mesophiles bacteria Growth on MRS agar at 30°C-48h					Thermophiles bacteria Growth on MRS agar at 42°C-48h			
		RM3	RM7	RM10	RM16	RM23	RM3	RM13	RM17	RM21
At species level										
Lactobacillus	*Lactobacillus delbrueckii*	x					x			x
	Lactobacillus paracasei			x						
Others species	*Leuconostoc lactis*			x						
	Streptococcus gallolyticus	x				x				
	Weisella viridescens	x			x					
At genus level										
	Lactobacillus	x	x				x	x	x	x
	Leuconostoc			x						
	Weisella				x					

1.5. Target: Escherichia coli

VRBD agar was used in order to cultivate *E.coli* species using incubation conditions of 24h at 37°C. However, after heat-treatment at 63°C for 30min of the milks (RM1 to RM30), no colonies grew in the VRBD agar.

1.6. Identification of total viable bacteria on mPCA

245 strains (growth at 30°C for 72h on mPCA agar) have been collected in Microbank™ and identified by MALDI-TOF MS. 243 strains were identified by using the Direct Transfer (DT) method. The two other strains were identified using the Formic acid Extraction (EX) method.

Results of identification using the DT method were:

- 102 strains identified at species level (score higher than 1.9)
- 56 identified at genus level (score between 1.7 and 1.9)
- 85 strains did not reliably identify (score lower than 1.7).

EX method has been used to identify 31 strains.
Identification results obtained with the EX method was:

- 15 strains identified at species level:
  - 8 strains which were identified at species level with DT method have been confirmed by EX method
  - 2 strains which were identified at a genus level with DT method have been well identified with the EX method. However, for one of these strains (RM14-103), the genus found by DT method was not the same than the result obtained by EX method.
  - 4 strains which were not previously reliably identified have been well identified by using this method.
  - 1 strain which was not tested by using the DT method.
- 6 strains identified at genus level:
  - 5 strains which were not reliably identified were identified at genus level
  - For one strain, the score obtained by EX method was lower than the score obtained by DT method. However, the genus identified was the same (*Microbacterium lacticum*).
- 10 strains with an unreliable identification:
  - For 7 strains, score did not increase. They were also not identified.
  - 3 other strains were identified at genus level by DT method. But with the EX method, these strains were not identified.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 13. Different microorganisms identified at species and genus level found in the heat-treated raw milks are shown in Table 13 below.

Table 13: MALDI-TOF MS identification of microorganisms collected from raw milks samples and isolated on mPCA. Identification was done by using the DT and EX methods.

	RM1	RM2	RM3	RM4	RM5	RM6	RM7	RM8	RM9	RM10	RM11	RM12	RM13	RM14	RM15	RM16	RM17	RM18	RM19	RM21	RM23	RM24	RM25	RM28	RM29	RM30
At species level																										
Bacillus pumilus														x											x	
Chryseobacterium gleum											x				x	x	x									
Pseudomonas monteilli											x		x	x	x	x	x	x	x							
Pseudomonas putida											x	x	x	x	x	x	x	x								
Staphylococcus epidermidis								x	x						x				x							
Staphylococcus capitis		x							x		x		x					x								
Enterobacter asburiae																										
Enterococcus faecium												x														
Kocuria rhizophila								x	x																	
Kocuria varians	x	x	x		x	x				x		x								x		x	x			x
Lactobacillus paracasei		x																								
Microbacterium lacticum							x					x		x		x					x			x		
Microbacterium oxydans																	x									
Micrococcus luteus				x																						
Sphingobacterium multivorum												x														
Staphylococcus warneri							x	x	x	x																
Streptococcus gallolyticus													x								x					
Stenotrophomonas maltophilia											x							x	x							
Moraxella_sg_Moraxella osloensis			x	x			x	x				x							x			x				

	RM1	RM2	RM3	RM4	RM5	RM6	RM7	RM8	RM9	RM10	RM11	RM12	RM13	RM14	RM15	RM16	RM17	RM18	RM19	RM21	RM23	RM24	RM25	RM28	RM29	RM30
At genus level																										
Bacillus																			x						x	
Pseudomonas											x															
Rhizobium					x						x			x												
Arthrobacter		x		x				x						x		x										
Staphylococcus							x	x	x																	
Enterococcus							x																			
Gordonia	x																									
Kocuria varians		x	x	x		x								x												
Microbacterium lacticum	x	x	x		x	x				x		x	x	x	x	x	x		x		x					
Streptococcus			x																							
Moraxella	x					x		x							x							x	x	x		x

2. Psychrotrophic bacteria in the commercialized milk at the end of the shelf-life

16 milks have been selected randomly for this study (called A to P). These milks came from three different producers:

- **Producer 1** for milks A, B, D, E, F, G, H, I, J, K.
- **Producer 2** for milks C and L.
- **Producer 3** for milks M, N, O, P

Different kind of milks had been used:

- Full fat milk (whole milk) which contains at least 3.5% of fat ,
- Semi-skimmed milk which contains at least 1.5% of fat and at most 1.8% of fat,
- Skimmed milks which contains between 0.5 to 0.7 % of fat.

Full fat milks used were milks J and N. Milks A, B, C, D, E, F, G, K, and L were semi-skimmed milk. Concerning these milks, milks C, G, L were ecological milks. Milk O and P were skimmed milks, containing 1% of fat. Extra light milks are milks H, I and M.

2.1. Identification of spore-formers bacteria, target: *Bacillus cereus*

From the milk at the end of the shelf-life, 72 strains isolated from MYP agar have been collected in Microbank™ and identified by MALDI-TOF MS.

All of these strains were selected by using the spread plate method (MYP agar plate) from 15 different milks. Among the 16 milks analyzed, one was not concerned by this kind of microorganisms.

All kind of colony were selected and not only the characteristic colony above the *Bacillus cereus* group. They can be Bacillus species or other Gram positive bacteria.

All of these strains have been identified by MALDI-TOF MS using the Direct Transfer (DT) method.

These results of identification using the DT method were:

- 49 strains identified at species level (score higher than 1.9)
- 16 strains identified at genus level (score between 1.7 and 1.9)
- 5 strains did not reliably identify (score lower than 1.7)
- 2 strains did not have any peaks found (score lower than 0).

Otherwise, 10 strains which have results of identification obtained at a genus level and one strains identified at species level by using the DT method have been identified again by using Extended Direct Transfer (eDT) method.

Results of identification obtained were:

- 8 strains identified at species level:
  - For 7 strains, the score has been improved.
  - For one strain which was identified as *Bacillus weihenstephanensis* (score= 2.119) with DT method, results of identification with eDT method was *Bacillus mycoides* (score= 2.241).
- 3 strains identified at genus level: same results of identification compared to the result obtained with DT method.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 14.

The different microorganisms identified at species and genus levels which have been found on the MYP agar from consumer milks at the end of the shelf-life are summarize in the Table 14 below.

Table 14: Microorganisms identified by MALDI-TOF MS at a strain or genus level, collected from MYP agar in the consumer milk at the end of shelf-life.

		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
At species level																	
B. cereus group	*Bacillus weihenstephanensis*			x					x		x	x	x	x	x	x	x
	Bacillus mycoides			x					x		x	x	x	x	x	x	x
	Bacillus thuringiensis				x												
Others species	*Bacillus pumilus*				x					x							
	Paenibacillus amylolyticus														x		
	Staphylococcus haemolyticus	x															
	Kocuria varians						x										
At genus level																	
	Bacillus			x	x					x						x	
	Staphylococcus					x	x										

2.2. Target: Enterobacteriaceae

From the milk at the end of the shelf-life, 12 strains isolated from VRBD agar plate have been collected in Microbank™ and identified by MALDI-TOF MS. These strains have been collected from the milk C only. No colony grew for other milks analyzed.

All of these strains have been identified by MALDI-TOF MS using the Direct Transfer (DT) method only.

These results of identification using the DT method were:

- 1 strains identified at species level (score higher than 1.9)
- 6 strains identified at genus level (score between 1.7 and 1.9)
- 5 strains did not reliably identify (score lower than 1.7)

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 15.

Different microorganisms identified at species and genus levels which have been found on the VRBD agar (incubated at 30°C for 1 day) from consumer milk at the end of the shelf-life are summarize in the Table 15 below. No members of the Enterobacteriaceae family have been found.

Table 15: Microorganisms identified by MALDI-TOF MS at a strain or genus level, collected from VRBD agar (target: Enterobacteriaceae) in the consumer milks at the end of shelf-life.

	Consumer milk « C » at the end of shelf life
At species level	
Bacillus mycoides	x
At genus level	
Pseudomonas	x

2.3. Target: *Pseudomonas*

From the milk at the end of the shelf-life, 15 strains isolated from the VRBD agar plate (incubated at 25°C for 3 days) have been collected in Microbank™ and identified by MALDI-TOF MS. These strains have been collected from the milk C and L only. From other milks analyzed, no colonies grew on this medium.

All of these strains have been identified by MALDI-TOF MS using the Direct Transfer (DT) method only.

The results of identification using the DT method were:

- 9 strains identified at genus level (score between 1.7 and 1.9)
- 6 strains did not reliably identify (score lower than 1.7)

Otherwise, 2 strains which have identification results at a genus level by using the DT method have been identified again by using Extended Direct Transfer (eDT) method.

Results of identification obtained were:

- Same identification result for one strain: identification at genus level with score lower than the score obtained by using the DT method.
- For the other strain, the score was lower than the score obtained by using the DT method. In fact, the strain was considered as “not reliable identification”.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 16.

Different microorganisms identified at genus level which have been found on the VRBD agar (incubated at 25°C for 3 days) from consumer milks at the end of the shelf-life are summarize in the Table 16 below. No colony identified at species level has been found.

Table 16: Microorganisms identified by MALDI-TOF MS at a strain or genus level, collected from VRBD agar (target: Enterobacteriaceae) in the consumer milks at the end of shelf-life.

	Consumer milk « C » at the end of shelf life	**Consumer milk « L » at the end of shelf life**
At genus level		
Pseudomonas	x	
Stenotrophomonas		x

2.4. Identification of total viable bacteria

From the milk at the end of the shelf-life, 85 strains isolated from TGEA medium have been collected in Microbank™ and identified by MALDI-TOF MS.

All of these strains were selected using the spread plate method (using TGEA medium) from 14 different milks. The milks concerned were milks: A,C, D, E, F, G, H, I, K, L M, N, O and P. Among the 16 milks analyzed, milk B was not affected by this kind of microorganisms, no colony grew. Colonies from Milk J were out of scope. Number of colonies enumerated on the TGEA medium was higher than 300. They were too closer of each other and difficult to take off.

Among these strains, 81 have been identified by MALDI-TOF MS using the Direct Transfer (DT) method.

These results of identification using the DT method were:

- 37 strains identified at species level (score higher than 1.9)
- 26 strains identified at genus level (score between 1.7 and 1.9)
- 18 strains did not reliably identify (score lower than 1.7)

Otherwise, 22 strains have been identified by using Extended Direct Transfer (eDT) method. Some of these strains were already identified using DT method to improve the score or to verify and confirm the results of identification

Results of identification obtained were:

- 9 strains identified at species level:
  - 3 strains which were identified with score >2.0 by using DT method are also identified with a score higher than 2.0. However, their identification is different:
    - 2 *Bacillus weihenstephanensis* identified by DT method and identified as *Bacillus mycoides* by eDT method. For one strain, the score obtained using the eDT method were higher than score of identification obtained using the DT method. However, for the other strain, the score was lower.
    - *Paenibacillus amylolyticus* identified by DT method and identified as *Bacillus mycoides* by eDT method. Score of identification has been increased by using the eDT method.
  - 3 strains which were identified with score between 1.7 and 1.9 using the DT method were identified at species level using eDT method (score higher than 2.0) :
    - 2 *Bacillus weihenstephanensis* identified by DT method and identified as *Bacillus mycoides* by eDT method.
    - 1 strain identified as *Microbacterium lacticum* for the 2 methods (DT and eDT).
  - 3 strains which were not reliably identified using the DT method were identified at species level using eDT method.

- 5 strains identified at genus level:
  - Same identification at genus level between the two methods tested (DT and eDT) for 4 strains.
  - One strain has been identified at genus level by using the eDT method (only this method tested).
- 5 strains not reliably identified:
  - 3 strains which were identified at genus level by using the DT method have not been reliably identified using the eDT method. Scores of these strains decreased.
  - One strain obtained the same results of identification between the two methods tested (DT and eDT). Score obtained by using the eDT method has been improved a little bit.
  - One strain has not been reliably identified by using the eDT method (only this method tested).

Moreover, 8 strains have been identified by using the Formic Acid Extraction (EX) method. Some of these strains were already identified by using the DT method and / or the eDT method in order to improve the score or to verify and/or confirm the results of identification
Results of identification obtained were:

- 4 strains identified at species level:
  - 1 strain was identified as *B. pumilus* at species level by using the DT method. By using the EX method, the same identification at species level has been found. The score was however a little bit lower by using the EX method.
  - 3 strains have been identified as *M. lacticum* at species level by using the EX method. By using the DT method, one strain was not reliably identified (score lower than 1.7), one other was identified as *M. lacticum* but at genus level. Scores of these 2 strains have been improved by using EX method. One other strain was already identified as *M. lacticum* with a score higher than 2.0 by using the eDT method. EX method allowed to confirm the identification results of this strain with a same score obtained.
- 2 strains identified at genus level:
  - 1 strain was identified as *Corynebacterium tuberculostearicum* by using the EX method. This strain has been identified by using only this method.
  - The other strain has been identified as *B. pumilus* at genus level. This identification was the same than the identification found by using DT method. However, the score was lower by using the EX method.
- 2 strains not reliably identified :
  - One strain was identified by using only the EX method and no identification result was given.
  - The other strain has been identified as *M. avium* at genus level by using DT method. EX method has been used in order to improve the score. However, the score decreased and not reliably identification has been given for this strain.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 17.

Different microorganisms identified at species and genus levels which have been found on the MYP agar from consumer milks at the end of the shelf-life are summarized in the Table 17 below.

Table 17: Microorganisms identified by MALDI-TOF MS (using DT, eDT or EX methods) at a specie or genus level, collected from TGEA agar in the consumer milks at the end of shelf-life.

		A	B	C	D	E	F	G	H	I	K	L	M	N	O	P
At species level																
***B. cereus group:* Rods, Gram +**	*Bacillus weihenstephanensis*								x		x	x	x	x	x	x
	Bacillus mycoides								x		x	x	x	x	x	x
Rod-shaped, Gram +	*Bacillus pumilus*										x					
	Corynebacterium tuberculostearicum	x														
	Corynebacterium accolens						x									
	Paenibacillus odorifer											x				
	Paenibacillus amylolyticus													x		
	Microbacterium lacticum									x	x					x
Cocci, Gram +	*Staphylococcus warneri*			x					x							
	Staphylococcus epidermidis					x										
	Kocuria varians				x											
	Kocuria kristinae		x													
	Micrococcus luteus					x		x								
At genus level																
Rod-shaped, Gram +	*Bacillus*										x			x	x	
	Corynebacterium	x														
	Mycobacterium	x						x		x						
	Microbacterium				x			x			x				x	
Cocci or rods, Gram +	*Gordonia*							x								
	Arthrobacter			x				x								
Coccobacilli, short rods, Gram -	*Moraxella*							x					x			

3. Identification of yeasts on the cheese surfaces by MALDI-TOF MS

After incubation, reading and enumeration of yeasts on the DRBCA contact plates is done in order to select colonies randomly as described in Material and Methods part. Finally, 135 yeasts samples have been purified and collected.

Cheeses information and the number of samples collected randomly from each are given in the Table 18 below.

Table 18: Number of yeasts collected on DRBCA contact plate from 4 different white type cheeses. Colonies were chosen randomly from different surface and then frozen in Microbank™ until their identification.

	Cheeses			
	G	**H**	**I**	**J**
Sampling date	18.09.2014	18.09.2014	18.09.2014	18.09.2014
Expiration date	21.01.2015	24.11.2015	25.11.2014	01.01.2015
Surface 1, plate 1	**N/A**	**8**	**N/A**	**5**
Surface 1, plate 2	**10**	**N/A**	**N/A**	**4**
Surface 2, plate 1	**N/A**	**14**	**1**	**4**
Surface 2, plate 2	**8**	**N/A**	**N/A**	**5**
	G	**H**	**I**	**J**
Sampling date	09.01.2015	09.01.2015	09.01.2015	09.01.2015
Expiration date	12.05.2015	15.01.2015	04.03.2015	23.03.2015
Surface 1, plate 1	**10**	**11**	**10**	**11**
Surface 2, plate 1	**11**	**12**	**1**	**10**

Concerning these 135 yeast samples collected, 114 stains have been identified by MALDI-TOF MS. From these stains, 74 yeasts have been identified by using Extended direct transfer (eDT) method and 30 yeasts identified by using Formic acid Extraction (EX) method.

Among the strains identified by using Formic acid Extraction (EX) method, 24 strains were tested in order to confirm the identification and improve the score of identification and 6 samples were identified only by using this method.

Results of identification obtained automatically by comparison to the Bruker database are given in Appendix 18.

The spectra obtained for 34 samples identified using the Extended direct transfer (eDT) method allowed the creation of MSP using the Maldi Biotyper 3.0 software.

After the MSP creation, 63 strains were compared to this collection of spectra. An identification and score were then given for each samples tested.

Of the 63 samples compared to the MSP, 30 samples were compared to Bruker database and 33 were not.

Different yeast species and genus were found on the cheese surface (Table 19 below). However, one sample has been identified as *Staphylococci warneri* in the milk H and 34 strains did not have a reliable identification where the score was lower than 1.7.

Table 19: Identification of yeast by MALDI-TOF MS at a strain level and genus level in function of the cheeses tested.

	Cheeses			
	G	**H**	**I**	**J**
At species level				
Candida intermedia	x		x	x
Candida_colliculosa[ana] (Torulaspora_delbrueckii[teleo]#)	x			x
Candida_sphaerica[ana] (Kluyveromyces_var_lactis [teleo])	x			x
Candida_sphaerica[ana] (Kluyveromyces_lactis [teleo])	x			x
Candida_pelliculosa[ana] (Pichia_anomala[teleo]#)		x	x	
Candida pelliculosa		x	x	
Candida_guilliermondii[ana]# (Pichia_guilliermondii [teleo])		x		
Candida lipolytica		x		
Candida zeylanoides		x		
Candida_lipolytica[ana] (Yarrowia_lipolytica[teleo]#)		x	x	
Geotrichum silvicola		x		
Debaryomyces hanseni		x	x	
Candida parapsilosis			x	
At genus level				
Debraryomyces	x	x		
Candida	x	x	x	

4. Identification of mastitis samples and detection of the β-lactamase resistance

4.1. Identification of bacteria collected from the mastitis laboratory in Molde

The Mastitis laboratory in Molde collected 330 strains from goat milk. These strains were identified as *Staphylococcus* coagulase negative by using phenotypical tests, morphologic recognizing and antibiotic testing using a diffusion disk. By using phenotypical tests to recognize *Streptococcus*, 132 strains from cow milk have been collected.

Identification through biochemical tests and morphology of colonies has been compared using identification results obtained by MALDI-TOF MS.

Results of identification by MALDI-TOF MS are given in Appendix 19 for strains identified as *Staphylococcus* coagulase negative by the mastitis laboratory and in Appendix 20 for microorganisms identified as *Streptococcus*.

MALDI-TOF MS allowed identifying at genus and species level the strains. In Figure 14 the different species found (genus level, species level) above the group of *Staphylococcus* coagulase negative described by Mastitis laboratory are given.

The different species found above the group of *Streptococcus* described by Mastitis laboratory are given in the Figure 15 below.

Among the strains collected as *Staphylococcus* coagulase negative, all of them were identified by using the using the Direct Transfer (DT) method.

These results of identification using the DT method were:

- 258 strains identified at species level (score higher than 1.9)
- 64 strains identified at genus level (score between 1.7 and 1.9)
- 8 strains did not reliably identify (score lower than 1.7).

Otherwise, 16 strains which were identified at genus level have been identified by using Formic acid Extraction (EX) method.

Results of these identifications were:

- 15 strains identified at species level
- 1 strain identified at genus level.

Finally, in this group classified as *Staphylococcus* coagulase negative, 319 strains were *Staphylococcus* genus including 256 strains identified at species level.
Other microorganisms identified in this collection were *Brevibacterium celere*, *Micrococcus luteus*, *Rothia amarae*.

Among the strains collected as *Streptococcus*, all of them were identified by using the using the Direct Transfer (DT) method.

These results of identification using the DT method were:

- 102 strains identified at species level (score higher than 1.9)
- 20 strains identified at genus level (score between 1.7 and 1.9)
- 10 strains did not reliably identify (score lower than 1.7).

Finally, in this group classified as *Streptococcus*, 70 strains were *Streptococcus* genus including 64 strains identified at species level.

Other microorganisms identified in this collection were *Arcanobacterium pluranimalium, Aerococcus viridans, Enterococcus faecalis, Enterococcus faecium, Lactococcus lactis and Lactococcus garvieae.*

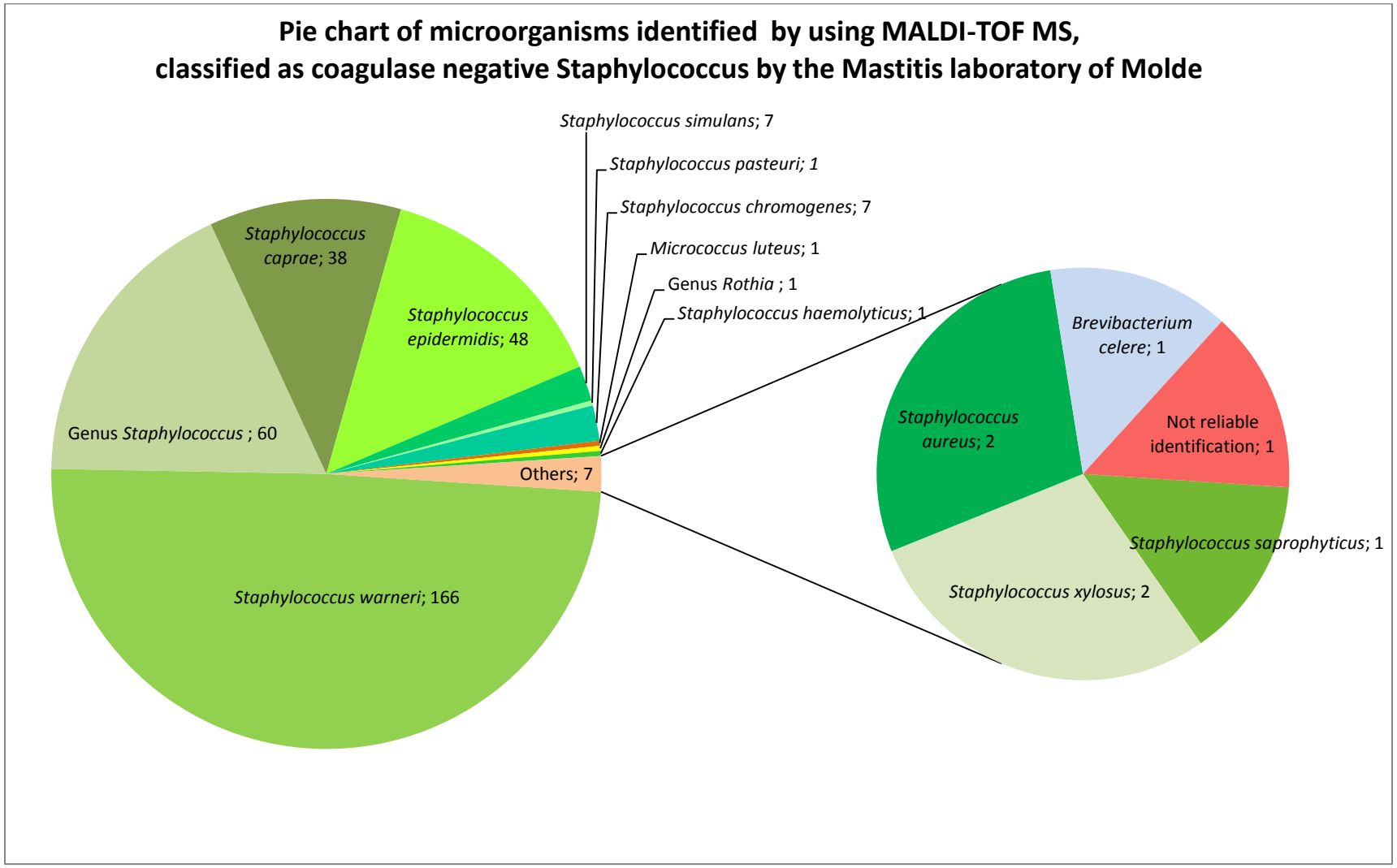


Figure 14: Microorganisms identified by MALDI-TOF MS at a strain or genus level: *Staphylococcus* group
Strains represented in this Figure were classified along the *Staphylococcus* coagulase negative by TINE Mastitis lab. On this Figure, first the name of species/genus is given followed by a number which represents the number of sample isolated identified by this species or genus.

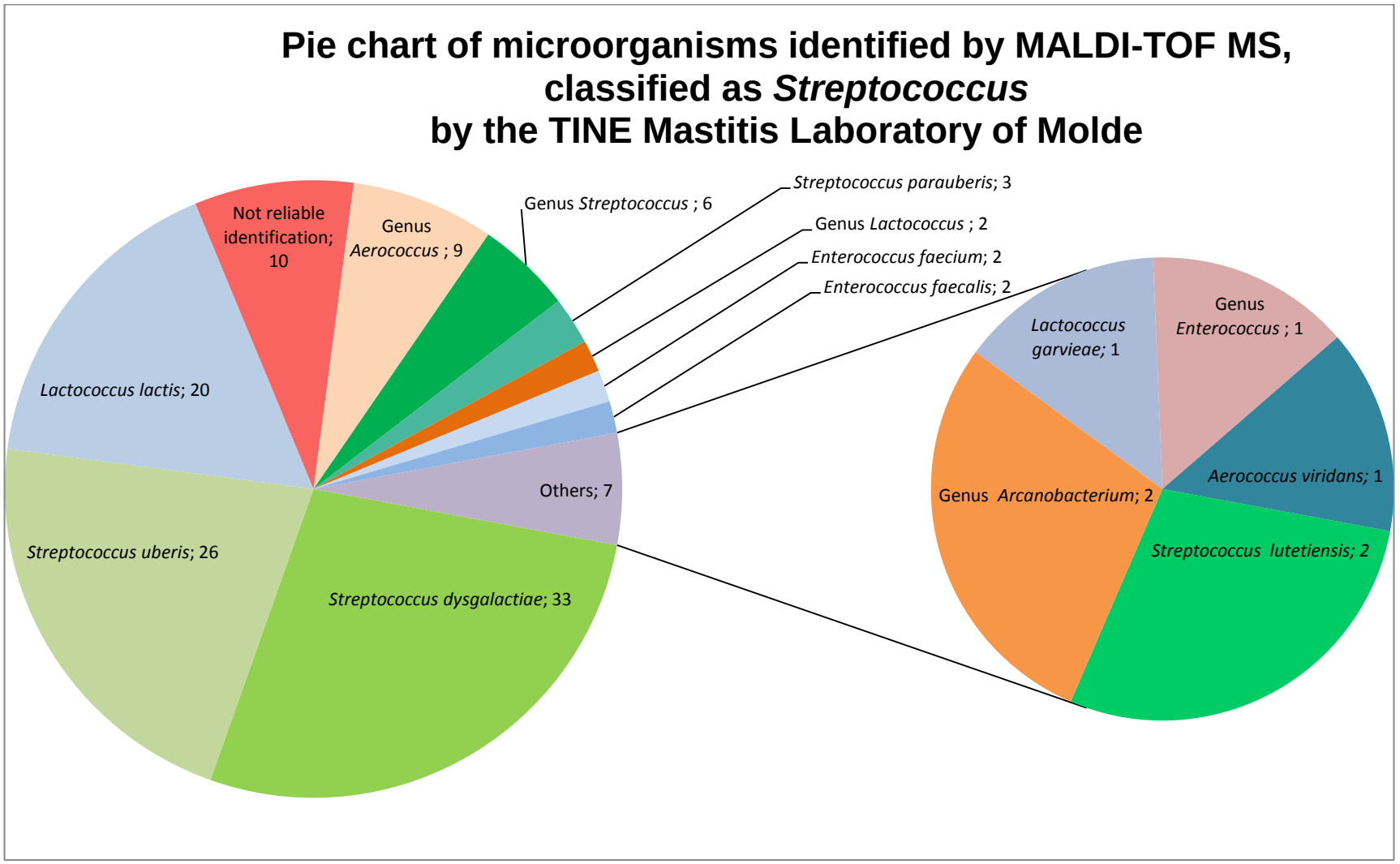


Figure 15: Microorganisms identified by MALDI-TOF MS at a strain or genus level: *Streptococcus* group
Strains represented in this Figure were classified as *Streptococcus* by TINE Mastitis lab. On this Figure, first the name of species/genus is given followed by a number which represents the number of sample isolated identified by this species or genus.

4.2. Determination of B-lactamase resistance

MS spectra were obtained from the supernatant of pure strains after incubation with Penicillin G (1mg/mL) for 3 hours.

As explained in Materials and Methods parts, molecular peaks of Penicillin G expected in case of sensivity pattern were:

- $[M + H]^+$ at 357.4 Da
- $[M + Na]^+$ at 379.4 Da
- $[M + 2Na]^+$ at 402.4 Da.

In case of strains producing β-lactamase resistant mechanism, peaks expected were:

- $[M_{hydrolyzed}+H]^+$ at 375.4 Da
- $[M_{hydrolyzed}+ Na]^+$ at 397.4 Da
- $[M_{hydrolyzed}+ 2Na]^+$ at 419.4 Da.

These peaks correspond to the Penicillin G hydrolyzed and also its adduct ions.

Concerning controls, Penicillin G applied on the MSP target without strain and overlaid by 1µL of HCCA matrix (control 1) or 1µL of 2.5-DHB (control 2) were done. The goal of these controls was to identified peaks of the antibiotic and peaks of the matrix. This was done in order to verify without strains that peaks of antibiotic are correctly detected with a same m/z ratio. These controls also allowed the verification of the peaks of the matrix and the possibility to distinguish them against peaks of the Penicillin G.

Concerning the control 1, different peaks were expected. Among them, peaks of Penicillin G at 357.4 Da, 379.4 Da and 402.4 Da but also peaks from the HCCA matrix found at 190.05 Da and 379.05 Da (Sparbier et al., 2012).

The results and identification of peaks from the Control 1 are given in Figure 16 below. With regards to this Control 1, four peaks were found. Peaks at 379 Da cannot be differentiated between HCCA matrix and Penicillin G, their m/z ratios are too close. This is because the identification of resistance against Penicillin G of strains tested by using the HCCA matrix was not appropriate.

Every sample was duplicate and overlaid by HCCA and 2.5-DHB matrices. Spectra using the HCCA matrix have been easily acquired. However, these spectra cannot be used due to the Control 1 (peaks at 379 Da not differentiate).

Results and identification of peaks from the Control 2 are given in Figure 17 below. Peaks of Penicillin G and peak from the 2.5-DHB protonated: $[2.5\text{-DHB}+ H]^+$ were expected and found. The peak of the 2,5-DHB is recognized at 155 Da (molecular weight of the 2,5-DHB is 154.12 g/mol).

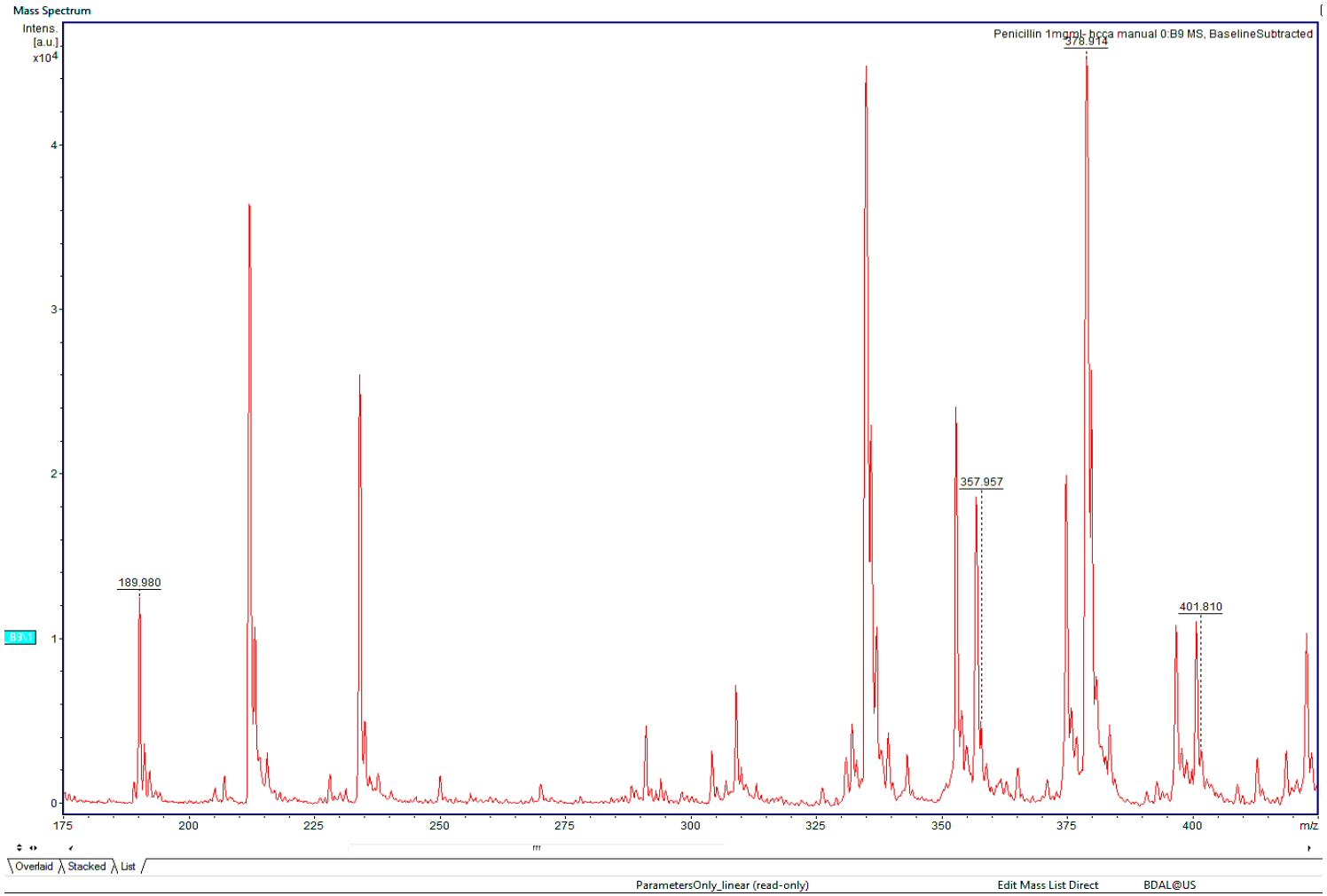


Figure 16: Determination of the peaks from the Control 1.
This control is done with Penicillin G at 1mg/mL and HCCA matrix. Fours peaks were identified. Peaks identified have been found at 189.980 Da, 357.957 Da, 378.914 Da and 401.810 Da.

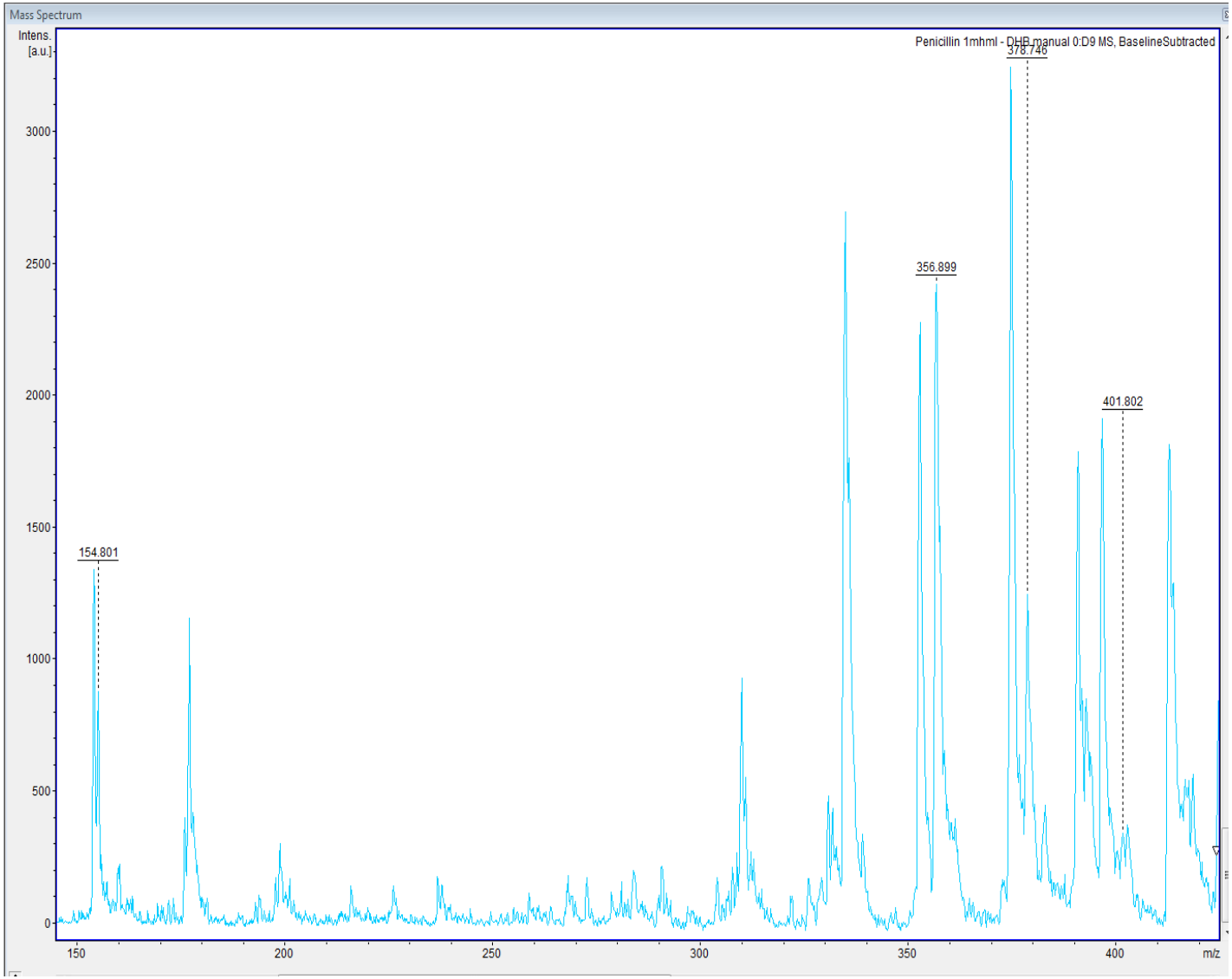


Figure 17: Determination of the peaks from the Control 2.
This control is done with Penicillin G at 1mg/mL and 2,5-DHB matrix. Fours peaks were identified. Peaks identified have been found at 154,801 Da, 356.899 Da, 378.746 Da and 401.802 Da.

Results of the determination of the resistance mechanism (β-lactamase production) of strains by using 2,5-DHB matrix are given in Appendix 21. For only five strains MALDI-TOF MS spectra have been detected (Figure 18 below). Actually, to compare to the mass spectra detected by using the HCCA matrix, the 2,5-DHB matrix seems more difficult to acquired spectra, the intensity of shot needs to be more modified and higher than 50%.

Spectra of two strains show peaks characteristic of the resistance pattern to the Penicillin G. Peaks obtained for two strains have shown a sensible pattern to the Penicillin G.

However, one strain cannot be confirmed with a resistance pattern against Penicillin G. In fact, the list of peaks found was not complete for the resistance pattern and for the sensible pattern. Three peaks for each kind of pattern were expected, only two were found.

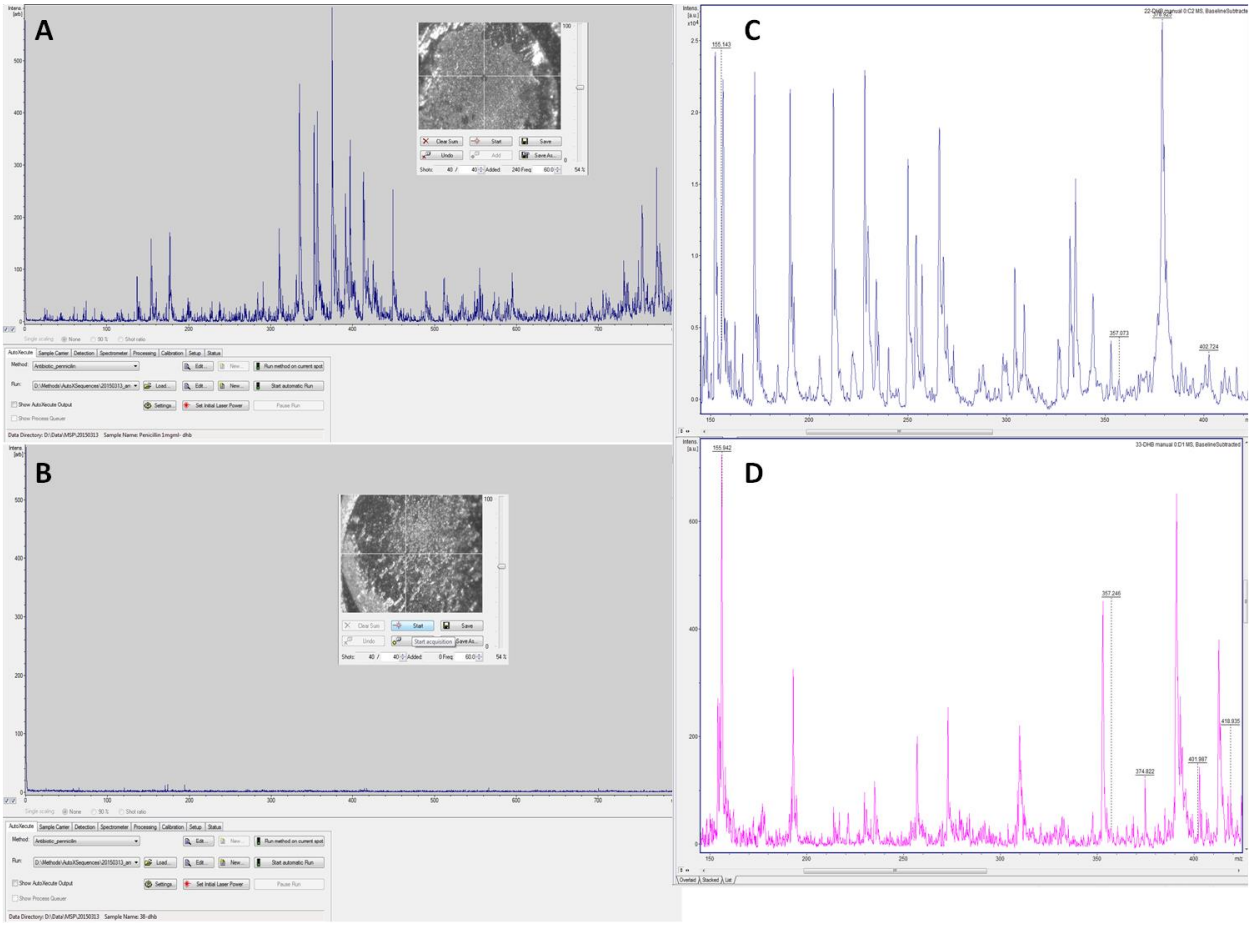


Figure 18: MALDI-TOF MS spectra to identify resistance of strains to Penicillin G
(A, B, C, D) MALDI-TOF MS spectra of Penicillin G after 2 hours of incubation (A), no spectra from a strain (38, ID: 15038-31) producing β-lactamase (B), spectra of a strain (22, ID: 13864-19) non-producing β-lactamase (C) and spectra of a producing β-lactamase strain (33, ID: 13857-5). FlexControl (Bruker) software was used to acquire spectra of all samples tested (pictures A and B). The software FlexAnalysis (Bruker) has been used to analyse spectra (picture C and D).

5. Bacterial stains comparison in different part of the value chain

Microorganisms identified in different part of the value chain were compared. Comparison of these results is given in Table 20 below.

Based on this Table 20, species and genus found on different products were highlighted. Species and Genus common to the raw milk heat treated and the consumer milk at the end of the shelf life were:

- **Genus:** *Pseudomonas, Staphylococcus* and *Bacillus,*
- **Species:** *Bacillus weihenstephanensis, B. mycoides, B. thuringiensis and B. pumilus*, *Kocuria varians* and *Paenibacillus amylolyticus.*

Otherwise, some species and genus of strains collected from the Mastitis Laboratory have been also found in the raw milk heat-treated:

- **Genus**: *Enterococcus, Staphylococcus* and *Streptococcus,*
- **Species**: *Enterococcus faecium Micrococcus luteus, Staphylococcus epidemidis, S. warneri* and *S. pasteuri.*

Table 20: Comparison of microorganisms identified by MALDI-TOF MS found in raw milk and other milk products. Genus are colored in red, species relative to genus are listed below. Species and genus found in different milk product are highlights in green.

	Milks studied		
	Raw milk heat-treated	**Raw milk from goats/ cows (mastitis samples)**	**Consumer milk at the end of shelf life**
Genus *Aerococcus*		x	
Aerococcus viridans		x	
Genus *Arcanobacterium*		x	
Genus *Arthrobacter*	x		
Genus *Bacillus*	x		x
Bacillus cereus	x		
Bacillus weihenstephanensis	x		x
Bacillus mycoides	x		x
Bacillus thuringiensis	x		x
Bacillus pumilus	x		x
Bacillus subtilis	x		
Bacillus thermoamylovarans	x		
Bacillus licheniformis	x		
Genus *Brevibacterium*			
Brevibacterium celere		x	
Genus *Clostridium*	x		
Clostridium perfringens	x		
Clostridium sporogenes	x		
Clostridium diolis	x		
Clostridium tyrobutyricum	x		

	Milks studied		
	Raw milk heat-treated	Raw milk from goats/ cows (mastitis samples)	Consumer milk at the end of shelf life
Genus *Chryseobacterium*			
Chryseobacterium gleum	x		
Genus *Enterobacter*			
Enterobacter asburiae	x		
Genus *Enterococcus*	x	x	
Enterococcus faecium	x	x	
Enterococcus faecalis		x	
Genus *Gordonia*	x		
Genus *Kocuria*	x		
Kocuria varians	x		x
Kocuria rhizophila	x		
Genus *Lactobacillus*	x		
Lactobacillus delbrueckii	x		
Lactobacillus paracasei	x		
Genus *Lactococcus*		x	
Lactococcus garvieae		x	
Lactococcus lactis		x	
Genus *Leuconostoc*	x		
Leuconostoc lactis	x		
Genus *Lysinibacillus*	x		
Lysinibacillus fusiformis	x		
Lysinibacillus sphaericus	x		
Genus *Microbacterium*	x		
Microbacterium lacticum	x		
Microbacterium oxydans	x		
Genus *Micrococcus*			
Micrococcus luteus	x	x	
Genus *Moraxella*	x		
Moraxella_sg_Moraxella osloensis	x		
Genus *Paenibacillus*	x		
Paenibacillus amylolyticus	x		x
Paenibacillus turicensis	x		
Paenibacillus brasilensis	x		
Paenibacillus polymyxa	x		
Genus *Propionobacterium*			
Propionobacterium acnes	x		
Genus *Pseudomonas*	x		x
Pseudomonas monteilli	x		
Pseudomonas putida	x		
Genus *Rhizobacterium*	x		
Genus *Sphingobacterium*			
Sphingobacterium multivorum	x		
Genus *Rothia*		x	
Genus *Saphylococcus*	x	x	x
Staphylococcus aureus		x	

	Milks studied		
	Raw milk heat-treated	**Raw milk from goats/ cows (mastitis samples)**	**Consumer milk at the end of shelf life**
Staphylococcus capitis	x		
Staphylococcus caprae		x	
Staphylococcus chromogenes		x	
Staphylococcus epidermidis	x	x	
Staphylococcus haemolyticus		x	X
Staphylococcus pasteuri	x	x	
Staphylococcus saprophyticus		x	
Staphylococcus simulans		x	
Staphylococcus warneri	x	x	
Staphylococcus xylosus		x	
Genus *Stenotrophomonas*			
Stenotrophomonas maltophilia	x		
Genus *Streptococcus*	x	x	
Streptococcus dysgalactiae		x	
Streptococcus gallolyticus	x		
Streptococcus lutetiensis		x	
Streptococcus parauberis		x	
Streptococcus uberis		x	
Genus *Weisella*	x		
Weisella viridescens	x		

Discussion

MALDI-TOF MS is a method mainly used for microbial identification in the clinical sector. This identification is based on the proteome. The analysis of the protein profile should be stable and only be influenced to a limited degree by the growth conditions. The stability of peptide mass fingerprint depends of the range of the chosen mass. In general, when using MALDI-TOF MS for microbial identification, the range selected is between 2,000 and 20,000 Da (Saenz et al., 1999).
The bacteria are composed largely of proteins (ribosomal proteins and chaperone proteins), which have proteins have a short generation time. Strains have to be in the exponential phase to growth to obtain better identification results. This is because the quality of the spectra can be decreased due to disturbance during the culture of the microorganisms. Williams, Andrezejewsk, Lay and Musser demonstrated that different parameters such as the cleaning the MSP target, instrumental parameters to generate spectra, type of matrix used, solvents and solvents in the matrix can influence the quality of the spectra (Williams et al., 2003).

In order to evaluate the MALDI Biotyper™ (Bruker Daltonik®) system for bacterial identification using mass spectrometry, the microfex instrument equipped of the Biotyper 3.1 software (Real Time Classification) has been loan from Bruker Daltonik to TINE R&D department. This is composed of a database of approximately 5,000 spectra and allows the identification of about 2,000 species.

In this study, the culture media used to purify the microorganisms prior to identification by MALDI-TOF MS were TSA and Heart Infusion agar with esculin and blood. The composition of nutriments in a culture media can modify the protein expression and the peptides contained in the culture media can be found in the spectra.
In the literature, it has been establish that the spectra profile can vary in terms of number and the intensity of the peaks (Bizzini et al., 2010;Bizzini and Greub, 2010;Wunschel et al., 2005).

The software, Biotyper ™ has been tested in this study with the aim of identifying different kind of microorganisms in the dairy value chain (yeasts and bacteria: Gram +, Gram -, aerobes and anaerobes).
MALDI-TOF MS commercialized systems used for microbial identification are based on simple calculation to produce rapid identification results across a large spectra of species. The identification result is bound to a score which is calculated by evaluation of the peak similarities between the strain studied and peaks of the mass spectrum of

reference (database). Moreover, intensity of peaks is correlated with the microbial identification. Scores higher than 1.9 are considered as reliable identifications. These systems however have some difficulties in differentiating closely related species (for example: strains among the *Bacillus cereus* group).

One of the goals of this study was to show that the software is able to identify bacteria species within the database. Certain strains have not been identified or only partially identified that can be explained because the database is based towards the clinical sector rather than the diary sector. MALDI-TOF MS allow rapid and accurate identification of microorganisms to compare to the conventional and the molecular methods which can be long, less precise for the phenotypical, biochemistry methods.

A part of strains identified at genus level using DT method (see Results part) have been identified again by using extraction methods (eDT or EX). These methods can require centrifugation steps and solvents such as formic acid, acetonitrile and TFA. These methods require additional preparation time which is important, even if these methods are faster than the conventional methods. The DT method allows very fast identifications. With the microflex system (Bruker Daltonik), 96 spots are identified in 45 minutes. Results of identification and score can be improved using the extraction methods. This means that these extraction methods can be used occasionally, not in routine identification.
In this study, 82 strains were tested using the DT and EX methods. The results of identification have been improved for 75.6% for these strains, the identification and score were approximately the same for 22% of these strains.
For strains with the same results of identification, at genus level, this can be explained by the composition of the Bruker library. These species are not included in their database, and therefore only identification at genus level is possible.
Moreover, the score and identification of 2.4% of these strains had decreased. These errors may be due to:

- Pellet missing during extraction process,
- Very low quantity of colonies in the extraction tube
- A loop of colonies was selected during extraction, containing more than one colony and the sample was contaminated.

The Biotyper software is also able to differentiate strains comparing different strains to each other, identify whether the peaks are common or not. This was demonstrated in the Results part for the identification of *Bacillus cereus* group and *Clostridium*.
The comparison of mass spectra at species of the strain of *Bacillus thuringiensis* against strains of *Bacillus cereus* demonstrates that species along the *B. cereus* group are very close to each other and that differentiating species can be difficult. It is also shown in the literature that species within the *B. cereus* group are very similar to each other (Priest et

al., 2004). Scores obtained with higher than 2 indicate that the identifications were good at species level. A conclusion could be that the strain RM10-002 is a *Bacillus cereus*, however the identification given by MALDI-TOF MS (Bruker database) identify this strain as *Bacillus thuringiensis* with a higher score. The higher score corresponds to the best identification. With regard to comparisons at strain level, in this study three strains of *Bacillus cereus* were compared. All of them were identified as *Bacillus cereus* by MALDI-TOF MS spectra compared to the Bruker database. This method demonstrates that species can be differentiated by the peaks (m/z) and their intensity.

As shown in this studied, thermoduric bacteria including species such as *Micrococcus, Microbacterium, Streptococcus, Lactobacillus, Pseudomonas* and spore-forming bacteria such as *Bacillus* and *Clostridium* have been isolated and identified.
Some bacteria (aerobes, non spore-forming and spore-forming bacteria) found in the raw milk were also found in consumer's milk. These microorganisms found in the consumer's milk can be survivors of the pasteurization process, fixed to the equipment used during the manufacturing process (post-pasteurization contamination). Consequently, these microorganisms have to be controlled during all the manufacturing process. They are spoilage bacteria and they can have negative impacts on the final product. An accurate process control has to be applied to ensure the quality during the manufacturing process and in the final products.
Research of *Pseudomonas* was performed on the consumer milk samples at the end of shelf life. These bacteria are the main source of PPC. Some microorganisms such as *Pseudomonas* are able to adhere to surfaces and constitute biofilm (Van Houdt and Michiels, 2010). The presence of biofilm on the surface of manufacturing equipment can cause residual contamination of the products. Consequently, all equipment used has to be correctly washed and decontaminated to avoid the biofilm production.
Concerning the yeasts identified on the surfaces of different hard white type cheeses, species found on the surface of the cheese from the same producer were often similar. The environmental flora during the ripening affects the flavor of the final product. Microorganisms found in this study on the cheese surfaces were also described in the literature (Corsetti et al., 2001).

MALDI-TOF MS analysis of the milk by their application directly on to the MSP target did not demonstrate that their identification were possible.
The results of this were ranked as "no peaks found" or "not reliable identification". These results demonstrate that identification directly from the milk cannot be applied in dairy industry because number of bacteria can be low (no peaks found) or that they are too many different microorganisms in the milk (not reliable identification).
Identification from colonies growth from a milk drop on MYP agar, however are identifiable. Identification at species level was not detected for all of them. As explained previously, the Bruker database was not intended for the dairy industry but more for

clinical identification. Consequently, strains which were identified at genus level or not identified cannot be implemented in this database. Unreliable identification results can be also explained if on the milk drop on MYP agar, different kind of microorganisms can grow and colony was not pure.

FlexAnalysis software can be used to identify the β-lactamase production by some microorganisms. Some studies proved that MALDI-TOF MS can be used for the identification of resistance pattern as the production of β-lactamase enzymes (Camara and Hays, 2007;Hrabák et al., 2013;Sparbier et al., 2012). The presence of resistance pattern is detected and identified in a different mass range than the microbial identification. The mass range employed is <1,000 Da and based on the β-lactam degradation using pre-defined peaks recognition. This method compared to the diffusion disks method can be used in the laboratory because it is a very fast determination, with an incubation time of only 2 hours. The method tested in this study however was not successful because production or not of the β-lactamase has been detected for only 12.5% of strains analyzed.

Conclusion

MALDI-TOF MS is useful tool: accurate, rapid. This system can be used for bacterial identification, but also for the research approaches such as the study of different components.
Concerning the microbial identification, MALDI-TOF MS systems commercialized are developed mainly for clinical sectors.
However, in this study, identification of microorganisms isolated from the raw milk and other milk products have been done. Microorganisms non identified or identified at genus level are mainly due to the database which is made for clinical used. However, this study has shown that own MSP can be used by dairy industry to identify strains. This own MSP can also be used to compare strains at strains level.

Perspectives

For further studies, it could be interesting to improve the database. This could be consisted to identify by another methods (molecular approaches) a part of non-identified strains or those identify at the “genus” level. Then, MALDI-TOF MS (Biotyper, Bruker Daltonik) could be used to produce the mass spectrum of each strain and then the own MSP could be done. Mass spectra of the stains non-identified in this study could be compared using Biotyper V3.0 to the other strains non-identified using mass spectra of this studied or from the strains collections.
Another approach could be to work with Bruker firm in order to make a database for food industry and why not especially for dairy industry.
Otherwise, identification of microorganisms by MALDI-TOF MS during the manufacturing process and compare them at the strain level could be interesting. This could be useful to determine if microorganisms identified are post-contaminations or survivors of the pasteurization process.
Further studies concerning the detection of β-lactamase should be undertaken in order to understand and improve mass spectra detection using the 2.5-DHB as for examples:

- Replace the sterile water used to incubate the strains by an incubation buffer like Ammonium Bicarbonate.
- Change intensity of the shots.

References

Anhalt, J. P., and C. Fenselau, 1975, Identification of bacteria using mass spectrometry: Analytical chemistry, v. 47, no. 2, p. 219-225.

Avaniss-Aghajani, E., K. Jones, D. Chapman, and C. Brunk, 1994, A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences: BioTechniques, v. 17, no. 1, p. 144-146.

Beresford, T. P., N. A. Fitzsimons, N. L. Brennan, and T. M. Cogan, 2001, Recent advances in cheese microbiology: International Dairy Journal, v. 11, no. 4, p. 259-274.

bioMerieux, 1998 L'Antibiogramme, Service Formation Produits, D036F, Version 2, Revision 28/12/98, p.: 13:14; 20-22; 92-94.

bioMerieux, 2004, Resistance and susceptibility testing - GCS Training Manual, TM 0022, Version 3, Revision 05/05/2004, p. 18; 42.

Bizzini, A., C. Durussel, J. Bille, G. Greub, and G. Prod'hom, 2010, Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory: Journal of clinical microbiology, v. 48, no. 5, p. 1549-1554.

Bizzini, A., and G. Greub, 2010, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification: Clinical Microbiology and Infection, v. 16, no. 11, p. 1614-1619.

Bockelmann, W., and T. Hoppe-Seyler, 2001, The surface flora of bacterial smear-ripened cheeses from cow's and goat's milk: International Dairy Journal, v. 11, no. 4, p. 307-314.

Borge, G. I. A., M. Skeie, T. Sørhaug, T. Langsrud, and P. E. Granum, 2001, Growth and toxin profiles of Bacillus cereus isolated from different food sources: International journal of food microbiology, v. 69, no. 3, p. 237-246.

Brendehaug,J., 2008. Anaerobe sporedannere (Clostridier) i råmelk og ystemelk, 9-rørs-prøve med RCM, Tine-internal method.

Bruker Daltonics, 2008. Application Note # MT-80: Microorganism identification and classification based on MALDI-TOF MS Fingerprinting with MALDI Biotyper.

Bruker Daltonics, 2012 MALDI Biotyper 3.1 - user manual. p. 179-212.

Camara, J. E., and F. A. Hays, 2007, Discrimination between wild-type and ampicillin-resistant Escherichia coli by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Analytical and bioanalytical chemistry, v. 389, no. 5, p. 1633-1638.

Chattopadhyay, S., A. Kaur, S. Jain, and H. Singh, 2013, Sensitive detection of food-borne pathogen Salmonella by modified PAN fibers-immunoassay: Biosensors and Bioelectronics, v. 45, no. 0, p. 274-280.

Chiang, Y. C., C. Y. Yang, C. Li, Y. C. Ho, C. K. Lin, and H. Y. Tsen, 2006, Identification of Bacillus spp., Escherichia coli, Salmonella spp., Staphylococcus spp. and Vibrio spp. with 16S ribosomal DNA-based oligonucleotide array hybridization: International journal of food microbiology, v. 107, no. 2, p. 131-137.

Christiansson, A., I. Andersson, K. Ekelund, L. Jönsson, and E. Mänsson, 1992, Två metoder för bestämning av Bacillus cereus-sporer i leverantörmjölk: SMR reg, no. 4883.

Christiansson, A., K. Ekelund, I. Ollermark, and I. Andersson, 1995, Utveckling av ny, snabbare, analysmetod för Bacillus cereus-sporer i leverantörmjölk: Mejerierna FoU Mjölkråvara reg, no. 4945.

Cirolini, A., M. Miotto, F. M. Machado, H. S. d. Silva, R. D. Ralyea, and C. R. W. Vieira, 2013, Evaluation of the PetrifilmTM EB and TEMPO-« EB systems with ISO 21528-2: 2004 method for the count of Enterobacteriaceae in milk: Brazilian Journal of Microbiology, v. 44, no. 3, p. 771-775.

Corsetti, A., J. Rossi, and M. Gobbetti, 2001, Interactions between yeasts and bacteria in the smear surface-ripened cheeses: International journal of food microbiology, v. 69, no. 1, p. 1-10.

Courvalin, P., R. Leclercq, and E. Bingen, 2006,Courvalin, P., R. Leclercq, and E. Bingen Antibiogramme: Eska.

Curk, M. C., J. C. Hubert, and F. Bringel, 1996, Lactobacillus paraplantarum sp. nov., a new species related to Lactobacillus plantarum: International Journal of Systematic Bacteriology, v. 46, no. 2, p. 595-598.

Daley, M., and P. Hayes, 1992, Mastitis: why is it so hard to cure?: The Cornell veterinarian, v. 82, no. 1, p. 1.

Demeter,KJ., 1952. Bakteriologische Untersuchungsmethoden der Milchwirtschaft, Eugen Ulmer. p.:144-148. 1952. Stuttgart.

Deshpande, W., 2007, Mastitis: Community practitioner: the journal of the Community Practitioners'& Health Visitors' Association, v. 80, no. 5, p. 44-45.

Di Cagno, R., M. De Angelis, A. Limitone, P. F. Fox, and M. Gobbetti, 2006, Response of Lactobacillus helveticus PR4 to heat stress during propagation in cheese whey with a gradient of decreasing temperatures: Applied and Environmental Microbiology, v. 72, no. 7, p. 4503-4514.

Dogan, B., and K. J. Boor, 2003, Genetic diversity and spoilage potentials among Pseudomonas spp. isolated from fluid milk products and dairy processing plants: Applied and Environmental Microbiology, v. 69, no. 1, p. 130-138.

Everitt, B., and A. Christiansson, 1996, Sporer i mjölk-omfattning, orsak och betydelse för vidareförädlingen: SLU Info rapporter, v. Del 1, 197, p. 1-8.

Fenselau, C., and P. A. Demirev, 2001, Characterization of intact microorganisms by MALDI mass spectrometry: Mass Spectrometry Reviews, v. 20, no. 4, p. 157-171.

Fox, A., 2006, Mass spectrometry for species or strain identification after culture or without culture: past, present, and future: Journal of clinical microbiology, v. 44, no. 8, p. 2677-2680.

Frank, J. F., G. L. Christen, and L. B. Bullerman, 1992, Tests for groups of microorganisms: Standard methods for the examination of dairy products, v. 17, no. 18, p. 227-248.

Frank, J. F., A. E. Yousef, and J. F. Frank, 2004, Chapter 08, Tests for Groups of Microorganisms, in JF Frank ed., Standard Methods for the Examination of Dairy Products: American Public Health Association, p. 189-191.

Fuchs, B., R. Süß, and J. Schiller, 2010, An update of MALDI-TOF mass spectrometry in lipid research: Progress in lipid research, v. 49, no. 4, p. 450-475.

Gleeson, D., A. O'Connell, and K. Jordan, 2013, Review of potential sources and control of thermoduric bacteria in bulk-tank milk: Irish Journal of Agricultural and Food Research, p. 217-227.

Granum, P. E., and T. Lund, 1997, Bacillus cereus and its food poisoning toxins: FEMS microbiology letters, v. 157, no. 2, p. 223-228.

Guillet, F., C. Bonnefoy, G. Leyral, and E. Verne-Bourdais, 2002,Guillet, F., C. Bonnefoy, G. Leyral, and E. Verne-Bourdais Microbiologie et qualite dans les industries agroalimentaires: Wolters Kluwer France.

Hayes, R., 1992,Hayes, R. Food microbiology and hygiene: Springer.

Hayes, S., 1981,Hayes, S. Dairy microbiology.

He, H., J. Dong, C. N. Lee, and Y. Li, 2009, Molecular analysis of spoilage-related bacteria in pasteurized milk during refrigeration by PCR and denaturing gradient gel electrophoresis: Journal of Food Protection-«, v. 72, no. 3, p. 572-577.

Holland, R. D., J. G. Wilkes, F. Rafii, J. B. Sutherland, C. C. Persons, K. J. Voorhees, and J. O. Lay, 1996, Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry: Rapid Communications in Mass Spectrometry, v. 10, no. 10, p. 1227-1232.

Hrabak, J., E. Chudackova, and R. Walkova, 2013, Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis: Clinical microbiology reviews, v. 26, no. 1, p. 103-114.

Hrabák, J., C. Eva, and W. Radka, 2013, Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis: Clinical microbiology reviews, v. 26, no. 1, p. 103-114.

ISO 4833, 2003, Microbiology of food and animal feeding stuff- horizontal method for the enumeration of microorganisms- Colony-count technique at 30°C.

ISO 7218:2007. Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations.

ISO 7954:1987. Microbiology-General guidance for enumeration of yeasts and moulds - Colony count technique at 25°C. 1-3. 2008.

Jay, J. M., M. J. Loessner, and D. A. Golden, 2005,Jay, J. M., M. J. Loessner, and D. A. Golden Modern food microbiology: Springer.

Kostrzewa, M., K. Sparbier, T. Maier, and S. Schubert, 2013, MALDI-TOF MS: an upcoming tool for rapid detection of antibiotic resistance in microorganisms: PROTEOMICS-Clinical Applications, v. 7, no. 11-12, p. 767-778.

Lavigne, J. P., P. Espinal, C. Dunyach-Remy, N. Messad, A. Pantel, and A. Sotto, 2013, Mass spectrometry: a revolution in clinical microbiology?: Clinical Chemistry and Laboratory Medicine, v. 51, no. 2, p. 257-270.

Marcadé, G., 2013, Tests de diagnostic rapide en bacteriologie: Immuno-analyse & Biologie Specialisee, v. 28, no. 4, p. 167-173.

Martin, N. H., M. L. Ranieri, S. C. Murphy, R. D. Ralyea, M. Wiedmann, and K. J. Boor, 2011, Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk: Journal of dairy science, v. 94, no. 3, p. 1211-1222.

Marvin, L. F., M. A. Roberts, and L. B. Fay, 2003, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry: Clinica chimica acta, v. 337, no. 1, p. 11-21.

McAuley, C. M., K. S. Gobius, M. L. Britz, and H. M. Craven, 2012, Heat resistance of thermoduric enterococci isolated from milk: International journal of food microbiology, v. 154, no. 3, p. 162-168.

Mortimore, S., and C. Wallace, 2013,Mortimore, S., and C. Wallace HACCP: A practical approach: Springer Science & Business Media.

Murphy, P. M., D. Lynch, and P. M. Kelly, 1999, Growth of thermophilic spore forming bacilli in milk during the manufacture of low heat powders: International Journal of Dairy Technology, v. 52, no. 2, p. 45-50.

Murray, P. R., 2010, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology: Clinical Microbiology and Infection, v. 16, no. 11, p. 1626-1630.

Nielsen, C., and U. Emanuelson, 2013, Mastitis control in Swedish dairy herds: Journal of dairy science, v. 96, no. 11, p. 6883-6893.

NMKL no.125, 2005, Termotolerant coliform bacteria and Escherichia coli. Enumeration in food and feed.: Nordic committee on food analysis, v. 4th Edition.

NMKL no.192, 2011, Gram-negative bacteria in pasteurized milk and cream. Detection of recontamination., Nordic Committee on Food Analysis: p. 1-5.

Pácová, Z., P. Švec, L. P. Stenfors, M. Vyletelová, and I. Sedlácek, 2003, Isolation of the psychrotolerant species Bacillus weihenstephanensis from raw cow's milk: Czech J Anim Sci, v. 48, p. 93-96.

Pavlovic, M., I. Huber, R. Konrad, and U. Busch, 2013, Application of MALDI-TOF MS for the identification of food borne bacteria: The open microbiology journal, v. 7, p. 135.

Perry, J. D., M. Ford, J. Taylor, A. L. Jones, R. Freeman, and F. K. Gould, 1999, ABC medium, a new chromogenic agar for selective isolation of Salmonella spp: Journal of clinical microbiology, v. 37, no. 3, p. 766-768.

Priest, F. G., M. Barker, L. W. Baillie, E. C. Holmes, and M. C. Maiden, 2004, Population structure and evolution of the Bacillus cereus group: Journal of bacteriology, v. 186, no. 23, p. 7959-7970.

Quintela-Baluja, M., K. Böhme, I. C. Fernandez-No, S. Morandi, M. E. Alnakip, S. Caamano-Antelo, J. Barros-Velazquez, and P. Calo-Mata, 2013, Characterization of different food-isolated Enterococcus strains by MALDI-TOF mass fingerprinting: Electrophoresis, v. 34, no. 15, p. 2240-2250.

Robinson, R. K., 2005,Robinson, R. K. Dairy microbiology handbook: the microbiology of milk and milk products: John Wiley & Sons.

Rowan,NJ, J G Anderson, 1998. Diarrhoeal enterotoxin production by psychrophic Bacillus cereus present in reconstituted milk-based infant formulae (MIF). Letter in Aplied Microbiology. 26[2], p.:161-165.

Ruegg, P. L., 2012,Ruegg, P. L. Mastitis in Dairy Cows, An Issue of Veterinary Clinics: Food Animal Practice: Elsevier Health Sciences.

Saenz, A. J., C. E. Petersen, N. B. Valentine, S. L. Gantt, K. H. Jarman, M. T. Kingsley, and K. L. Wahl, 1999, Reproducibility of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for replicate bacterial culture analysis: Rapid Communications in Mass Spectrometry, v. 13, no. 15, p. 1580-1585.

Seng, P., M. Drancourt, F. Gouriet, B. La Scola, P. E. Fournier, J. M. Rolain, and D. Raoult, 2009, Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: Clinical Infectious Diseases, v. 49, no. 4, p. 543-551.

Setlow, P., 2006, Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals: Journal of Applied Microbiology, v. 101, no. 3, p. 514-525.

Smit, G., 2003,Smit, G. Dairy processing: Improving quality: Woodhead Publishing Limited.

Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows, 1972, API system: a multitube micromethod for identification of Enterobacteriaceae: Applied microbiology, v. 24, no. 3, p. 449.

Sogawa, K., M. Watanabe, K. Sato, S. Segawa, C. Ishii, A. Miyabe, S. Murata, T. Saito, and F. Nomura, 2011, Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms: Analytical and bioanalytical chemistry, v. 400, no. 7, p. 1905-1911.

Sørhaug, T., and L. Stepaniak, 1997, Psychrotrophs and their enzymes in milk and dairy products: quality aspects: Trends in Food Science & Technology, v. 8, no. 2, p. 35-41.

Sparbier, K., S. Schubert, U. Weller, C. Boogen, and M. Kostrzewa, 2012, Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Functional Assay for Rapid Detection of Resistance against -Lactam Antibiotics: Journal of clinical microbiology, v. 50, no. 3, p. 927-937.

Van Houdt, R., and C. W. Michiels, 2010, Biofilm formation and the food industry, a focus on the bacterial outer surface: Journal of Applied Microbiology, v. 109, no. 4, p. 1117-1131.

Van Veen, S. Q., E. C. J. Claas, and E. J. Kuijper, 2010, High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories: Journal of clinical microbiology, v. 48, no. 3, p. 900-907.

Whist, A. C., O. Østerås, and L. Sølverød, 2009, Association between isolation of Staphylococcus aureus one week after calving and milk yield, somatic cell count, clinical mastitis, and culling through the remaining lactation: Journal of dairy research, v. 76, no. 01, p. 24-35.

Wieckowska-Szakiel, M., A. Bubert, M. Rózalski, U. Krajewska, W. Rudnicka, and B. Rózalska, 2002, Colony-blot assay with anti-p60 antibodies as a method for quick identification of Listeria in food: International journal of food microbiology, v. 72, no. 1-2, p. 63-71.

Williams, T. L., D. Andrzejewski, J. O. Lay, and S. M. Musser, 2003, Experimental factors affecting the quality and reproducibility of MALDI TOF mass spectra obtained from whole bacteria cells: Journal of the American Society for Mass Spectrometry, v. 14, no. 4, p. 342-351.

Wunschel, D. S., E. A. Hill, J. S. McLean, K. Jarman, Y. A. Gorby, N. Valentine, and K. Wahl, 2005, Effects of varied pH, growth rate and temperature using controlled fermentation and batch culture on matrix assisted laser desorption/ionization whole cell protein fingerprints: Journal of microbiological methods, v. 62, no. 3, p. 259-271.

Xu, S., T. P. Labuza, and F. Diez-Gonzalez, 2006, Thermal inactivation of Bacillus anthracis spores in cow's milk: Applied and Environmental Microbiology, v. 72, no. 6, p. 4479-4483.

Yang, Y., B. A. Rasmussen, and D. M. Shlaes, 1999, Class A B-lactamases - enzyme-inhibitor interactions and resistance: Pharmacology & therapeutics, v. 83, no. 2, p. 141-151.

Reference Websites

[1] http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/popular.html

Appendices

Table of contents

Appendix 1: Preparation of the culture media

Preparation of the Dichloran-Rose Bengal Chloramphenicol (DRBC) agar

This medium is a selective agar used for the enumeration of yeasts and fungi in food spoiling.

It follows the recommendations of the ISO21527-1.

Reagents:

- Dichloran-Rose Bengal Chloramphenicol (DRBC) agar, Merck 100466
- Distilled water

Preparation:

The volume of medium for each RODAC plate is 12-13mL.

The volume to prepare was estimated, and then the quantity of DRBC agar powder for the volume necessary was calculated by following the table below:

Distilled water (L)	DRBC Agar (g)
1	31,6

DRBCA powder was dissolved in the demineralized water under stirring and heating.

Autoclave and fill the contact plates

The medium was autoclaved 20 minutes at 121°C in glass bottle of 500mL (filled maximum until 400mL).

Then, bottles were cooled at 50°C and plates will be filled under aseptic condition.

Plates have to be store at 4°C in the dark less than 1 week before using.

Preparation of the Tryptic Soy Agar (TSA)

This medium is a non-selective agar.

Reagents:

- Tryptic Soy Agar, Merck 105458
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of TSA powder for the volume necessary was calculated by following the table below:

Distilled water (L)	TSA (g)
1	40

TSA powder was dissolved in the demineralized water under stirring and heating.

Autoclave and fill the contact plates

The medium was autoclaved 20minutes at 121°C in glass bottle of 500mL (filled maximum until 400mL).

Then, bottles were cooled at 50°C and plates will be filled under aseptic condition. Plates have to be store at 4°C. Bottles were also kept at 4°C for further used.

Nota: TSA bottles can be stored at 4°C until 6 month.

Preparation of the Tryptone Glucose Extract agar (TGEA)

This medium is a non-selective agar. It is used for enumeration of total aerobic viable count by using the pour-plate inoculation method.

Reagents:

- Tryptone Glucose Extract agar, Oxoid CM 0127.
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of TGEA powder for the volume necessary was calculated by following the table below:

Distilled water (L)	TGEA(g)
1	24

TGEA powder was dissolved in the demineralized water under stirring and heating.

Autoclave

The medium was autoclaved 20 minutes at 121°C in glass bottle of 500mL (filled maximum until 400mL).

Then, bottles were cooled at 50°C and pour plate method was used.

Preparation of the Violet Red Bile Dextrose (VRBD) agar

This medium is a selective agar. It is used for enumeration of the microorganisms above the Enterobacteriaceae family in food products.

Reagents:

- Violet Red Bile Dextrose agar, Merck 110275
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of VRBD powder for the volume necessary was calculated by following the table below:

Distilled water (L)	VRBD(g)
1	39.5

Then, the preparation is heat to boiling under stirring in order to dissolve the powder. This medium is thermo-sensible, and then it cannot be autoclave. Moreover, the medium cannot be boiled more than 2 minutes.

Preparation of Mannitol Egg Yolk Polymixin agar (MYP)

This medium is a selective agar. It is used for enumeration detection and isolation of *Bacillus cereus* in food products.

Reagents:

- Cereus selective agar base acc. to Mossel, Merck 105267
- Egg yolk Emulsion, Merck 103784
- Bacillus Cereus Selective Supplement 109875
- Distilled water

For the preparation of 500 mL of medium:

- *Egg-yolk emulsion……..……..……..……50mL*
- *Polymyxin B sulfate 50,000 IU suspends in 1 mL of distilled water……..1mL*

Preparation:

The volume to prepare was estimated, and then the quantity of MYP powder for the volume necessary was calculated by following the table below:

Distilled water (L)	MYP(g)
1	21.5

21,5g of MYP powder and add 450 mL of distilled water. Under stirring and heating, the powder was dissolved.

Autoclave

Bottle was autoclaved at 121°C for 20 minutes, and then they were cooled down to 50°C.

After that, 50mL of sterile egg-yolk emulsion and 1 vial of *Bacillus cereus* selective supplements (Polymixin B, 50 000UI) were added and mixed in the bottle.

Preparation of milk Plate Count Agar (mPCA)

This medium is a non-selective agar. It is used for enumeration of total aerobic viable count in the dairy products.

Reagents:

- Milk Plate Count Agar, Oxoid CM 0681
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of mPCA powder for the volume necessary was calculated by following the table below:

Distilled water (L)	mPCA(g)
1	20

mPCA powder was dissolved in the demineralized water under stirring and heating.

Autoclave

The medium was autoclaved 20minutes at 121°C.

Then, bottles were cooled at 50°C and pour plate method was used.

Bottles of mPCA medium can be stored at 4°C until 3 month.

Preparation of DE MAN, ROGOSA, SHARPE agar (MRS)

This medium is non-selective agar. It is used for enumeration, cultivation, isolation, and enrichment species above the *Lactobacillus* family.

Reagents:

- MRS Agar: Lactobacillus agar acc. To DE MAN, ROGOSA, SHARPE agar, Merck 110660
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of mPCA powder for the volume necessary was calculated by following the table below:

Distilled water (L)	MRS agar(g)
1	68.2

MRS agar powder was dissolved in the demineralized water under stirring and heating.

Autoclave

The medium was autoclaved 20 minutes at 121°C.

Then, bottles were cooled at 50°C and pour plate method was used.

Preparation of RCM broth

This broth is non-selective. It is used for enumeration and cultivation of *Clostridium* species.

Reagents:

- Reinforced Clostridial Medium, Oxoid CM 0149
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of RCM broth powder for the volume necessary was calculated by following the table below:

Distilled water (L)	RCM broth (g)
1	38

RCM broth powder was dissolved in the distilled water under stirring and heating.

Preparation of tubes of RCM with Paraffin/Vaseline mixture

For each milk samples analyzed:
3 tubes have to be prepared per milk sample to test. These tubes were previously sterilized by using dry heat sterilization process (160°C-4h).

Each tube was filled with Paraffin/Vaseline mixture (1cm in the tube) and then 5mL of RCM not autoclaved was added.
Tubes were autoclaved at 121°C for 20 minutes.

RCM agar (RCM)

This medium is non-selective. It is used for enumeration and cultivation of *Clostridium* species.

Reagents:

- Reinforced Clostridial Agar, Oxoid CM 0151
- Distilled water

Preparation:

The volume to prepare was estimated, and then the quantity of RCM agar powder for the volume necessary was calculated by following the table below:

Distilled water (L)	RCM agar (g)
1	52.5

RCM agar powder was dissolved in the distilled water under stirring and heating.

Autoclave

The medium was autoclaved 20minutes at 121°C.

Then, bottles were cooled at 50°C and plates will be filled under aseptic condition.

Heart Infusion agar with esculin and blood

This medium is non-selective medium used for the growth of microorganisms from the raw milk. Preparation of this medium has been done by TINE Mastitis Laboratory in Molde.

Composition of the Heart Infusion agar with esculin and blood:

- *Brain Heart Infusion agar:……..…….……..…..40g/L*
  - *Brain infusion solids (12.5g/L)*
  - *Beef heart infusion solids (5g/L)*
  - *Proteose peptone (10g/L)*
  - *Sodium chloride (5g/L)*
  - *Glucose (2g/L)*
  - *Disodium phosphate (2.5g/L)*
  - *Agar (10g/L)*
- *Esculin:……..……..……..……..0.5g/L*
- Groomedcattle blood*:……..……..……..….50 mL/L*

pH= 7.4 at 25°C

Preparation:

Brain infusion agar is dissolve in distilled water by heating under stirring.

Autoclave

The medium was autoclaved 15 minutes at 121°C.
Other bottles containing the agar are cool down until 45-50°C. Then, the esculin is added, mixed and bottles were kept at 45-50°C.
Bovine blood (heated at 45°C°) was added and mixed by swirling the bottles.
Sterile petri dishes were filled with 10-15mL.

Appendix 2: Protocol to sample the milk sample from the cow

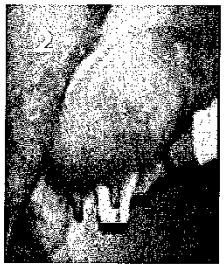
Uttak av speneprøver

TINE Mastittlaboratoriet Molde
Postboks 2038
6402 Molde
Telefon: 918 66 600
E-post: mastittlab.molde@tine.no

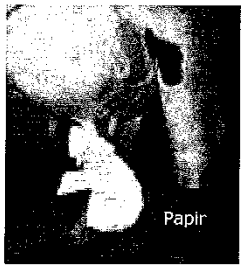
Prøvene tas slik:
Glass 1: Høyre framspene
Glass 2: Venstre framspene
Glass 3: Høyre bakspene
Glass 4: Venstre bakspene

OBS:
- Ta prøve fra spene med synlig mastitt til slutt for å hindre "smitte" fra glass til glass.
- Sørg for at jur og spener er tørre under prøvetakingen!

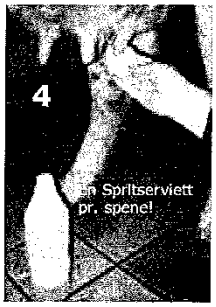
- Grundig håndvask!
- Rene og TØRRE hender



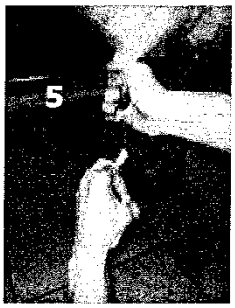
- Rene og TØRRE spener!



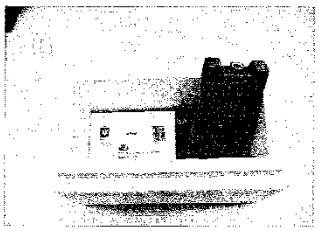
- Melk ut 10-15 ml i prøvekopp



- Gni spenespiss med 70% sprit e.l.



- Hold glasset på skrå og pass på at strålen går rett i glasset uten søl. Melkesøl tørkes straks!
- Fyll glasset maks ¾ fullt
- Nedre del av korken og kanten av glasset MÅ IKKE komme i kontakt med fingre, gulv, ku e.l.



- Legg prøven kjølig så fort som mulig. Den kan med fordel fryses før sending
- Prøver tatt fredag, lørdag eller søndag fryses før sending mandag.

- **Husk frimerke på esken** ☺

GODT JUR!

HELSETJENESTEN FOR STORFE

Ta alltid speneprøver:

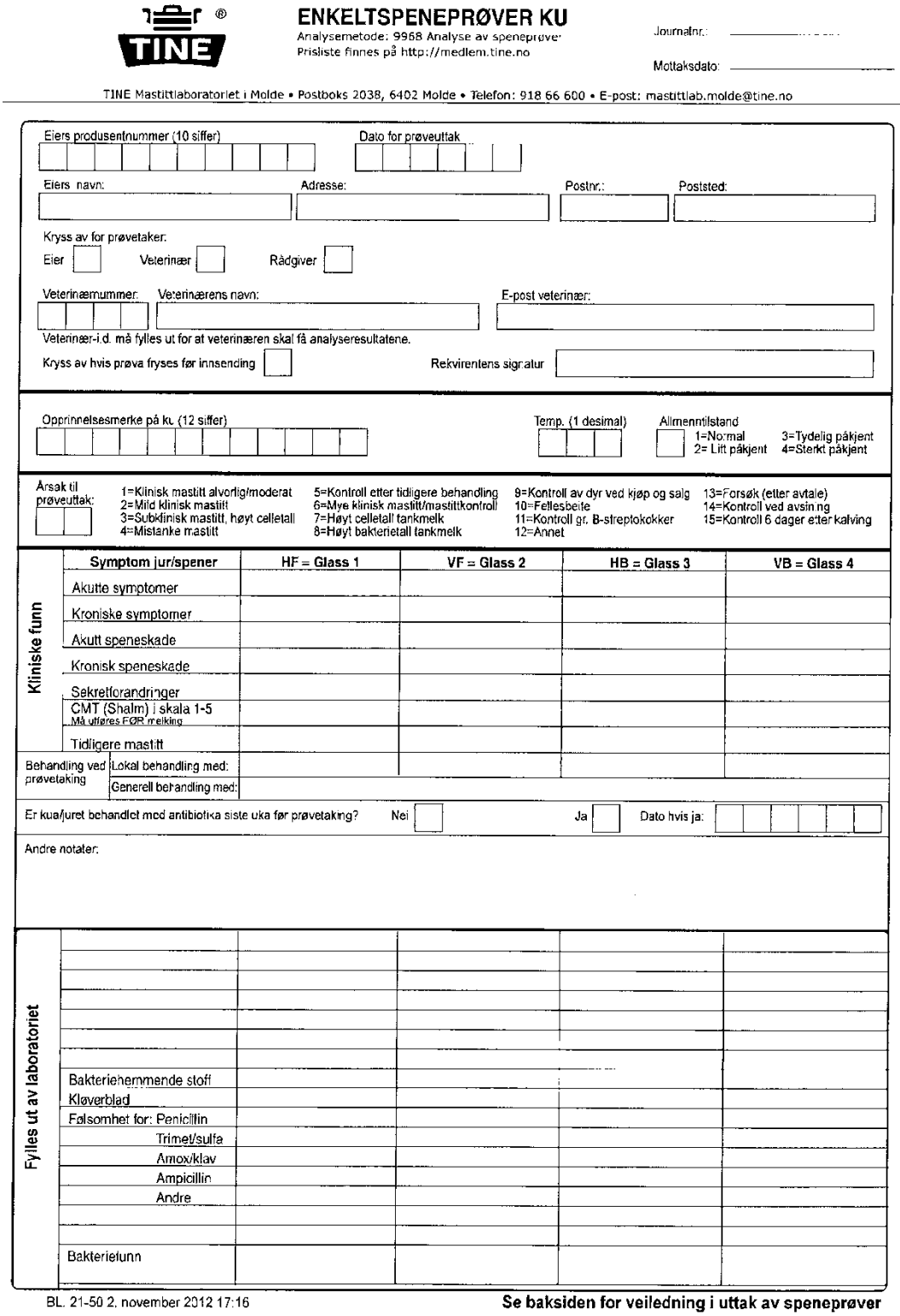
- **Før avsining av alle kyr med geometrisk middel over 100.000**
- **Av sinbehandla kyr 6 dager etter kalving**
- **Av alle synlige mastitter**
- **Av alle mastitter som behandles**
- **Ved kjøp og salg**

Resultater av analyse av speneprøver finner du på Helseutskriften og http://medlem.tine.no

Helseoversikten – verdens beste mastittstyringsverktøy!

03.04.2009

Appendix 3: Roadmap of cow milk sample in mastitis laboratory in Molde



TINE

ENKELTSPENEPRØVER KU

Analysemetode: 9968 Analyse av spenepröver
Prisliste finnes på http://medlem.tine.no

Journalnr.: _____

Mottaksdato: _____

TINE Mastittlaboratoriet i Molde • Postboks 2038, 6402 Molde • Telefon: 918 66 600 • E-post: mastittlab.molde@tine.no

Eiers produsentnummer (10 siffer) | Dato for prøveuttak

Eiers navn: | Adresse: | Postnr.: | Poststed:

Kryss av for prøvetaker:
Eier ☐ Veterinær ☐ Rådgiver ☐

Veterinærnummer: | Veterinærens navn: | E-post veterinær:

Veterinær-i d. må fylles ut for at veterinæren skal få analyseresultatene.

Kryss av hvis prøva fryses før innsending ☐ | Rekvirentens signatur

Opprinnelsesmerke på ku (12 siffer) | Temp. (1 desimal) | Allmenntilstand
1=Normal 3=Tydelig påkjent
2= Litt påkjent 4=Sterkt påkjent

Årsak til prøveuttak:
1=Klinisk mastitt alvorlig/moderat
2=Mild klinisk mastitt
3=Subklinisk mastitt, høyt celletall
4=Mistanke mastitt
5=Kontroll etter tidligere behandling
6=Mye klinisk mastitt/mastittkontroll
7=Høyt celletall tankmelk
8=Høyt bakterietall tankmelk
9=Kontroll av dyr ved kjøp og salg
10=Fellesbeite
11=Kontroll gr. B-streptokokker
12=Annet
13=Forsøk (etter avtale)
14=Kontroll ved avsetning
15=Kontroll 6 dager etter kalving

Kliniske funn	Symptom jur/spener	HF = Glass 1	VF = Glass 2	HB = Glass 3	VB = Glass 4
	Akutte symptomer				
	Kroniske symptomer				
	Akutt speneskade				
	Kronisk speneskade				
	Sekretforandringer				
	CMT (Shalm) i skala 1-5 Må utføres FØR melking				
	Tidligere mastitt				
Behandling ved prøvetaking	Lokal behandling med:				
	Generell behandling med:				

Er kua/juret behandlet med antibiotika siste uka før prøvetaking? Nei ☐ Ja ☐ Dato hvis ja:

Andre notater:

Fylles ut av laboratoriet					
	Bakteriehemmende stoff				
	Kløverblad				
	Følsomhet for: Penicillin				
	Trimet/sulfa				
	Amox/klav				
	Ampicillin				
	Andre				
	Bakterielunn				

BL. 21-50 2. november 2012 17:16

Se baksiden for veiledning i uttak av spenepröver

Appendix 4: Preparation of the Heart Infusion Broth with glycerol for freezing of strains from Mastitis laboratory in Molde

This preparation is used for freezing of bacteria at -70°C.

Composition of the Heart Infusion Broth (HIB) with glycerol 18%:

- *HIB:……..…..…….…….……...2.5g*
- *Glycerol 87%:……..……..……..18mL*
- *Distilled water:……..……....100mL*

Protocol:

The HIB is dissolved in water
Then, the glycerol is added.
Dissolve bouillon in water.
The preparation of HIB with glycerol is store in bottles to be ready to use.

Autoclave

The medium is autoclaved 15 minutes at 121°C.

Pour vials:

Vials were Nalgene® Cryogenic vials of 1.2mL. They were filled with 1mL of HIB.

Appendix 5: Preparation of the MALDI matrix solutions

1/ Matrix ***α-cyano-4-hydroxycinnamic acid (HCCA)***

$HCCA_{portioned}$ (# 8255344, Bruker Daltonics GmbH) is stored in vial and was used as matrix for identification of all samples tested with the MALDI Biotyper system.

Standard solution is prepared previously (volume of 1mL) and it is used to suspend the $HCCA_{portioned}$. This solution is composed of:

- 50% Acetronitrile,
- 47.5% of distilled water,
- 2.5% of trifluoroacetic acid.

250µL of standard solution was transferred to the $HCCA_{portioned}$ vial (HCCA concentration = 10 mg/mL). Then, vial was mixed by using vortex to dissolve completely the HCCA.

Matrix vial prepared was kept at room temperature. Every week, a new matrix has to be use.

2/ Matrix ***2,5-Dihydroxybenzoic acid (2.5-DHB)***

2.5-DHB (# 8201346, Bruker Daltonics GmbH) 2is stored in vial of 200mg. This matrix has been used only for the detection of resistance mechanisms from the strains isolated from Molde.

In an Eppendorf tube, 0.010 mg of 2.5-DHB was added and dissolved in 1 mL of 50% Ethanol.
The final concentration of this vial was 10 mg/mL.

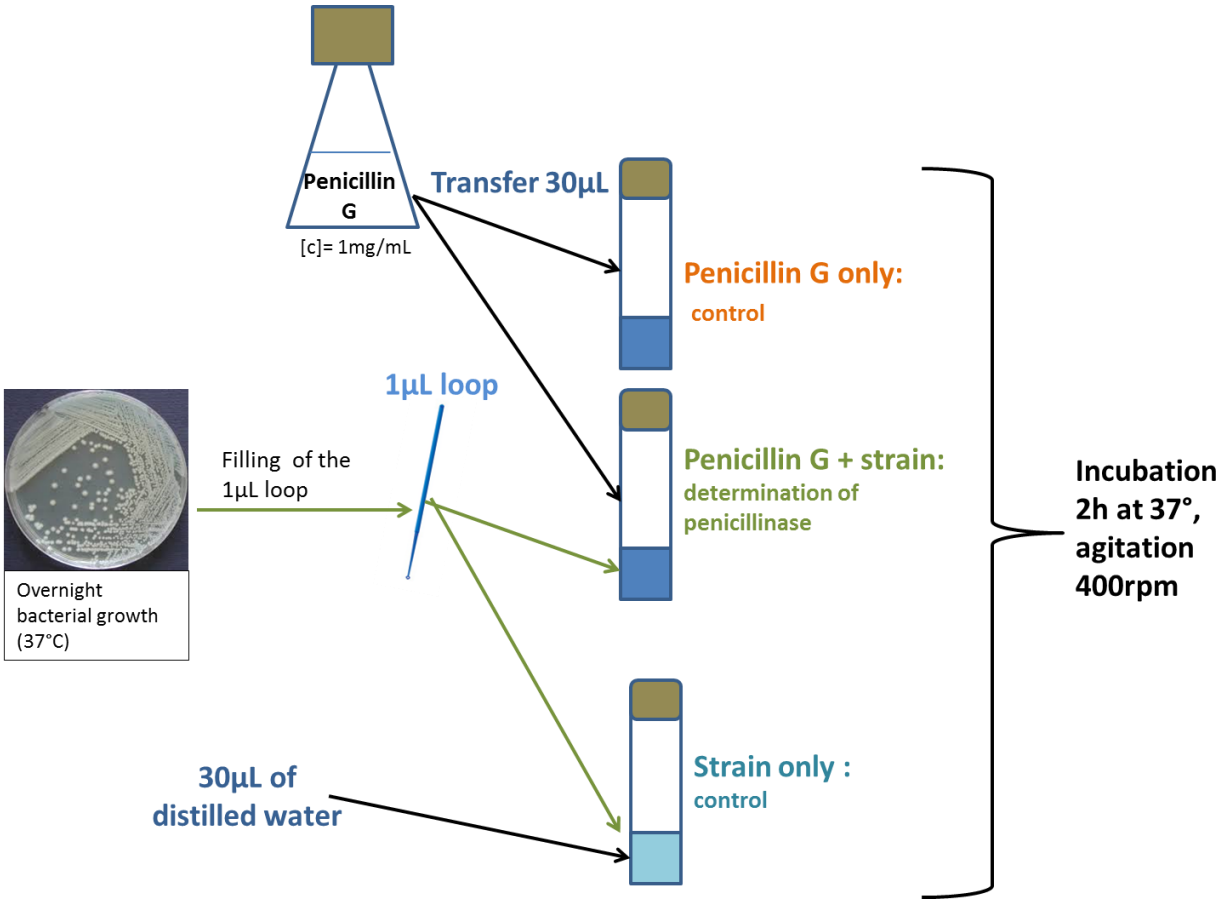
Then, the matrix was diluted 1:10 to obtain a final concentration of 1 mg/mL.

Appendix 6: List of the strains selected for the determination of the penicillin's resistance by MALDI-TOF MS

	Strains reference	Isolated from	Identification by MALDI-TOF MS results	Susceptibility testing (Pencillin G diffusion test)
1	M214-14409-15	goat	*Staphylococcus warneri*	sensible
2	M214-14410-18	goat	*Staphylococcus epidermidis*	**resistant**
3	M214-14408-14	goat	*Staphylococcus epidermidis*	**resistant**
4	M214-14555-32	goat	*Staphylococcus aureus*	**resistant**
5	M214-14411-19	goat	*Staphylococcus epidermidis*	**resistant**
6	M214-15016-27	goat	*Staphylococcus caprae*	**resistant**
7	M214-15100-35	goat	*Staphylococcus chromogenes*	sensible
8	M214-15015-25	goat	*Staphylococcus warneri*	sensible
9	M214-15102-40	goat	*Staphylococcus warneri*	**resistant**
10	M214-14494-12	goat	*Staphylococcus haemolyticus*	**resistant**
11	M214-14911-18	goat	*Staphylococcus chromogenes*	**resistant**
12	M214-14845-12	goat	*Staphylococcus warneri*	sensible
13	M214-15125-6	goat	*Staphylococcus warneri*	sensible
14	M214-13857-6	goat	*Staphylococcus epidermidis*	sensible
15	M214-14523-7	goat	*Staphylococcus caprae*	sensible
16	M214-14526-14	goat	*Staphylococcus warneri*	sensible
17	M214-14884-3	goat	*Staphylococcus warneri*	**resistant**
18	M214-14886-8	goat	*Staphylococcus epidermidis*	**resistant**
19	M214-15271-3	goat	*Staphylococcus warneri*	**resistant**
20	M214-15272-1	goat	*Staphylococcus warneri*	**resistant**
21	M214-15272-2	goat	*Staphylococcus warneri*	**resistant**
22	M214-13864-19	goat	*Staphylococcus epidermidis*	**resistant**
23	M214-14404-6	goat	*Staphylococcus epidermidis*	**resistant**
24	M214-14486-36	goat	*Staphylococcus caprae*	sensible
25	M214-15057-30	goat	*Staphylococcus warneri*	**resistant**

	Strains reference	Isolated from	Identification by MALDI-TOF MS results	Susceptibility testing (Pencillin G diffusion test)
26	M214-14549-20	goat	*Staphylococcus saprophyticus*	sensible
27	M214-15071-17	goat	*Staphylococcus simulans*	sensible
28	M214-15092-19	goat	*Staphylococcus warneri*	sensible
29	M214-15084-4	goat	*Staphylococcus caprae*	sensible
30	M214-13706-16	goat	*Staphylococcus simulans*	sensible
31	M214-14536-33	goat	*Staphylococcus caprae*	sensible
32	M214-14481-26	goat	*Staphylococcus caprae*	sensible
33	M214-13857-5	goat	*Staphylococcus epidermidis*	**resistant**
34	M214-14547-15	goat	*Staphylococcus xylosus*	**resistant**
35	M214-14533-27	goat	*Staphylococcus caprae*	sensible
36	M214-13833-2	goat	*Staphylococcus warneri*	sensible
37	M214-14480-23	goat	*Staphylococcus caprae*	**resistant**
38	M214-15038-31	goat	*Staphylococcus chromogenes*	**resistant**
39	M214-14489-2	goat	*Staphylococcus caprae*	sensible
40	M214-14534-30	goat	*Brevibacterium celere*	sensible

Appendix 7: Preparation of samples to determine the penicillin's resistance by MALDI-TOF MS



Appendix 8: Results of identification of *Bacillus cereus* group and others microorganisms collected from the raw milk samples using MALDI-TOF MS

Sample name	Isolated from…		Date	***RESULTS: identification by MALDI-TOF MS using DT method (best match)***	Score	Date	***RESULTS: identification by MALDI-TOF MS using EX method (best match)***	Score
RM19-301	From MYP plate (spot test), spot number 1-1		24/2	*Bacillus pumilus*	2.169			
RM24-001	From MYP plate (spot test), spot number 3-1		30/1	*Bacillus pumilus*	2.184			
RM26-001	From MYP plate (spot test), spot number 1-1 **Characteristic colony of *B. cereus* group**		30/1	*Bacillus weihenstephanensis*	2.236			
RM26-002	From MYP plate (spot test), spot number 1-2 **Characteristic colony of *B. cereus* group**		30/1	*Bacillus mycoides*	2.22			
RM27-006	From MYP plate (spot test), spot number 1-2		30/1	*Bacillus pumilus*	1.956	30/1	*Bacillus pumilus*	2.087
RM27-005	From MYP plate (spot test), spot number 1-1		30/1	*Bacillus subtilis*	2.178			
RM27-003	From MYP plate (spot test), spot number 3-1		30/1	*Bacillus subtilis*	2.217			
RM27-004	From MYP plate (spot test), spot number 3-2		30/1	*Bacillus pumilus*	1.937	30/1	*Bacillus altitudinis*	2.134
RM27-002	From MYP plate (spot test), spot number 2-2		30/1	*Lysinibacillus fusiformis*	2.068			
RM27-001	From MYP plate (spot test), spot number 2-1		23/1	*Lysinibacillus fusiformis*	1.985	27/2	*Lysinibacillus boronitolerans*	1.92
RM28-001	From MYP plate (spot test),		30/1	*Bacillus pumilus*	1.967	30/1	*Bacillus pumilus*	2.116

Sample name	Isolated from…		Date	*RESULTS: identification by MALDI-TOF MS using DT method (best match)*	Score	Date	*RESULTS: identification by MALDI-TOF MS using EX method (best match)*	Score
	spot number 2-1							
RM1-001	From MYP plate (spot test), spot number 1-1		24/2	*Paenibacillus polymyxa*	1.973	24/2	*Paenibacillus polymyxa*	1.888
RM1-002			30/1	*Lysinibacillus sphaericus*	2.26			
RM1-004	From MYP plate (spot test), spot number 1-2		30/1	*Paenibacillus polymyxa*	2.006	30/1	*Paenibacillus polymyxa*	2.05
RM1-005			30.01.2015	*Paenibacillus polymyxa*	1.962	30/1	*Paenibacillus polymyxa*	1.976
RM1-006	From MYP plate (spot test), spot number 2-1		24/2	*Paenibacillus polymyxa*	1.988	24/2	*Paenibacillus polyxyma*	1.86
RM1-008	From MYP plate (spot test), spot number 3-1		24/2	*Paenibacillus terrae*	1.987	30/1	**not reliable identification**	1.599
RM1-010	From MYP plate (spot test), spot number 2-2		30/1	*Paenibacillus brasilensis*	2.003	30/1	*Paenibacillus polymyxa*	1.959
RM1-009	From MYP plate (spot test), spot number 3-2		30/1	**not reliable identification**	1.661			
RM2-003	From MYP plate (spot test), spot number 3-1		30/1	**not reliable identification**	1.566			
RM2-001	From MYP plate (spot test), spot number 1-1		30/1	*Kocuria varians*	2.072			
RM3-003	From MYP plate (spot test), spot number 3-1		30/1	*Microbacterium lacticum*	2.057			
RM3-001	From MYP plate (spot test), spot number 1-1		30/1	*Microbacterium lacticum*	2.025			
RM3-002	From MYP plate (spot test), spot number 1-2		24/2	*Microbacterium lacticum*	1.969	24/2	*Microbacterium lacticum*	2.038
RM5-001	From MYP plate (spot test), spot number 1-1		30/1	**not reliable identification**	1.47	?	**not reliable identification**	1.631
RM7-001	From MYP plate (spot test), spot number 1-1		30/1	*Bacillus cereus*	2.274			

Sample name	Isolated from…		Date	*RESULTS: identification by MALDI-TOF MS using DT method (best match)*	Score	Date	*RESULTS: identification by MALDI-TOF MS using EX method (best match)*	Score
RM7-003	From MYP plate (spot test), spot number 2-2		30/1	*Bacillus pumilus*	1.847	30/1	*Bacillus pumilus*	2.173
RM7-004	From MYP plate (spot test), spot number 3-1		30/1	*Bacillus cereus*	2.281			
RM8-003	From MYP plate (spot test), spot number 3-1		30/1	*Bacillus cereus*	2.322			
RM8-002	From MYP plate (spot test), spot number 2-1		30/1	**not reliable identification**	1.444	*?*	**no peaks found**	<0
RM9-001	From MYP plate (spot test), spot number 1-1		30/1	**not reliable identification**	1.517			
RM10-001	From MYP plate (spot test), spot number 1-1		30/1	*Bacillus weihenstephanensis*	1.887	30/1	*Bacillus mycoides*	2.163
RM10-002	From MYP plate (spot test), spot number 3-1		30/1	*Bacillus thuringiensis*	2.157			
RM10-003	From MYP plate (spot test), spot number 2-1		30/1	*Bacillus subtilis*	2.194			
RM10-004	From MYP plate (spot test), spot number 2-1		30/1	*Bacillus subtilis*	2.339			
RM10-005	From MYP plate (spot test), spot number 2-2		30/1	*Bacillus subtilis*	2.19			

Appendix 9: Milk analysis (deposit of milk drop on the target) and identification of colonies isolated from MYP medium using MALDI-TOF MS

In this appendix, different sample preparations have been used. In the column **"Number of milk and sample preparation used",** the milk number is written and then an annotation as example * or *[1]. These annotations correspond to the sample preparation used. The meaning of each annotation is given below:

*: Sterile tubes filled by 4mL of raw milk only.

*[1]: Tube prepared before the heat-treatment with 3.5mL milk+0.5mL of *B .cereus* (2 McF).

*[2]: Tube prepared before the heat-treatment with 3.5mL milk+0.5mL of *B. cereus* (0.5 McF).

*[3]: Tube prepared before the heat-treatment with 4mL milk+1 colony of *B. cereus.*

After filling of the sterile tubes, they were heat-treated at 72°C for 5 minutes. Then, tubes were incubated 24h at 20°C. Drop of milk were tested directly from the tube. However, from each tube, one drop was inoculated on the surface of MYP agar. After incubation of these plates, colonies were identified directly by MALDI TOF MS (Direct Transfer method).

Milk samples	Comments	Tubes	Sample name	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Drop of milk identified directly	Score	Sopts uesd from MYP agar plate	RESULTS: identification by MALDI-TOF MS using DT method (best match) Colony from MYP identified	Score	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Confirmation from MYP	Score
milk 1 (*)	no colony on the milk drop	1	1-1	17/2	no peaks found	<0						
	no colony on the milk drop	2	1-2	17/2	no peaks found	<0						
	no colony on the milk drop	3	1-3	17/2	no peaks found	<0						
milk 2 (*)	n/a	1	2-1	17/2	no peaks found	<0		not reliable identification	1.619			
	n/a	2	2-2	17/2	no peaks found	<0		*Micrococcus luteus*	2.007			
	no colony on the milk drop	3	2-3	17/2	no peaks found	<0						
milk 3 (*)	n/a	1	3-1	17/2	not reliable identification	1.52		*Bacillus pumilus*	2.12			
	n/a	2	3-2	17/2	not reliable identification	1.284		*Bacillus pumilus*	1.827			
	n/a	3	3-3	17/2	not reliable identification	1.384		*Bacillus pumilus*	2.051			

Milk samples	Comments	Tubes	Sample name	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Drop of milk identified directly	Score	Sopts uesd from MYP agar plate	RESULTS: identification by MALDI-TOF MS using DT method (best match) Colony from MYP identified	Score	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Confirmation from MYP	Score
milk 4 (*)	n/a	1	4-1	17/2	not reliable identification	1.396		*Bacillus pumilus*	2.028			
	n/a	2	4-2	17/2	not reliable identification	1.243		not reliable identification	1.421			
	no colony on the milk drop	3	4-3	17/2	not reliable identification	1.259						
milk 5 (*)	no colony on the milk drop	1	5-1	17/2	not reliable identification	1.457						
	no colony on the milk drop	2	5-2	17/2	not reliable identification	1.401						
	no colony on the milk drop	3	5-3	17/2	not reliable identification	1.444						
milk 6 (*)	no colony on the milk drop	1	6-1	17/2	not reliable identification	1.444						
	no colony on the milk drop	2	6-2	17/2	not reliable identification	1.444						
	no colony on the milk drop	3	6-3	17/2	not reliable identification	1.307						
milk 7 (*)	no colony on the milk drop	1	7-1	17/2	not reliable identification	1.365						
	no colony on the milk drop	2	7-2	17/2	not reliable identification	1.549						
	no colony on the milk drop	3	7-3	17/2	no peaks found	<0						
milk 8 (*)	n/a	1	8-1	17/2	not reliable identification	1.457	spot 1-1	***not reliable identification***	1.68			
	n/a	1		17/2	not reliable identification	1.457	spot 1-2	*Micrococcus luteus*	2.31			
	n/a	2	8-2	17/2	not reliable identification	1.42		*Staphylococcus capitis*	2.066			
	n/a	3	8-3	17/2	not reliable identification	1.431		*Kocuria varians*	1.877			
milk 9 (*)	n/a	1	9-1	17/2	not reliable identification	1.386		***not reliable identification***	1.632			
	no colony on the milk drop	2	9-2	17/2	not reliable identification	1.51						
	no colony on the milk drop	3	9-3	17/2	not reliable identification	1.531						

Milk samples	Comments	Tubes	Sample name	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Drop of milk identified directly	Score	Sopts uesd from MYP agar plate	RESULTS: identification by MALDI-TOF MS using DT method (best match) Colony from MYP identified	Score	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) Confirmation from MYP	Score
milk 10 (*)	no colony on the milk drop	1	10-1	17/2	**not reliable identification**	1.384						
	no colony on the milk drop	2	10-2	17/2	**not reliable identification**	1.51						
	no colony on the milk drop	3	10-3	17/2	**not reliable identification**	1.302						
milk 11 (*)	yellow colony	1	M1S1	4/3				*Bacillus pumilus*	1.876			
	yellow colony	2	M1S2	4/3				*Bacillus pumilus*	2.078			
	yellow colony	3	M1S3	4/3				**no peaks found**	<0			
milk 12 (*[1])	no colony on the milk drop	1	M2S1									
	no colony on the milk drop	2	M2S2									
	no colony on the milk drop	3	M2S3									
milk 13 (*[2])	After 1 day of incubation: On MYP, presence of pink halo around the drop of milk	1	M3S1	4/3	**not reliable identification**	1.377		**not reliable identification**	1.535	5/3	*Lysinibacillus sphaericus*	*1.867*
	yellow colony	2	M3S2	4/3	**no peaks found**	<0		*Bacillus pumilus*	1.917			
	yellow colony	3	M3S3	4/3	**no peaks found**	<0		*Bacillus pumilus*	1.994			
milk 14 (*)	no colony on the milk drop	1	M4S1									
	no colony on the milk drop	2	M4S2									
	no colony on the milk drop	3	M4S3									
milk 15 (*)	no colony on the milk drop	1	M5S1									
	no colony on the milk drop	2	M5S2									
	no colony on the milk drop	3	M5S3									

Milk samples	Comments	Tubes	Sample name	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) / Drop of milk identified directly	Score	Sopts uesd from MYP agar plate	RESULTS: identification by MALDI-TOF MS using DT method (best match) / Colony from MYP identified	Score	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match) / Confirmation from MYP	Score
milk 16 (*[3])	no colony on the milk drop	1	M6S1									
	no colony on the milk drop	2	M6S2									
	no colony on the milk drop	3	M6S3									
milk 17 (*)	yellow colony	1	M7S1	4/3				*Bacillus pumilus*	1.754			
	yellow colony	2	M7S2	4/3				*Bacillus pumilus*	2.016			
	yellow colony	3	M7S3	4/3				*Bacillus pumilus*	1.713			
milk 18 (*[3])	yellow colony	1	M8S1	4/3				**no peaks found**	<0			
	yellow colony	2	M8S2	4/3				**no peaks found**	<0			
	yellow colony	3	M8S3	4/3				**not reliable identification**	1.683			

Appendix 10: Results of anaerobes identification (especially spore-formers) by using MALDI-TOF MS

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Comparison with own MSP (*C. tyrobutyricum ATCC 25755*)	Score
RM16-601	a	3/2	*Clostridium perfringens*	2.43					
	b	3/2	*Clostridium perfringens*	2.453					
RM16-602		3/2	*Clostridium perfringens*	2.376					
RM16-603		4/3	*Clostridium perfringens*	1.996	5/3	*Clostridium perfringens*	2.18		
RM16-604		4/3	*Clostridium perfringens*	2.304					
RM16-605		4/3	*Clostridium perfringens*	2.105					
RM18-301		3/2	**not reliable identification**	1.612					
RM19-301		3/2	*Bacillus licheniformis*	2.057	27/2	*Bacillus licheniformis*	2.365		
RM19-302		5/2	**not reliable identification**	1.683					
RM19-303		5/2	*Bacillus licheniformis*	1.79					
		5/2	*Bacillus licheniformis*	1.707					
		5/2	*Bacillus licheniformis*	1.71					
RM19-304		5/2	*Bacillus thermoamylovarans*	2.249					
RM21-203		3/2	**not reliable identification**	1.517				*Clostridium tyrobutyricum*	2.150
RM21-201		27/2	*Clostridium sporogenes*	2.053	27/2	*Clostridium sporogenes*	2.015		
RM21-202		27/2	*Clostridium sporogenes*	1.999	27/2	*Clostridium sporogenes*	2.12		
RM24-101	a	5/2	**not reliable identification**	1.424				*Clostridium tyrobutyricum*	1.726
	b	5/2	**not reliable identification**	1.459					
RM24-102	a	5/2	**not reliable identification**	1.348				*Clostridium tyrobutyricum*	2.231
	b	5/2	**not reliable identification**	1.47				*Clostridium tyrobutyricum*	1.825
RM29-001	a	3/2	*Clostridium sporogenes*	2.185					
	b	3/2	*Clostridium sporogenes*	2.105					
RM29-003		2/2	*Lactobacillus delbrueckii*	2.125				*Clostridium tyrobutyricum*	2.845
RM29-002		3/2	*Clostridium sporogenes*	1.903				*Clostridium tyrobutyricum*	2.127
RM1-302		27/2	*Clostridium sporogenes*	2.17	27/2	*Clostridium sporogenes*	2.376		
RM1-303		3/2	**not reliable identification**	1.578					

		Date	RESULTS: identification by MALDI-TOF MS using DT method	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method	Score	Comparison with own MSP (*C. tyrobutyricum ATCC 25755*)	Score
RM1-301		3/2	*Clostridium sporogenes*	1.912	5/2	*Clostridium sporogenes*	2.237		
RM1-304		3/2	*Clostridium sporogenes*	2.334					
RM1-305		3/2	*Clostridium sporogenes*	2.231					
RM2-401		3/2	*Clostridium sporogenes*	2.243					
RM4-301	a	3/2	**not reliable identification**	1.438					
	b	3/2	**no peaks found**	<0					
	c	3/2	*Bacillus sonorensis*	1.993					
RM4-302		3/2	*not reliable identification*	1.639					
RM4-303		3/2	*Bacillus sonorensis*	1.866	5/2	*Bacillus sonorensis*	1.921		
RM4-203		30/1	*Moraxella_sg_Moraxella osloensis*	2.048					
RM7-202		3/2	*Clostridium diolis*	1.947	5/2	*Clostridium diolis*	2.215		
RM7-205		3/2	*Clostridium sporogenes*	2.104					
RM7-203	a	5/2	*Clostridium sporogenes*	1.759					
	b	5/2	*Staphylococcus pasteuri*	2.03					
RM7-204		3/2	*not reliable identification*	1.434				*Clostridium tyrobutyricum*	1.974
RM7-201	a	5/2	*Bacillus licheniformis*	2.144					
	b	5/2	*Bacillus licheniformis*	2.102					
	c	5/2	**no peaks found**	<0					
RM10-301		3/2	*Clostridium perfringens*	2.487					
RM10-302	a	3/2	*Clostridium sporogenes*	2.463					
	b	3/2	*Clostridium perfringens*	2.365					
RM10-303	a	3/2	*Clostridium sporogenes*	2.438					
	b	3/2	*Clostridium sporogenes*	2.319					
RM11-301		5/2	**not reliable identification**	1.424				*Clostridium tyrobutyricum*	2.030
RM11-302		5/2	*Bacillus licheniformis*	1.877					
RM11-303		4/3	**not reliable identification**	1.364				*Clostridium tyrobutyricum*	2.168
RM11-304		5/2	*Bacillus licheniformis*	2.19					
RM11-305	a	4/3	*Propionibacterium acnes*	2.064					
	b	4/3	*Paenibacillus turicensis*	1.949	5/3	*Paenibacillus turicensis*	1.893		
	c	4/3	*Paenibacillus turicensis*	1.952	5/3	*Paenibacillus turicensis*	2.081		

		Date	RESULTS: identification by MALDI-TOF MS using DT method	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method	Score	Comparison with own MSP (*C. tyrobutyricum ATCC 25755*)	Score
RM14-301		27/2	*Clostridium sporogenes*	2.052					
RM14-302	a	3/2	*Clostridium sporogenes*	2.038	3/2	*Clostridium sporogenes*	2.062		
	b	5/2	*Bacillus sonorensis*	1.77					
RM14-303	a	5/2	*Bacillus cereus*	2.147					
	b	5/2	*Bacillus licheniformis*	2.252					
	c	5/2	**no peaks found**	<0					
	d				27/2	*Bacillus licheniformis*	2.286		
RM14-304	a	3/2	**not reliable identification**	1.64					
	b	3/2	*Bacillus licheniformis*	2.124					
	c	3/2	*Bacillus licheniformis*	2.046					
	d	3/2	**no peaks found**	<0					
RM14-305		5/2	**not reliable identification**	1.475				*Clostridium tyrobutyricum*	2.125
RM15-301		5/2	**not reliable identification**	1.428				*Clostridium tyrobutyricum*	2.242

Appendix 11: Results of mesophiles bacteria identification (target: *Lactobacillus*) collected from MRS agar at 30°C for 48h using MALDI-TOF MS

Sample name	Date	***RESULTS: identification by MALDI-TOF MS using DT method (best match)***	Score	Date	***RESULTS: identification by MALDI-TOF MS using EX method (best match)***	Score
RM16-001	2/2	*Weissella viridescens*	2.256			
RM16-002	2/2	*Weissella viridescens*	2.081			
RM16-101	2/2	*Weissella viridescens*	2.343			
RM16-102	2/2	*Weissella viridescens*	1.952			
RM23-001	2/2	*Streptococcus gallolyticus*	1.948	2/2	*Streptococcus gallolyticus*	2.132
RM3-102	2/2	*Streptococcus gallolyticus*	2.001			
RM3-103	2/2	*Weissella viridescens*	2.157			
RM3-101	2/2	*Lactobacillus delbrueckii*	2.076			
RM3-202	2/2	*Lactobacillus delbrueckii*	1.916			
RM3-201	2/2	**not reliable identification**	1.61			
RM7-301	2/2	*Lactobacillus delbrueckii*	1.752			
RM10-101	2/2	*Lactobacillus paracasei*	2.409			
RM10-105	2/2	*Lactobacillus paracasei*	2.379			
RM10-107	2/2	**not reliable identification**	1.541			
RM10-102	2/2	*Lactobacillus paracasei*	2.4			
RM10-109	2/2	*Leuconostoc lactis*	1.741			
RM10-103	2/2	**not reliable identification**	1.54			
RM10-108	2/2	*Leuconostoc lactis*	1.933	2/2	*Leuconostoc lactis*	1.709
RM10-110	2/2	*Leuconostoc lactis*	1.827	27/2	*Leuconostoc lactis*	2.355
RM10-106	2/2	*Leuconostoc lactis*	1.861			
RM10-104	2/2	*Leuconostoc lactis*	1.891			

Appendix 12: Results of thermophile bacteria identification (target: *Lactobacillus*) collected from MRS agar at 42°C for 48h using MALDI-TOF MS

Vials name	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM17-002	2/2	*Lactobacillus delbrueckii*	1.985	2/2	*Lactobacillus delbrueckii*	2.045
RM17-001	2/2	*Lactobacillus delbrueckii*	1.833			
RM21-002	2/2	*Lactobacillus delbrueckii*	2.08			
RM21-001	2/2	*Lactobacillus delbrueckii*	1.956			
RM21-004	2/2	*Lactobacillus delbrueckii*	1.95			
RM21-006	2/2	not reliable identification	1.646			
RM21-010	2/2	*Lactobacillus delbrueckii*	1.763			
RM21-009	2/2	*Lactobacillus delbrueckii*	2.035			
RM21-003	2/2	*Lactobacillus delbrueckii*	1.994	2/2	*Lactobacillus delbrueckii*	1.886
RM21-005	2/2	*Lactobacillus delbrueckii*	1.864			
RM21-007	2/2	not reliable identification	1.598	2/2	*Lactobacillus delbrueckii*	1.746
RM21-008	2/2	*Lactobacillus delbrueckii*	2.125			
RM3-304	2/2	*Lactobacillus delbrueckii*	1.988			
RM3-305	2/2	*Lactobacillus delbrueckii*	2.025			
RM3-310	2/2	*Lactobacillus delbrueckii*	1.702			
RM3-009	2/2	*Lactobacillus delbrueckii*	1.888			
RM13-005	2/2	*Lactobacillus delbrueckii*	1.791			
RM3-306	2/2	*Lactobacillus delbrueckii*	1.786			
RM3-303	2/2	*Lactobacillus delbrueckii*	1.836			
RM3-307	2/2	*Lactobacillus delbrueckii*	1.881	2/2	not reliable identification	1.694
RM3-308	2/2	*Lactobacillus delbrueckii*	1.789	2/2	*Lactobacillus delbrueckii*	1.77
RM3-309	2/2	*Lactobacillus delbrueckii*	1.891			
RM13-004	2/2	not reliable identification	1.562			
RM13-006	2/2	*Lactobacillus delbrueckii*	1.903			
RM13-003	2/2	not reliable identification	1.688	2/2	*Lactobacillus delbrueckii*	1.763
RM13-010	2/2	*Lactobacillus delbrueckii*	1.758			
RM13-008	2/2	*Lactobacillus delbrueckii*	1.821	2/2	*Lactobacillus delbrueckii*	1.771

Appendix 13: Results of identification of total viable bacteria collected on mPCA using MALDI-TOF MS (incubation condition: 30°C for 1-3days)

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM16-302		4/2	*Pseudomonas monteilii*	2.083			
RM16-410		4/2	**not reliable identification**	1.607	6/2	*Microbacterium aurum*	1.885
RM16-401		4/2	*Microbacterium lacticum*	2.000			
RM16-405		4/2	*Chryseobacterium gleum*	2.145			
RM16-404		6/2	**not reliable identification**	1.688			
RM16-403		4/2	*Pseudomonas monteilii*	2.055			
RM16-409		6/2	*Microbacterium lacticum*	1.827			
RM16-408		4/2	*Pseudomonas monteilii*	2.065			
RM16-407		4/2	*Pseudomonas putida*	2.188			
RM16-414		4/2	*Arthrobacter polychromogenes*	1.728			
RM17-203		6/2	*Chryseobacterium gleum*	2.408			
RM17-302		4/2	*Pseudomonas putida* and *Pseudomonas monteilli*	2.138 2.063			
RM17-202		6/2	**no peaks found**	<0			
RM17-201		4/2	*Microbacterium oxydans*	2.328			
RM17-101		5/2	**not reliable identification**	1.424	6/2	**not reliable identification**	1.686
RM17-102		5/2	*Microbacterium lacticum*	1.763	6/2	**not reliable identification**	1.445
RM17-207		4/2	*Pseudomonas putida*	2.084			
RM18-105		4/2	*Enterobacter asburiae*	2.377			
RM18-103		4/2	*Pseudomonas putida*	2.481			
RM18-101		4/2	*Pseudomonas putida*	2.396			
RM18-001		5/2	*Pseudomonas monteilii*	2.002			
RM18-003		4/2	*Pseudomonas monteilii*	2.07			
RM18-006		4/2	*Pseudomonas monteilii*	2.017			
RM18-102		4/2	*Enterobacter asburiae*	2.248			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM18-104		4/2	*Pseudomonas putida*	2.071			
RM18-009		4/2	*Stenotrophomonas maltophilia*	2.156			
RM19-101		5/2	*Pseudomonas monteilii*	2.025			
RM19-105		5/2	**not reliable identification**	1.503			
RM19-103		6/2	*Mycobacterium avium*	1.728			
RM19-104		27/2	*Staphylococcus epidermidis*	2.002			
RM19-102		5/2	*Pseudomonas monteilii*	2.036			
RM19-115					27/2	*Moraxella_sg_Moraxella osloensis*	2.189
RM19-006		27/2	*Stenotrophomonas maltophilia*	2.159	27/2	*Stenotrophomonas maltophilia*	2.127
RM19-112		5/2	**no peaks found**	<0			
RM19-109		27/2	*Bacillus licheniformis*	1.716	27/2	**not reliable identification**	1.677
RM19-113		05/2 27/2	*Bacillus licheniformis* *Moraxella_sg_Moraxella osloensis*	1.786 2.135			
RM19-110		5/2	*Moraxella_sg_Moraxella osloensis*	2.333			
RM19-108		27/2	**not reliable identification**	1.687	27/2	**not reliable identification**	1.563
RM21-104		5/2	**no peaks found**	<0			
RM21-103		5/2	**not reliable identification**	1.536			
RM21-101		5/2	**no peaks found**	<0			
RM21-110		5/2	**not reliable identification**	1.39			
RM21-102		5/2	**no peaks found**	<0			
RM21-107		5/2	*Kocuria varians*	2.079			
RM21-111		5/2	**no peaks found**	<0			
RM21-108		5/2	**not reliable identification**	1.511			
RM23-001		5/2	**not reliable identification**	1.599	6/2	**not reliable identification**	1.556
RM23-003		6/2	**not reliable identification**	1.641			
RM23-005		6/2	*Microbacterium lacticum*	2.138			
RM23-004		5/2	*Microbacterium lacticum*	1.764			
RM23-002		6/2	*Streptococcus gallolyticus*	2.144			
RM24-205		5/2	*Kocuria varians*	2.157			
RM24-201		5/2	**not reliable identification**	1.379			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM24-210		5/2	*Moraxella_sg_Moraxella osloensis*	2.027			
RM24-204		5/2	**not reliable identification**	1.406			
RM24-207		5/2	*Moraxella_sg_Moraxella osloensis*	1.997			
RM24-208		5/2	*Kocuria varians*	2.334			
RM25-006		5/2	*Kocuria varians*	2.231			
RM25-009		5/2	**not reliable identification**	1.413			
RM25-004		5/2	*Moraxella_sg_Moraxella osloensis*	1.741			
RM25-002		5/2	**no peaks found**	<0			
RM25-010		5/2	**not reliable identification**	1.587			
RM25-001		5/2	*Moraxella_sg_Moraxella osloensis*	1.842			
RM25-008		5/2	**no peaks found**	<0			
RM26-101		5/2	**not reliable identification**	1.575			
RM26-102		5/2	**not reliable identification**	1.272			
RM26-104		5/2	**not reliable identification**	1.591			
RM26-105		5/2	**not reliable identification**	1.596			
RM26-103		5/2	**not reliable identification**	1.457			
RM28-104		5/2	**not reliable identification**	1.67	6/2	*Microbacterium lacticum*	2.185
RM28-102		5/2	**not reliable identification**	1.518	6/2	*Microbacterium lacticum*	2.078
RM28-103		5/2	**not reliable identification**	1.526			
RM28-105		5/2	**not reliable identification**	1.377			
RM28-106		5/2	**not reliable identification**	1.32			
RM28-101		5/2	*Moraxella_sg_Moraxella osloensis*	1.74			
RM29-107		5/2	**no peaks found**	<0			
RM29-106		5/2	*Bacillus pumilus*	2.077			
RM29-101		5/2	**no peaks found**	<0			
RM29-105		5/2	**not reliable identification**	1.331			
RM29-104		5/2	*Bacillus pumilus*	2.057			
RM29-109		5/2	*Bacillus pumilus*	1.847			
RM30-003		5/2	*Kocuria varians*	2.103			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM30-002		6/2	not reliable identification	1.61			
RM30-004		5/2	not reliable identification	1.607			
RM30-005		5/2	*Moraxella_sg_Moraxella osloensis*	1.792			
RM30-001		5/2	*Kocuria varians*	2.306			
RM1-103		30/1	not reliable identification	1.355			
RM1-203		30/1	*Moraxella_sg_Moraxella osloensis*	1.79			
RM1-105		30/1	not reliable identification	1.672			
RM1-108		30/1	*Microbacterium lacticum*	1.781			
RM1-204		30/1	*Microbacterium lacticum*	1.914			
RM1-205		30/1	not reliable identification	1.391			
RM1-113		30/1	*Moraxella_sg_Moraxella osloensis*	1.91			
RM1-201		30/1	not reliable identification	1.523			
RM1-102		30/1	not reliable identification	1.427			
RM1-111		30/1	not reliable identification	1.669			
RM1-110		30/1	*Kocuria varians*	2.159			
RM1-114		30/1	not reliable identification	1.637			
RM1-202		30/1	*Gordonia rubripertincta*	1.726			
RM1-101		30/1	*Microbacterium lacticum*	1.786			
RM1-112		30/1	not reliable identification	1.677			
RM2-101		30/1	*Arthrobacter polychromogenes*	1.767			
RM2-103		30/1	not reliable identification	1.503			
RM2-105		30/1	*Microbacterium lacticum*	1.895			
RM2-201		30/1	*Kocuria varians*	1.962			
RM2-204		30/1	*Kocuria varians*	2.107			
RM2-302		30/1	*Kocuria varians*	2.345			
RM2-102		30/1	*Lactobacillus paracasei*	2.151			
RM2-203		30/1	not reliable identification	1.369			
RM2-104		30/1	*Arthrobacter polychromogenes*	1.803			
RM2-205		30/1	not reliable identification	1.549			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM2-107		30/1	not reliable identification	1.62			
RM2-110		30/1	*Kocuria varians*	2.138			
RM2-108		30/1	*Staphylococcus capitis*	2.239			
RM2-106		30/1	*Kocuria varians*	1.954			
RM3-401		30/1	*Moraxella_sg_Moraxella osloensis*	2.066			
RM3-402		30/1	*Moraxella_sg_Moraxella osloensis*	2.05			
RM3-501		30/1	*Kocuria varians*	1.948			
RM3-504		30/1	*Microbacterium lacticum*	1.996			
RM3-408		30/1	*Kocuria varians*	2.197			
RM3-502		30/1	not reliable identification	1.657			
RM3-406		30/1	not reliable identification	1.695			
RM3-410		30/1	not reliable identification	1.564			
RM3-407		30/1	not reliable identification	1.697			
RM3-409		30/1	*Streptococcus gallolyticus*	1.968			
RM4-110		30/1	not reliable identification	1.554			
RM4-106		30/1	*Kocuria varians*	1.945			
RM4-205		30/1	*Arthrobacter polychromogenes*	1.7			
RM4-101		30/1	*Kocuria varians*	1.928			
RM4-102		30/1	**no peaks found**	<0			
RM4-104		30/1	*Micrococcus luteus*	2.156			
RM4-105		30/1	*Moraxella_sg_Moraxella osloensis*	2.053			
RM4-201		30/1	not reliable identification	1.669			
RM5-101		30/1	not reliable identification	1.474			
RM5-201		27/2	not reliable identification	1.58	27/2	not reliable identification	1.645
RM5-103		30/1	*Kocuria varians*	2.155			
RM5-104		27/2	not reliable identification	1.636			
RM5-110		30/1	*Microbacterium lacticum*	1.92			
RM5-109		30/1	not reliable identification	1.515			
RM6-103		30/1	*Moraxella_sg_Moraxella osloensis*	1.977			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM6-101		30/1	*Kocuria varians*	2.288			
RM6-105		30/1	*Kocuria varians*	2.029			
RM6-104		30/1	*Kocuria varians*	2.203			
RM6-102		30/1	**not reliable identification**	1.596			
RM6-110		30/1	**not reliable identification**	1.696			
RM6-109		30/1	*Kocuria varians*	1.98			
RM6-111		30/1	*Kocuria varians*	2.11			
RM6-108		30/1	*Mycobacterium avium*	1.77			
RM6-107		30/1	**not reliable identification**	1.678			
RM6-106		2/2	**not reliable identification**	1.568			
RM7-102		30/1	*Enterococcus faecium*	1.72			
RM7-104		30/1	*Staphylococcus epidermidis*	1.917			
RM7-103		30/1	**not reliable identification**	1.538			
RM7-101		30/1	**not reliable identification**	1.633			
RM7-109		30/1	*Moraxella_sg_Moraxella osloensis*	2.039			
RM7-106		30/1	**not reliable identification**	1.53			
RM7-108		27/2	**not reliable identification**	1.674	27/2	*Microbacterium lacticum*	2.144
RM7-110		30/1	*Staphylococcus warneri*	2.105			
RM7-107		30/1	**not reliable identification**	1.579			
RM7-105		30/1	**not reliable identification**	1.333			
RM8-104		30/1	*Moraxella_sg_Moraxella osloensis*	2.048			
RM8-102		30/1	**not reliable identification**	1.422			
RM8-105		30/1	*Staphylococcus warneri*	2.211			
RM8-201		30/1	*Kocuria rhizophila*	2.032			
RM8-203		30/1	*Staphylococcus epidermidis*	1.809			
RM8-301		30/1	*Staphylococcus epidermidis*	2.085			
RM8-302		30/1	*Staphylococcus warneri*	1.985			
RM8-305		30/1	*Moraxella_sg_Moraxella osloensis*	1.843			
RM8-101		30/1	*Arthrobacter polychromogenes*	1.703			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM8-205		30/1	**not reliable identification**	1.482			
RM8-209		30/1	*Kocuria rhizophila*	2.032			
RM8-208		30/1	*Staphylococcus epidermidis*	1.971			
RM8-210		30/1	**not reliable identification**	1.397			
RM8-207		30/1	**not reliable identification**	1.508			
RM9-105		30/1	*Staphylococcus warneri*	2.143			
RM9-201		30/1	*Staphylococcus capitis*	2.195			
RM9-204		30/1	*Staphylococcus epidermidis*	1.971			
RM9-101		30/1	*Staphylococcus epidermidis*	2.153			
RM9-103		30/1	*Kocuria rhizophila*	2.101			
RM9-202		30/1	*Staphylococcus warneri*	2.199			
RM9-104					27/2	*Staphylococcus epidermidis*	*1.941*
RM9-107		27/2	*Staphylococcus warneri*	2.08	27/2	*Staphylococcus warneri*	*2.178*
RM10-205		4/2	**no peaks found**	<0			
RM10-201		27/2	*Staphylococcus warneri*	2.028	27/2	*Staphylococcus warneri*	2.251
RM10-202		4/2	**no peaks found**	<0			
RM10-212		5/2	*Microbacterium lacticum*	1.908	6/2	*Microbacterium lacticum*	1.772
RM10-210		4/2	*Staphylococcus warneri*	2.187			
RM10-208		4/2	*Staphylococcus warneri*	2.216			
RM10-207		4/2	*Kocuria varians*	2.266			
RM11-003	a	4/2	*Pseudomonas putida*	2.083			
	b	4/2	*Pseudomonas putida*	1.986			
RM11-102	a	4/2	*Pseudomonas pseudoalcaligenes*	1.736			
	b	5/2	*Stenotrophomonas maltophilia*	2.192			
RM11-101		4/2	*Pseudomonas graminis*	1.709			
RM11-007		30/1	*Enterobacter asburiae*	2.291			
RM11-008		30/1	*Pseudomonas putida*	2.054			
RM11-006		4/2	*Rhizobium radiobacter*	1.879			
RM11-001		4/2	*Chryseobacterium gleum*	2.358			
RM11-109		4/2	**not reliable identification**	1.564	6/2	**not reliable identification**	1.433

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM11-006	a	4/2	*Pseudomonas putida*	2.161			
	b	4/2	*Pseudomonas putida*	2.137			
	c	5/2	*Rhizobium radiobacter*	1.806			
RM11-012		4/2	*Stenotrophomonas maltophilia*	2.167			
RM11-009		4/2	*Pseudomonas putida*	2.505			
RM11-004		4/2	*Pseudomonas monteilii*	2.117			
RM11-110		4/2	*Pseudomonas asplenii*	1.724	6/2	**not reliable identification**	1.373
RM12-004		27/2	*Pseudomonas putida*	2.44	27/2	*Pseudomonas putida*	2.286
RM12-001		27/2	*Microbacterium lacticum*	1.895	27/2	*Microbacterium lacticum*	2.118
RM12-002		27/2	**not reliable identification**	1.365	27/2	**not reliable identification**	1.258
RM12-006		27/2	**not reliable identification**	1.498	27/2	*Microbacterium aurum*	1.918
RM12-007		27/2	**not reliable identification**	1.441	27/2	**not reliable identification**	1.409
RM12-010		27/2	**not reliable identification**	1.549	27/2	*Microbacterium lacticum*	1.945
RM12-003		27/2	*Enterococcus faecium*	2.013	27/2	*Enterococcus faecium*	2.091
RM12-105		4/2	*Kocuria varians*	2.177			
RM12-104		4/2	*Moraxella_sg_Moraxella osloensis*	2.008			
RM12-103		4/2	*Kocuria varians*	2.177			
RM12-102		4/2	**not reliable identification**	1.687			
RM12-101		4/2	*Sphingobacterium multivorum*	2.14			
RM13-103		4/2	*Pseudomonas putida*	2.088			
RM13-207		4/2	*Pseudomonas putida*	2.493			
RM13-205		4/2	*Microbacterium lacticum*	1.777			
RM13-206		27/2	*Enterobacter asburiae*	2.277	27/2	*Enterobacter asburiae*	2.058
RM13-101	a	4/2	*Pseudomonas monteilii*	2.121			
	b	4/2	*Pseudomonas monteilii* and *Pseudomonas putida*	2.083 2.08			
RM13-111		4/2	*Pseudomonas putida*	2.105			
RM13-102		4/2	*Streptococcus gallolyticus*	2.139			
RM14-001		4/2	*Bacillus pumilus*	2.043			

Sample name		Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
RM14-103		04.02.2015 27.02.2015	*Rhizobium radiobacter* *Arthrobacter polychromogenes*	1.718 1.845	27/2	*Microbacterium lacticum*	2.113
RM14-107		27/2	**not reliable identification**	1.481	27/2	*Kocuria varians*	2.407
RM14-002		4/2	*Bacillus pumilus*	2.063			
RM14-003		27/2	*Pseudomonas putida*	2.024	27/2	*Pseudomonas monteilii*	2.184
RM14-101		4/2	*Microbacterium lacticum*	1.935			
RM14-108		4/2	*Pseudomonas monteilii*	2.112			
RM14-013		4/2	*Bacillus pumilus*	2.212			
RM14-005		4/2	*Bacillus pumilus*	2.168			
RM15-102		4/2	*Pseudomonas putida*	*2.491*			
RM15-104		4/2	*Chryseobacterium gleum*	*2.531*	27/2	*Staphylococcus epidermidis*	2.064
RM15-101		4/2	**not reliable identification**	*1.56*	6/2	*Microbacterium aurum*	1.939
RM15-105		5/2	*Moraxella_sg_Moraxella osloensis*	*1.884*			
RM15-009		4/2	*Chryseobacterium gleum*	*2.449*			
RM15-006		4/2	**not reliable identification**	*1.584*			
RM15-008		4/2	*Pseudomonas monteilii*	*2.071*			
RM15-007		5/2	*Rhizobium radiobacter*	1.878			
RM15-005		6/2	*Pseudomonas monteilii*	*2.138*			

Appendix 14: Identification results of bacteria isolated on MYP agar (target: *Bacillus cereus* group) from consumers milks using MALDI-TOF MS

Sample name	Isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score
MA001	Colonies isolated from MYP plates, isolated from dilution 10^{-2} at 40h of incubation	17/2	*Staphylococcus haemolyticus*	2.054			
MA002	Colonies isolated from MYP plates, isolated from dilution 10^{-2} at 40h of incubation	17/2	*Staphylococcus haemolyticus*	2.056			
MC401	From MYP, first plate (1), dilution 10^{0} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.216/ 2.178			
	From MYP, first plate (1), dilution 10^{0} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.179 / 2.178			
MC403	From MYP, first plate (1), dilution 10^{0} ==> On MYP plate, colony selected was characteristic of Bacillus cereus group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.286/ 2.27			
MC402	From MYP, first plate (1), dilution 10^{0} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.326/ 2.183			
MC 405	From MYP, first plate (1), dilution 10^{0} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.244/ 2.119			
MC501	From MYP, second plate (2), dilution 10^{0}	17/2	**not reliable identification**	1.487			
	From MYP, second plate (2), dilution 10^{0}	17/2	*Bacillus licheniformis*	1.817			
MC502	From MYP, second plate (2), dilution 10^{0}	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.343 / 2.218			
MD101	From MYP, second plate (2), dilution 10^{0}	17/2	*Bacillus licheniformis*	1.803			
MD102	From MYP, second plate (2), dilution 10^{0}	17/2	*Bacillus pumilus*	1.909			
MD103	From MYP, second plate (2), dilution 10^{0}	17/2	*Bacillus thuringiensis*	2.073			
MD001	From MYP, first plate (1), dilution 10^{0}	17/2	*Bacillus pumilus*	2.142			
ME001	From MYP, first plate (1), dilution 10^{0}	17/2	**no peaks found**	<0			

Sample name	Isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score
ME002	From MYP, first plate (1), dilution 10^0	17/2	*Staphylococcus haemolyticus*	1.859			
ME003	From MYP, first plate (1), dilution 10^0	17/2	**no peaks found**	<0			
ME101	From MYP, second plate (2), dilution 10^0	17/2	**not reliable identification**	1.544			
	From MYP, second plate (2), dilution 10^0	17/2	**not reliable identification**	1.474			
ME102	From MYP, second plate (2), dilution 10^0	17/2	**not reliable identification**	1.476			
MF001	From MYP, first plate (1), dilution 10^0	17/2	*Kocuria varians*	2.107			
MF003	From MYP, first plate (1), dilution 10^0	17/2	*Staphylococcus warneri*	1.877			
MF101	From MYP, second plate (2), dilution 10^0	17/2	*Kocuria varians*	2.268			
MF103	From MYP, second plate (2), dilution 10^0	17/2	**not reliable identification**	1.552			
MH102	From MYP, plate 2, dilution 10^{-2} ==> On MYP plate, colony selected was characteristic of Bacillus cereus group	17/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.299/ 2.241			
		17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.186/ 2.158			
MH101	From MYP, plate 2, dilution 10^{-2} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.294/ 2.15			
MH001	From MYP, plate 1, dilution 10^{-2} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.128/ 2.079			
MH002	From MYP, plate 1, dilution 10^{-2} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.303/ 2.27			
MH103	From MYP, plate 2, dilution 10^{-2} ==> On MYP plate, colony selected was characteristic of *Bacillus cereus* group	17/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.067/ 2.031			
MI001	From MYP, plate1, dilution 10^0	18/2	*Bacillus pumilus*	1.986	20/2	*Bacillus pumilus*	2.127
MI101	From MYP, plate 2, dilution 10^0	18/2	*Bacillus pumilus*	1.908	20/2	*Bacillus pumilus*	1.924
MI102	From MYP, plate 2, dilution 10^0	19/2	*Bacillus pumilus*	1.844	20/2	*Bacillus pumilus*	1.915
MI103	From MYP, plate 2, dilution 10^0	18/2	*Bacillus pumilus*	1.874			
MI104	From MYP, plate 2, dilution 10^0	18/2	*Bacillus pumilus*	1.854	20/2	*Bacillus pumilus*	2.012
MI105	From MYP, plate 2, dilution 10^0	19/2	*Bacillus pumilus*	2.021			
MJ-401	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis*	1.999	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.367/ 2.224
MJ-402	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis*	2.094			

Sample name	Isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score
MJ-403	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.205/ 2.087			
MJ-404	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.132/ 2.101			
MJ-405	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis*	2.099			
MK-301	From MYP, second plate (2), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis*	2.076			
MK-101	From MYP, second plate (2), dilution 10^{-1}	18/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.266 / 2.176			
MK-001	From MYP, first plate (1), dilution 10^{-1}	18/2	*Bacillus weihenstephanensis*	2.1			
MK-004	From MYP, first plate (1), dilution 10^{-1}	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.231/ 2.211			
MK-201	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.288/ 2.279			
MK-202	From MYP, first plate (1), dilution 10^{-2}	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.132/ 2.087			
ML-001	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.249/ 2.209			
ML-003	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	1.96	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.278/ 2.033
ML-005	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	2.065			
ML-102	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	2.017			
ML-104	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	1.977	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.273/ 2.041
MM-001	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.119/ 2.087	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.241/ 2.064
MM-002	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.139 / 2.112			
MM-003	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.224/ 2.191			
MM-101	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	2.013			
MM-105	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.075/ 2.021			
MN-101	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.173/ 2.113			
MN-103	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.196/ 2.059			
MN-104	From MYP, second plate (2), dilution 10^{-2}	19/2	*Paenibacillus amololyticus*	2.044			

Sample name	Isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score
MN-001	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	1.707	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.239/ 2.036
MN-003	From MYP, first plate (1), dilution 10^{-2}	19/2	*Paenibacillus amylolyticus*	2.113			
MO-001	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.125/ 2.086			
MO-003	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides*	1.964	20/2	*Bacillus mycoides*	1.975
MO-101	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus mycoides/ Bacillus weihenstephanensis*	2.3/ 2.276			
MO-102	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis*	2.166			
MO-104	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.247/ 2.177			
MP-101	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.159/ 2.066			
MP-103	From MYP, second plate (2), dilution 10^{-2}	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.232/ 2.212			
MP-001	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.191/ 2.044			
MP-003	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.221/ 2.187			
MP-005	From MYP, first plate (1), dilution 10^{-2}	19/2	*Bacillus mycoides*	1.953	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.096/ 2.083

Appendix 15: Identification results of bacteria isolated on VRBD agar (incubation condition: 30°C-24h; target: Enterobacteriaceae) from consumers milks using MALDI-TOF MS

Sample name	Isolated from…	Date	*RESULTS: identification by MALDI-TOF MS using DT method (best match)*	Score
MC101	From VRBD (30°C-1d), second plate (2), dilution 10^{0}	17/02/2015	*Pseudomonas fuscovaginae*	1.831
MC102	From VRBD (30°C-1d), second plate (2), dilution 10^{0}	17/02/2015	**not reliable identification**	1.575
MC202	From VRBD (30°C-1d), first plate (1), dilution 10^{-1}	17/02/2015	*Pseudomonas graminis*	1.709
MC201	From VRBD (30°C-1d), first plate (1), dilution 10^{-1}	17/02/2015	*Pseudomonas fuscovaginae*	1.734
MC301	From VRBD (30°C-1d), second plate (2), dilution 10^{-1}	17/02/2015	*Bacillus mycoides* / *Bacillus weihenstephanensis*	2.33 / 2.174
MC001	From VRBD (30°C-1d), first plate (1), dilution 10^{0}	17/02/2015	*Pseudomonas fuscovaginae*	1.753
MC005	From VRBD (30°C-1d), first plate (1), dilution 10^{0}	17/02/2015	*Pseudomonas graminis*	1.759
MC302	From VRBD (30°C-1d), second plate (2), dilution 10^{-1}	17/02/2015	**not reliable identification**	1.686
MC002	From VRBD (30°C-1d), first plate (1), dilution 10^{0}	17/02/2015	*Pseudomonas graminis*	1.732
MC003	From VRBD (30°C-1d), first plate (1), dilution 10^{0}	17/02/2015	**not reliable identification**	1.565
MC304	From VRBD (30°C-1d), second plate (2), dilution 10^{-1}	17/02/2015	**not reliable identification**	1.685
MC305	From VRBD (30°C-1d), second plate (2), dilution 10^{-1}	17/02/2015	**not reliable identification**	1.686

Appendix 16: Identification results of bacteria isolated on VRBD agar (incubation condition: 25°C-3 days; target: *Pseudomonas*) from consumers milks using MALDI-TOF MS

Sample name	Isolated from…	Date	*RESULTS: identification by MALDI-TOF MS using DT method (best match)*	Score	Date	*RESULTS: identification by MALDI-TOF MS using eDT method (best match)*	Score
MC-701	From VRBD (25°C-3d), second plate (2), dilution 10^0	17/2	*Pseudomonas viridiflava*	1.725			
MC-702	From VRBD (25°C-3d), second plate (2), dilution 10^0	17/2	**not reliable identification**	1.673			
MC-703	From VRBD (25°C-3d), second plate (2), dilution 10^0	17/2	**not reliable identification**	1.696			
MC-605	From VRBD (25°C-3d), first plate (1), dilution 10^0	17/2	**not reliable identification**	1.635			
MC-905	From VRBD (25°C-3d), second plate (2), dilution 10^{-1}	17/2	*Pseudomonas graminis*	1.75			
MC-801	From VRBD (25°C-3d), first plate (1), dilution 10^{-1}	17/2	**not reliable identification**	1.677			
MC-802	From VRBD (25°C-3d), first plate (1), dilution 10^{-1}	17/2	**not reliable identification**	1.625			
MC001	From VRBD (30°C-1d), first plate (1), dilution 10^0	17/2	*Pseudomonas fuscovaginae*	1.753			
MC601	From VRBD (25°C-3d), first plate (1), dilution 10^0	17/2	**not reliable identification**	1.655			
MC602	From VRBD (25°C-3d), first plate (1), dilution 10^0	17/2	*Pseudomonas graminis*	1.77			
ML-501	From VRBD (25°C-3d), second plate (2), dilution 10^0	19/2	*Stenotrophomonas rhizophila*	1.774	20/2	*Stenotrophomonas rhizophila*	1.734
ML-502	From VRBD (25°C-3d), second plate (2), dilution 10^0	19/2	*Stenotrophomonas rhizophila*	1.881			
ML-401	From VRBD (25°C-3d), first plate (1), dilution 10^0	19/2	*Stenotrophomonas rhizophila*	1.764	20/2	**not reliable identification**	1.698
ML-403	From VRBD (25°C-3d), first plate (1), dilution 10^0	19/2	*Stenotrophomonas rhizophila*	1.715			
ML-405	From VRBD (25°C-3d), first plate (1), dilution 10^0	19/2	*Stenotrophomonas rhizophila*	1.764			

Appendix 17: Identification results of bacteria isolated on TGEA agar (incubation condition: 30°C-3 days; target: total viable count) from consumers milks using MALDI-TOF MS

In this appendix, samples have been isolated and purified to make a collection. Samples were isolated on TGEA from different dilutions: 10^{-1}, 10^{-2} or 10^{-3}. In the column "Sample name, isolated from…", the sample name is written and then an annotation as example * or *[1]. These annotations correspond to the dilution used to pick the colony. The meaning of each annotation is given below:

* : From TGEA, dilution 10^{-2}

*[1]: From TGEA, dilution 10^{-3}

*[2]: From TGEA, dilution 10^{-1}

Sample name, isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
MA-004 *	18/2	*Mycobacterium avium*	1.707						
MA-008 *							20/2	*Corynebacterium tuberculostearicum*	1.708
MA-005 *							20/2	**not reliable identification**	1.468
MA-007 *	19/2	*Mycobacterium avium*	1.738						
MA-003 *	18/2	**not reliable identification**	1.623						
MA-006 *	18/2	*Corynebacterium tuberculostearicum*	1.767						
MA-010 *	18/2	*Corynebacterium tuberculostearicum*	2.038						
MB-001 *	18/2	**not reliable identification**	1.464	19/2	*Kocuria kristinae*	2.45			
MC-2003 *	17/2	*Arthrobacter polychromogenes*	1.767						
MC-2002 *	17/2	**not reliable identification**	1.626						
MC-1001 *	18/2	*Arthrobacter polychromogenes*	1.746						
MC-1006 *	18/2 19/2	*Arthrobacter polychromogenes*/ **not reliable identification**	1.729 / 1.695	20/2	**not reliable identification**	1.69			
MC-1005 *	17/2	**not reliable identification**	1.638						

Sample name, isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
MC-1007 *	17/2	*Staphylococcus warneri*	2.197						
MC-2001 *	17/2	not reliable identification	1.638						
MD-201 *	18/2	*Kocuria varians*	2.238						
MD-301 *	18/2	*Microbacterium lacticum*	1.834						
ME-201 *	17/2	*Micrococcus luteus*	2.278						
ME-301 *[1]	18/2	*Staphylococcus epidermidis*	2.224						
MF-201 *	18/2	not reliable identification	1.587						
MF-301 *	18/2	*Corynebacterium accolens*	2.162						
MG201 *	17/2	*Micrococcus luteus*	2.269						
MG105 *[2]	17/2	*Moraxella_sg_Moraxella osloensis*	1.876						
MG001 *[2]	18/2	*Microbacterium lacticum*	1.935						
MG002 *[2]	18/2	not reliable identification	1.452						
MG003 *[2]	18/2	not reliable identification	1.659						
MG202 *	18/2	*Microbacterium flavum*	1.724						
	18/2	*Microbacterium flavum*	1.764						
MG101 *[2]	18/2	*Gordomia rubripertincta*	1.701						
MG102 *[2]	18/2 19/2	*Arthrobacter polychromogenes/ Mycobacterium avium* *Microbacterium lacticum*	1.8/ 1.725 1.799	20/2	not reliable identification	1.425			
MH501 *	17/2	*Bacillus weihenstephanensis*	2.21						
MH503*	17/2	*Bacillus mycoides*	2.198						
MH401 *	17/2	*Bacillus mycoides*	2.227						

Sample name, isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
MH402*	17/2	*Staphylococcus warneri*	1.962						
	17/2	*Staphylococcus warneri*	2.155						
MH201*[1]	17/2	*Bacillus weihenstephanensis*	2.114						
MH301 *[1]	17/2	*Bacillus mycoides*	2.253						
MI401 *	19/2	*Mycobacterium avium*	1.729				20/2	not reliable identification	1.654
MI402 *	19/2	not reliable identification	1.537						
MI501 *	19/2	not reliable identification	1.584	20/2	not reliable identification	1.695			
MI502 *	18/2	*Microbacterium lacticum*	2.077						
MI203 *	19/2	not reliable identification	1.672	20/2	*Microbacterium lacticum*	2.06			
MK-401	19/2	*Microbacterium lacticum*	1.912	20/2	*Microbacterium lacticum*	1.755			
MK-702 *[2]	19/2	not reliable identification	1.645				20/2	*Microbacterium lacticum*	2.024
	19/2	*Microbacterium lacticum*	1.747				20/2	*Microbacterium lacticum*	2.024
MK-504 *	19/2	*Microbacterium lacticum*	1.747	20/2	not reliable identification	1.566			
MK-602 *[2]	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.174/ 2.099						
MK-601 *[2]	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.197/ 2.147						
MK-502 *[2]	19/2	not reliable identification	1.366						
MK-503 *[2]	18/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.347 / 2.29						
MK-604 *[2]	18/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.144/ 2.107						
MK-603 *[2]	19/2	*Microbacterium lacticum*	1.915	20/2	*Microbacterium lacticum*	1.938			
MK-701 *[2]	26/2	*Bacillus pumilus*	2.07				26/2	Bacillus pumilus	2.008
	26/2	*Bacillus pumilus*	1.883				26/2	Bacillus pumilus	1.751
MK-404 *	18/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.283 / 2.236						
MK-405	18/2	*Bacillus weihenstephanensis /*	2.208/						

Sample name, isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
*		*Bacillus mycoides*	2.166						
ML-704 *	19/2	*Paenibacillus odorifer*	2.054						
ML-701 *	19/2	*Bacillus weihenstephanensis*	2.08						
ML-702 *	19/2	*Bacillus weihenstephanensis*	1.927	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.322 / 2.206			
ML-703 *	23/1	*Bacillus weihenstephanensis*	2.119	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.322 / 2.131			
ML-705 *	23/1	*Bacillus mycoides*	2.222						
ML-602 *	19/2	*Bacillus weihenstephanensis*	1.954	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.289 / 2.05			
ML-604 *				20/2	***not reliable identification***	1.671			
MM-204 *	19/2	*Moraxella_sg_Moraxella osloensis*	1.931	20/2	*Moraxella_sg_Moraxella osloensis*	1.799			
MM-301 *	19/2	*Bacillus weihenstephanensis*	2.154						
MM-305 *	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.178						
MM-202 *	20/2	***not reliable identification***	1.573						
MM-303 *	20/2	***not reliable identification***	1.695						
MN-302 *	20/2	***not reliable identification***	1.524						
MN-201 *	19/2	*Paenibacillus amylolyticus*	2.005	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.241 / 2.064			
MN-203 *	19/2	*Bacillus simplex*	1.999	20/2	*Bacillus simplex*	1.983			
MN-204 *	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.205/ 2.062						
MN-301 *	19/2	***not reliable identification***	1.692	20/2	*Bacillus mycoides / Bacillus weihenstephanensis*	2.254 / 2.045			
MO-201 *	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.123/ 2.016						
MO-203 *				20/2	*Bacillus pumilus*	1.955			
MO-204*	19/2	*Bacillus weihenstephanensis*	2.129	20/2	*Bacillus mycoides*	2.033			

Sample name, isolated from…	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
MO-305 *	19/2	*Bacillus weihenstephanensis/ Bacillus mycoides*	2.1/ 2.088						
MO-301 *	19/2	*Bacillus weihenstephanensis*	2.048						
MO-202 *	20/2	***not reliable identification***	1.462						
MO-302 *	20/2	*Microbacterium lacticum*	1.914						
MP-201 *	19/2	*Microbacterium lacticum*	2.131						
MP-302 *	19/2	*Microbacterium lacticum*	1.781	20/2	*Microbacterium lacticum*	2.024	20/2	*Microbacterium lacticum*	2.024
MP-205 *	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.087/ 2.064						
MP-303 *	19/2	*Bacillus weihenstephanensis / Bacillus mycoides*	2.109 / 2.046						
MP-305 *	19/2	*Bacillus weihenstephanensis*	2.072						

Appendix 18: Results of yeasts identification from the surface of cheese using MALDI-TOF MS

The Table below summarizes the results of identification of yeasts from the cheeses surfaces. The (*) mark in the column "Comments" indicates that the colony selected were the dominating colony on the contact plate (DRBCA).

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
1B02-1	G								28/1	***5S01-5*** ***8B01-2***	2.569 2.572
1B02-2	G		25/2	not reliable identification	1.6	25/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	1.955	28/1	***5B01-6*** ***8B01-2*** ***5B01-10*** ***1S02-9***	2.43 2.426 2.376 2.37
1B02-3	G								28/2	***8S01-4*** ***1S02-7*** ***5B01-10***	2.247 2.334 2.328
1B02-4	G								28/2	***8S01-4*** ***8B01-2*** ***1S02-7*** ***5B01-10***	2.387 2.389 2.383 2.38
1B02-5	G		25/2	*Candida_colliculosa[ana] (Torulaspora_delbrueckii[teleo]#)*	1.78	25/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	2.225	28/1	***8B01-2*** ***5S01-5***	2.553 2.513
1B02-6	G		25/2	*spot 1: Candida_sphaerica [ana] (Kluyveromyces_lactis_var lactis [teleo])* *spot 2: Candida_sphaerica[ana] (Kluyveromyces_lactis [teleo])*	2.13 2.087	25/2	*Candida_sphaerica [ana] (Kluyveromyces_lactis [teleo])*	2.557	28/1	***1S02-5*** ***1S02-6***	2.469 2.401
1B02-7	G								28/1	***5S01-6*** ***5B01-7***	2.479 2.48
1B02-8	G								28/2	***8S01-4*** ***5S01-6*** ***5S01-5*** ***1S02-7***	2.375 2.424 2.399 2.508

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
1S02-1	G								28/2	***1S02-7*** ***8B01-2*** ***5S01-5*** ***5B01-10*** ***8S01-4***	2.508 2.478 2.436 2.361 2.375
1S02-2	G		25/2	*Candida_sphaerica [ana]* *(Kluyveromyces_lactis [teleo])*	2.228	25/2	*Candida_sphaerica [ana]* *(Kluyveromyces_lactis [teleo])*	2.511			
1S02-3	G								28/2	***5B01-10*** ***1S02-9*** ***5S01-5***	2.561 2.505 2.504
1S02-4	G								28/2	***8B01-2*** ***8S01-4*** ***5S01-5*** ***5S01-6***	2.568 2.261 2.552 2.206
1S02-5	G			*Candida_sphaerica [ana]* *(Kluyveromyces_lactis [teleo])*	2.254						
1S02-6	G		26/1	*Candida_sphaerica [ana]* *(Kluyveromyces_lactis_var_lactis [teleo])*	2.197						
1S02-7	G		26/1	**not reliable identification**	1.693	25/2	*Candida_colliculosa [ana]* *(Torulaspora_delbrueckii [teleo]#)*	2.135			
1S02-8	G		26/1	*Candida_sphaerica [ana]* *(Kluyveromyces_lactis_var_lactis [teleo])*	2.073						
1S02-9	G		26/1	**not reliable identification**	1.448	25/2	*Candida_colliculosa [ana]* *(Torulaspora_delbrueckii [teleo]#)*	2.268			
1S02-10	G		26/1	*Candida intermedia*	2.258						
2S01-1	H		24/2	*Candida_lipolytica [ana]* *(Yarrowia_lipolytica [teleo]#)*	2.091				28/1	***6B01-3*** ***6B01-5***	2.426 2.159
2S01-3	H		28/1	*Candida_lipolytica [ana]* *(Yarrowia_lipolytica[teleo]#)*	2.093				28/1	***7S01-5***	2.252
2S01-4	H								28/1	***6B01-7*** ***6B01-3***	2.061 2.045

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
2S01-5	H		28/1	*Candida_guilliermondii [ana]# (Pichia_guilliermondii[teleo])*	2.099				28/1	***6S01-11*** ***2B02-5***	2.472 2.486
2S01-6	H								28/1	***2B02-8*** ***6S01-7***	2.225 2.134
2S01-7	H		28/1	**not reliable identification**	1.61				28/1	***6B01-5*** ***6B01-3*** ***1S02-8***	2.426 2.42 1.79
2S01-8	H		28/1	*Candida_guilliermondii [ana]# (Pichia_guilliermondii[teleo])*	1.963						
2B02-1	H					26/2	*Debraryomyces hansenii*	1.723	28/1	***2B02-5*** ***6S01-11*** ***3B02-1***	2.313 2.321 2.239
2B02-2	H					26/2	*Candida zeylanoides*	2.236	28/1	***2B02-8***	2.265
2B02-3	H								28/2	***6S01-7*** ***2B02-8***	2.232 2.229
2B02-4	H		25/2	*Candida zeylanoides*	2.112	25/2	*Candida zeylanoides*	2.384	28/1	***2B02-8***	2.394
2B02-5	H		26/1	*Debaryomyces hansenii*	1.914	26/2	*Debraryomyces hansenii*	1.824			
2B02-6	H								28/1	***2B02-8*** ***6S01-7*** ***1S02-8*** ***8S01-4***	2.298 1.983 1.808 1.742
2B02-7	H					26/2	*Candida zeylanoides*	2.085	28/1	***2B02-8*** ***6S01-7***	2.332 2.276
2B02-8	H		26/1	*Candida zeylanoides*	1.941	25/2	*Candida zeylanoides*	2.367			
2B02-9	H		26/1	*Geotrichum silvicola*	1.972	25/2	*Geotrichum silvicola*	2.048			
2B02-10	H								28/1	***2B02-8*** ***6S01-7***	2.293 2.027

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
2B02-11	H		28/1	*Candida_pelliculosa[ana] (Pichia_anomala[teleo]#)*	1.913				28/1	***6S01-4***	2.332
2B02-12	H								28/1	***2B02-5*** ***6S01-11*** ***2B02-5*** ***3B02-1***	2:373 2.467 2.592 2.2
2B02-13	H		28/1	*Candida_pelliculosa[ana] (Pichia_anomala[teleo]#)*	2.121				28/1	***6S01-4***	2.358
2B02-14	H								28/1	***6S01-11*** ***3B02-1*** ***2B02-5***	2.584 2.188 2.45
3B02-1	I		26/1	*Debraryomyces hansenii*	2.198						
4S01-1	J		26/1	**not reliable identification**	1.342						
4S01-2	J								28/1	***4B01-4***	2.395
4S01-3	J		26/2	**not reliable identification**	1.273	26/2	**not reliable identification**	1.384	28/1	***4S01-1***	2.403
4S01-5	J		26/2	**not reliable identification**	1.371	26/2	**not reliable identification**	1.415	28/1	***4S01-1*** ***4B02-1***	2.663 2.643
4S01-6	J								28/1	***4B01-4*** ***4S01-1***	2.487 2.339
4S02-1	J		26/2	**not reliable identification**	1.658	26/2	**not reliable identification**	1.414	28/1	***4S01-1*** ***4B02-1***	2.659 2.574
4S02-3	J								28/1	***4B02-1*** ***4B01-4*** ***4S01-1***	2.341 2.336 2.271
4B01-1	J								28/1	***1S02-8***	2.264
4B01-2	J		26/1. 28/1	**not reliable identification**	1.367	26/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii[teleo]#)*	2.437	26.01. 2015 28.01. 2015	***8B01-2*** ***5B01-6*** ***5S01-5*** ***5B01-7***	2.648 2.502 2.485 2.469

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
4B01-3	J		26/1	not reliable identification	1.465						
4B01-4	J		26/1	not reliable identification	1.456	26/2	not reliable identification	1.422			
4B02-1	J		26/1	not reliable identification	1.346						
4B02-2	J		28/1						28/1	***4B01-4***	2.452
4B02-3	J		28/1			26/2	not reliable identification	1.347	28/1	***4B01-4***	2.48
4B02-4	J		28/1	not reliable identification	1.464						
4B02-6	J								28/1	***4S01-1*** ***4B01-4***	2.442 2.474
4S02-1	J								28/1	***4S01-1*** ***4B02-1***	2.697 2.591
5S01-1	G		28/1	not reliable identification	1.48	26/2	*Debaryomyces hansenii*	1.793	28/1	***5S01-10***	2.079
5S01-2	G								28/2	***8S01-6***	2.177
5S01-4	G								28/1	***5B01-6*** ***1S02-7*** ***8B01-2*** ***8S01-4***	2.426 2.403 2.419 2.379
5S01-5	G	*	26/1	*Candida_colliculosa[ana] (Torulaspora_delbrueckii [teleo]#)*	1.714	26/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	2.341			
5S01-6	G		26/1	not reliable identification	1.443						
5S01-7	G		26/1	not reliable identification	1.492						
5S01-8	G		26/1	not reliable identification	1.643						

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
5S01-10	G		26/1	not reliable identification	1.372						
5B01-1	G								28/1	***5S01-5*** ***8S01-4*** ***8B01-2***	2.671 2.295 2.567
5B01-2	G								28/1	***5B01-10*** ***5B01-7*** ***8S01-4*** ***5S01-6*** ***1S02-7***	2.53 2.522 2.371 2.426 2.343
5B01-3	G		26/1	not reliable identification	1.675	26/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	2.288			
5B01-4	G								28/1	***8S01-4*** ***5S01-6***	2.372 2.336
5B01-6	G		26/1	not reliable identification	1.667						
5B01-7	G		26/1	not reliable identification	1.559						
5B01-10	G	*	26/1	not reliable identification	1.624	25/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	2.41			
5B01-11	G								28/1	***5S01-5*** ***8B01-2*** ***5S01-6***	2.423 2.695 2.535
6S01-1	H	*							28/2	***6B01-37S01-56B01-76B01-5***	2.262.1 462.19 2.068
6S01-2	H								28/1	***6S01-11*** ***2B02-5*** ***3B02-1***	2.619 2.308 2.5
6S01-3	H		22/1	*Debaryomyces hansenii*	2.241				28/1	***3B02-1*** ***2B02-5*** ***6S01-11***	2.428 2;514 2.405
6S01-4	H		26/1	*Candida pelliculosa*	2.219						

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
6S01-6	H								28/1	***1S02-10***	2.375
6S01-7	H		26/1	*Candida zeylanoides*	2.079						
6S01-10	H		23/1	not reliable identification	1.483						
6S01-11	H		26/1	*Debraryomyces hansenii*	2.265						
6B01-1	H		28/2	*Candida_pelliculosa[ana] (Pichia_anomala[teleo]#)*	2.223				28/2	***6S01-4***	2.496
6B01-2	H	*							28/1	***6S01-11*** ***2B02-5***	2.523 2.519
6B01-3	H		26/1	*Candida lipolytica*	2.008						
6B01-4	H		28/1	*Candida _pelliculosa[ana] (Pichia_anomala[teleo]#)*	1.803	26/2	*Candida _pelliculosa[ana] (Pichia_anomala[teleo]#)*	1.766	28/1	***6S01-4***	2.478
6B01-5-1	H					26/2	*Debraryomyces hansenii*	1.892			
6B01-5-2	H		24/2	*Candida_lipolytica [ana] (Yarrowia_lipolytica [teleo]#)*	2.035						
6B01-7	H		24/2	*Candida_lipolytica [ana] (Yarrowia_lipolytica [teleo]#)*	2.117						
6B01-8	H		28/1	*Staphylococcus warneri*	2.071						
6B01-9	H		23/1	*Debaryomyces hansenii*	2.075						
7S01-1	I	*	23/1 28/15	*Candida_pelliculosa[ana] (Pichia_anomala[teleo]#)* *Candida pararugosa*	1.952 1.802						
7S01-3	I		28/1	*Candida parapsilosis*	2.17				28/1	***1S02-8***	1.77

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
7S01-4	I		28/1	*Candida _pelliculosa[ana] (Pichia_anomala[teleo]#)*	2.114				28/1	***6S01-4***	2.47
7S01-5	I		24/2	*Candida_lipolytica [ana] (Yarrowia_lipolytica [teleo]#)*	2.035						
7S01-6	I		28/1	**not reliable identification**	1.638				28/1	***1S02-8***	1.81
7S01-7	I		28/1	*Candida _pelliculosa[ana] (Pichia_anomala[teleo]#)*	1.954				28/1	***6S01-4***	2.48
7S01-8	I		23/1	*Candida intermedia*	2.314						
7S01-10	I		23/1	*Candida_lipolytica [ana] (Yarrowia_lipolytica [teleo]#)*	1.934						
8S01-1	J					26/2	**no peaks found**	<0	28/1	***8B01-2*** 2.426 ***5S01-5*** 2.442 ***5B01-3*** 2.43 ***1SO2-7*** 2.39 ***8S01-4*** 2.299	
8S01-4	J		26/1	**not reliable identification**	1.579						
8S01-5	J		26/2	**not reliable identification**	1.682	26/2	*Candida_colliculosa[ana] (Torulaspora_delbrueckii [teleo]#)*	2.093	28/1	***8B01-2*** 2.455 ***1S02-9*** 2.351 ***1S02-9*** 2.352 ***8S01-4*** 2.331	
8S01-6	J		26/1	**not reliable identification**	1.34						
8S01-7	J		23/1	**not reliable identification**	1.542						
8S01-8	J		23/1	**not reliable identification**	1.428						
8S01-10	J		23/1	**not reliable identification**	1.463						
8S01-11	J		26/1	**not reliable identification**	1.397						

Sample name	Cheese s	Comments	Date	RESULTS: identification by MALDI-TOF MS using eDT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score	Date	Similarities	Score
8B01-1	J					26/2	*Candida_sphaerica [ana] (Kluyveromyces_lactis [teleo])*	2.431	28/1	***1S02-5*** ***1S02-8*** ***1S02-6***	2.302 2.446 2.269
8B01-2	J		26/1	**not reliable identification**	1.661						
8B01-3	J								28/1	***5B01-3*** ***5S01-6*** ***5B01-6***	2.466 2.431 2.48
8B01-4	J	*	26/2	**not reliable identification**	1.629	26/2	*Candida_colliculosa [ana] (Torulaspora_delbrueckii [teleo]#)*	2.08	28/1	***8B01-2*** ***1S02-9*** ***8S01-4***	2.288 2.37 2.387
8B01-5	J		28/1	**not reliable identification**	1.517	26/2	**not reliable identification**	1.437			
8B01-7	J		23/1	**not reliable identification**	1.573						
8B01-9	J		23/1	**not reliable identification**	1.656						
8B01-10	J		23/1	*Candida_colliculosa[ana] (Torulaspora_delbrueckii[teleo]#)*	1.761						

Appendix 19: Identification of strains by using MALDI-TOF MS and classified as the *Staphylococcus* coagulase negative by TINE Mastitis lab

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
1	M214-12608-8	goat	S	10/2	*Staphylococcus warneri*	2.025			
2	M214-12610-12	goat	S	10/2	*Staphylococcus warneri*	2.081			
3	M214-12612-15	goat	S	10/2	*Staphylococcus warneri*	2.233			
4	M214-12612-16	goat	S	10/2	*Staphylococcus warneri*	1.961			
5	M214-12637-1	goat	S	10/2	*Staphylococcus caprae*	2.198			
6	M214-13711-25	goat	S	10/2	*Staphylococcus warneri*	2.08			
7	M214-13711-26	goat	S	10/2	*Staphylococcus warneri*	2.203			
8	M214-13705-13	goat	S	10/2	*Staphylococcus warneri*	2.23			
9	M214-13706-16	goat	S	10/2	*Staphylococcus simulans*	1.753			
10	M214-13699-1	goat	S	10/2	not reliable identification	1.681	*4/3*	*Staphylococcus warneri*	2.011
11	M214-13505-21	goat	S	10/2	*Staphylococcus caprae*	2.091			
12	M214-13815-10	goat	S	10/2	*Staphylococcus caprae*	2.194			
13	M214-13816-12	goat	S	10/2	*Staphylococcus warneri*	2.134			
14	M214-13817-14	goat	S	10/2	*Staphylococcus caprae*	1.742			
15	M214-13831-1	goat	R	10/2	not reliable identification	1.632	*4/3*	*Staphylococcus lentus*	1.78
16	M214-13833-2	goat	S	10/2	*Staphylococcus warneri*	2.126			
17	M214-13857-5	goat	R	10/2	*Staphylococcus epidermidis*	2.174			
18	M214-13857-6	goat	R	10/2	*Staphylococcus epidermidis*	2.044			
19	M214-13858-7	goat	S	10/2	*Staphylococcus caprae*	1.861			
20	M214-13858-8	goat	S	10/2	*Staphylococcus caprae*	1.855			
21	M214-13864-19	goat	R	10/2	*Staphylococcus epidermidis*	2.081			
22	M214-13864-20	goat	S	10/2	*Staphylococcus caprae*	1.892			
23	M214-14404-6	goat	R	10/2	*Staphylococcus epidermidis*	2.026			
24	M214-14408-13	goat	S	10/2	*Staphylococcus warneri*	1.853			
25	M214-14408-14	goat	R	10/2	*Staphylococcus epidermidis*	2.116			
26	M214-14409-15	goat	S	10/2	*Staphylococcus warneri*	2.252			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
27	M214-14410-17	goat	S	10/2	*Staphylococcus warneri*	1.754	10/2	*Staphylococcus warneri*	2.261
28	M214-14410-18	goat	R	10/2	*Staphylococcus epidermidis*	1.993	10/2	*Staphylococcus epidermidis*	2.172
29	M214-14411-19	goat	R	10/2	*Staphylococcus epidermidis*	1.992	10/2	*Staphylococcus epidermidis*	2.137
30	M214-14411-20	goat	R	10/2	*Staphylococcus epidermidis*	2.252			
31	M214-14481-26	goat	S	10/2	*Staphylococcus caprae*	2.091			
32	M214-14482-27	goat	S	10/2	*Staphylococcus caprae*	2.141			
33	M214-14486-36	goat	S	10/2	*Staphylococcus caprae*	2.119			
34	M214-14487-37	goat	S	10/2	*Staphylococcus epidermidis*	2.23			
35	M214-14510-24	goat		10/2	*Staphylococcus equorum*	1.979			
36	M214-14500-1	goat		10/2	*Staphylococcus caprae*	2.105			
37	M214-14496-15	goat	S	10/2	*Staphylococcus warneri*	2.247			
38	M214-14496-16	goat	S	10/2	*Staphylococcus warneri*	2.047			
39	M214-14494-12	goat	R	10/2	*Staphylococcus haemolyticus*	2.03			
40	M214-14483-30	goat	S	10/2	*Staphylococcus caprae*	1.894			
41	M214-14480-23	goat	R	10/2	*Staphylococcus caprae*	2.194			
42	M214-14476-15	goat	S	10/2	*Staphylococcus caprae*	2.042			
43	M214-14473-10	goat	S	10/2	*Staphylococcus warneri*	1.985	10/2	*Staphylococcus warneri*	2.456
44	M214-14474-11	goat	S	10/2	*Staphylococcus warneri*	2.138			
45	M214-14549-20	goat	S	10/2	*Staphylococcus saprophyticus*	1.991	10/2	*Staphylococcus saprophyticus*	2.446
46	M214-14547-15	goat	R	10/2	*Staphylococcus xylosus*	2.046			
47	M214-14542-7	goat		10/2	*Staphylococcus warneri*	2.159			
48	M214-14544-10	goat	S	10/2	*Staphylococcus warneri*	1.929			
49	M214-14536-34	goat	S	10/2	*Staphylococcus caprae*	2.058			
50	M214-14533-27	goat	S	10/2	*Staphylococcus caprae*	2.05			
51	M214-14533-28	goat	S	10/2	*Staphylococcus caprae*	1.963	10/2	*Staphylococcus caprae*	2.202
52	M214-14528-17	goat	S	10/2	*Staphylococcus warneri*	1.971			
53	M214-14526-13	goat	S	10/2	*Staphylococcus warneri*	1.877			
54	M214-14526-14	goat	S	10/2	*Staphylococcus warneri*	1.976	10/2	*Staphylococcus warneri*	2.144

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
55	M214-14521-3	goat	S	10/2	*Staphylococcus caprae*	1.974	10/2	*Staphylococcus caprae*	2.158
56	M214-14523-7	goat	S	10/2	*Staphylococcus caprae*	2.05			
57	M214-14558-37	goat	S	10/2	*Staphylococcus warneri*	2.049			
58	M214-14558-38	goat	S	10/2	*Staphylococcus warneri*	2.064			
59	M214-14556-34	goat	S	10/2	*Staphylococcus warneri*	2.103			
60	M214-14554-30	goat	S	10/2	*Staphylococcus warneri*	2.049			
61	M214-14555-32	goat	R	10/2	*Staphylococcus aureus*	2.208			
62	M214-14551-24	goat	S	10/2	*Staphylococcus warneri*	2.072			
63	M214-14552-26	goat	S	10/2	*Staphylococcus warneri*	1.85			
64	M214-14489-2	goat	S	10/2	*Staphylococcus caprae*	1.798			
65	M214-14534-30	goat	S	10/2	*Brevibacterium celere*	2.155			
66	M214-14536-33	goat	S	10/2	*Staphylococcus caprae*	2.082			
67	M214-14543-8	goat	S	10/2	not reliable identification	1.537	*04/03*	*Staphylococcus warneri*	1.947
68	M214-14550-22	goat	S	10/2	not reliable identification	1.554	*04/03*	*Staphylococcus warneri*	1.91
69	M214-14844-19	goat	R	10/2	not reliable identification	1.54	*04/03*	*Staphylococcus xylosus*	2.097
70	M214-14845-12	goat	S	10/2	*Staphylococcus warneri*	1.94			
71	M214-14884-3	goat	R	10/2	*Staphylococcus warneri*	2.128			
72	M214-14884-4	goat	R	10/2	*Staphylococcus warneri*	2.041			
73	M214-14886-8	goat	R	10/2	*Staphylococcus epidermidis*	2.184			
74	M214-15271-3	goat	R	10/2	*Staphylococcus warneri*	2.067			
75	M214-15272-1	goat	R	10/2	*Staphylococcus warneri*	1.954			
76	M214-15272-2	goat	R	10/2	*Staphylococcus warneri*	2.063			
77	M214-14887-9	goat	R	10/2	*Staphylococcus warneri*	2.03			
78	M214-14887-10	goat	R	10/2	*Staphylococcus epidermidis*	2.159			
79	M214-14891-17	goat		10/2	*Staphylococcus epidermidis*	1.902			
80	M214-14891-18	goat	R	10/2	*Staphylococcus warneri*	1.959			
81	M214-14894-23	goat	R	10/2	*Staphylococcus epidermidis*	2.05			
1	M214-14894-24	goat	R	11/2	*Staphylococcus epidermidis*	2.053			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
S	M214-14898-31	goat	R	11/2	*Staphylococcus epidermidis*	2.211			
3	M214-14898-32	goat	R	11/2	*Staphylococcus warneri*	2.245			
4	M214-14897-29	goat	S	11/2	*Staphylococcus simulans*	2.253			
5	M214-14897-30	goat	R	11/2	*Staphylococcus warneri*	2.061			
6	M214-14899-33	goat	R	11/2	*Staphylococcus warneri*	2.015			
7	M214-14900-36	goat		11/2	*Staphylococcus aureus*	2.294			
8	M214-14902-40	goat	R	11/2	*Staphylococcus epidermidis*	2.246			
9	M214-14905-5	goat	R	11/2	*Staphylococcus warneri*	2.128			
10	M214-14906-8	goat	R	11/2	*Staphylococcus warneri*	2.257			
11	M214-14907-9	goat	R	11/2	*Staphylococcus warneri*	2.121			
12	M214-14907-10	goat	R	11/2	*Staphylococcus warneri*	2.203			
13	M214-14908-12	goat	R	11/2	*Staphylococcus warneri*	2.145			
14	M214-14911-18	goat	R	11/2	*Staphylococcus chromogenes*	2.483			
15	M214-14913-21	goat	R	11/2	*Staphylococcus warneri*	2.014			
16	M214-14914-24	goat	R	11/2	*Staphylococcus warneri*	2.096			
17	M214-14916-27	goat	R	11/2	*Staphylococcus warneri*	2.243			
18	M214-14917-29	goat	R	11/2	*Staphylococcus warneri*	2.161			
19	M214-14921-38	goat	R	11/2	*Staphylococcus warneri*	2.187			
20	M214-14928-11	goat	R	11/2	*Staphylococcus warneri*	2.166			
21	M214-14928-12	goat	R	11/2	*Staphylococcus warneri*	1.72			
22	M214-14932-20	goat	R	11/2	*Staphylococcus warneri*	2.142			
23	M214-14933-22	goat	R	11/2	*Staphylococcus warneri*	2.128			
24	M214-14934-23	goat	S	11/2	*Staphylococcus simulans*	2.211			
25	M214-14935-25	goat	R	11/2	*Staphylococcus warneri*	2.032			
26	M214-14937-29	goat	R	11/2	*Staphylococcus warneri*	2.146			
27	M214-14937-30	goat	S	11/2	*Staphylococcus simulans*	2.298			
28	M214-14940-36	goat	R	11/2	*Staphylococcus warneri*	2.254			
29	M214-14945-5	goat	R	11/2	*Staphylococcus warneri*	2.097			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
30	M214-14948-12	goat	R	11/2	*Staphylococcus warneri*	2.019			
31	M214-14949-14	goat	R	11/2	*Staphylococcus warneri*	2.154			
32	M214-14951-17	goat	R	11/2	*Staphylococcus warneri*	2.116			
33	M214-14951-18	goat	R	11/2	*Staphylococcus epidermidis*	2.155			
34	M214-14953-21	goat	R	11/2	*Staphylococcus warneri*	2.072			
35	M214-14953-22	goat	R	11/2	*Staphylococcus warneri*	2.017			
36	M214-14954-23	goat	R	11/2	*Staphylococcus warneri*	2.067			
37	M214-14956-28	goat	R	11/2	*Staphylococcus epidermidis*	2.247			
38	M214-14957-29	goat	R	11/2	*Staphylococcus warneri*	2.006			
39	M214-14960-35	goat	R	11/2	*Staphylococcus epidermidis*	2.207			
40	M214-14960-36	goat	R	11/2	*Staphylococcus warneri*	2.041			
41	M214-14963-1	goat	R	11/2	*Staphylococcus epidermidis*	2.225			
42	M214-14970-15	goat	R	11/2	*Staphylococcus epidermidis*	2.118			
43	M214-14972-19	goat	R	11/2	*Staphylococcus warneri*	2.086			
44	M214-14972-20	goat	R	11/2	*Staphylococcus warneri*	2.062			
45	M214-14973-21	goat	R	11/2	*Staphylococcus simulans*	2.216			
46	M214-14973-22	goat	R	11/2	*Staphylococcus warneri*	2.166			
47	M214-14975-26	goat	R	11/2	*Staphylococcus warneri*	2.115			
48	M214-14980-36	goat	R	11/2	*Staphylococcus warneri*	2.093			
49	M214-14983-2	goat	R	11/2	*Staphylococcus warneri*	2.141			
50	M214-14986-7	goat	R	11/2	*Staphylococcus warneri*	2.043			
51	M214-14988-11	goat	S	11/2	*Staphylococcus warneri*	1.821			
52	M214-14988-12	goat	S	11/2	*Staphylococcus warneri*	1.923			
53	M214-14989-13	goat	R	11/2	*Staphylococcus warneri*	2.132			
54	M214-14989-14	goat	R	11/2	*Staphylococcus warneri*	2.17			
55	M214-14991-17	goat	R	11/2	*Staphylococcus warneri*	2.238			
56	M214-14992-19	goat	R	11/2	*Staphylococcus warneri*	2.153			
57	M214-14992-20	goat	R	11/2	*Staphylococcus warneri*	2.312			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
58	M214-14996-27	goat	R	11/2	*Staphylococcus warneri*	2.122			
59	M214-14997-29	goat	R	11/2	*Staphylococcus warneri*	2.236			
60	M214-14997-30	goat	R	11/2	*Staphylococcus epidermidis*	2.074			
61	M214-14998-31	goat	R	11/2	*Staphylococcus warneri*	2.127			
62	M214-14998-32	goat	R	11/2	*Staphylococcus warneri*	2.038			
63	M214-15003-1	goat	R	11/2	*Staphylococcus warneri*	2.117			
64	M214-15003-2	goat	R	11/2	*Staphylococcus epidermidis*	2.205			
65	M214-15006-7	goat	R	11/2	*Staphylococcus warneri*	2.157			
66	M214-15010-15	goat	R	11/2	*Staphylococcus warneri*	2.117			
67	M214-15011-17	goat	R	11/2	*Staphylococcus warneri*	2.041			
68	M214-15011-18	goat	R	11/2	*Staphylococcus warneri*	2.076			
69	M214-15015-25	goat	S	11/2	*Staphylococcus warneri*	2.147			
70	M214-15016-27	goat	R	11/2	*Staphylococcus chromogenes*	2.341			
71	M214-15018-31	goat	R	11/2	*Staphylococcus warneri*	2.07			
72	M214-15021-38	goat	R	11/2	*Staphylococcus epidermidis*	2.158			
73	M214-15023-1	goat	R	11/2	*Staphylococcus warneri*	2.092			
74	M214-15025-5	goat	R	11/2	*Staphylococcus warneri*	2.117			
75	M214-15026-8	goat	R	11/2	*Staphylococcus epidermidis*	2.219			
76	M214-15027-9	goat	R	11/2	*Staphylococcus warneri*	2.012			
77	M214-15028-12	goat	R	11/2	*Staphylococcus warneri*	2.058			
78	M214-15030-16	goat	R	11/2	*Staphylococcus warneri*	2.12			
79	M214-15032-19	goat	R	11/2	*Staphylococcus warneri*	2.177			
80	M214-15032-20	goat	R	11/2	*Staphylococcus epidermidis*	2.174			
81	M214-15033-21	goat	R	11/2	*Staphylococcus warneri*	2.078			
1	M214-15035-25	goat	R	12/2	*Staphylococcus warneri*	2.141			
S	M214-15036-27	goat	R	12/2	*Staphylococcus warneri*	1.895			
3	M214-15038-31	goat	R	12/2	*Staphylococcus chromogenes*	2.507			
4	M214-15039-33	goat	R	12/2	*Staphylococcus chromogenes*	2.406			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
5	M214-15040-35	goat	R	12/2	*Staphylococcus warneri*	2.17			
6	M214-15042-40	goat	R	12/2	*Staphylococcus warneri*	2.032			
7	M214-15044-3	goat	R	12/2	*Staphylococcus warneri*	2.077			
8	M214-15044-4	goat	R	12/2	*Staphylococcus warneri*	2.128			
9	M214-15046-7	goat	R	12/2	*Staphylococcus warneri*	2.116			
10	M214-15050-16	goat	R	12/2	*Staphylococcus warneri*	2.024			
11	M214-15052-20	goat	R	12/2	*Staphylococcus warneri*	2.043			
12	M214-15057-30	goat	R	12/2	*Staphylococcus warneri*	2.103			
13	M214-15059-34	goat	R	12/2	*Staphylococcus warneri*	2.13			
14	M214-15061-37	goat	R	12/2	*Staphylococcus warneri*	2.11			
15	M214-15062-39	goat	R	12/2	*Staphylococcus warneri*	2.155			
16	M214-15064-3	goat	R	12/2	*Staphylococcus warneri*	2.08			
17	M214-15067-10	goat	R	12/2	*Staphylococcus chromogenes*	2.29			
18	M214-15070-15	goat	R	12/2	*Staphylococcus epidermidis*	2.117			
19	M214-15071-17	goat	S	12/2	*Staphylococcus simulans*	2.331			
20	M214-15072-19	goat	R	12/2	*Staphylococcus warneri*	2.027			
21	M214-15075-25	goat	R	12/2	*Staphylococcus warneri*	2.076			
22	M214-15076-27	goat	R	12/2	*Staphylococcus warneri*	2.19			
23	M214-15077-29	goat	R	12/2	*Staphylococcus epidermidis*	2.128			
24	M214-15078-32	goat	R	12/2	*Staphylococcus epidermidis*	2.167			
25	M214-15079-34	goat	R	12/2	*Staphylococcus warneri*	2.205			
26	M214-15081-38	goat	R	12/2	*Staphylococcus warneri*	1.987			
27	M214-15084-4	goat	S	12/2	*Staphylococcus caprae*	2.166			
28	M214-15087-9	goat	S	12/2	*Staphylococcus caprae*	2.133			
29	M214-15088-11	goat	R	12/2	*Staphylococcus warneri*	2.179			
30	M214-15088-12	goat	R	12/2	*Staphylococcus warneri*	2.061			
31	M214-15089-14	goat	S	12/2	*Staphylococcus caprae*	1.887			
32	M214-15091-17	goat	S	12/2	*Staphylococcus caprae*	2.09			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
33	M214-15092-19	goat	S	12/2	*Staphylococcus warneri*	1.957			
34	M214-15092-20	goat	S	12/2	*Staphylococcus warneri*	2.011			
35	M214-15098-31	goat	S	12/2	*Staphylococcus warneri*	2.236			
36	M214-15098-32	goat	R	12/2	*Staphylococcus warneri*	2.189			
37	M214-15099-33	goat	R	12/2	*Staphylococcus warneri*	2.087			
38	M214-15099-34	goat	R	12/2	*Staphylococcus warneri*	2.226			
39	M214-15100-35	goat	S	12/2	*Staphylococcus chromogenes*	2.358			
40	M214-15102-40	goat	R	12/2	*Staphylococcus warneri*	2.091			
41	M214-15105-6	goat	S	12/2	*Staphylococcus caprae*	2.14			
42	M214-15107-10	goat	R	12/2	*Staphylococcus warneri*	2.175			
43	M214-15114-23	goat	S	12/2	*Staphylococcus caprae*	2.052			
44	M214-15114-24	goat	R	12/2	*Staphylococcus warneri*	2.236			
45	M214-15119-33	goat	R	12/2	*Staphylococcus warneri*	2.111			
46	M214-15119-34	goat	R	12/2	*Staphylococcus warneri*	2.228			
47	M214-15123-1	goat	S	12/2	*Staphylococcus warneri*	1.968			
48	M214-15125-6	goat	S	12/2	*Staphylococcus warneri*	2.146			
49	M214-15126-8	goat	R	12/2	*Staphylococcus warneri*	2.219			
50	M214-15127-10	goat	R	12/2	*Staphylococcus warneri*	1.863			
51	M214-15128-11	goat	S	12/2	*Staphylococcus warneri*	1.837			
52	M214-14900-35	goat	R	12/2	*Staphylococcus caprae*	2.048			
53	M214-14890-16	goat	R	12/2	*Staphylococcus caprae*	2.088			
54	M214-14930-16	goat	R	12/2	*Staphylococcus warneri*	2.065			
55	M214-14963-2	goat	R	12/2	*Staphylococcus caprae*	2.054			
56	M214-14967-9	goat	R	12/2	*Staphylococcus caprae*	1.716			
57	M214-15009-14	goat	R	12/2	*Staphylococcus caprae*	1.812			
58	M214-15014-23	goat	R	12/2	*Staphylococcus caprae*	2.047			
59	M214-15014-24	goat	R	12/2	*Staphylococcus caprae*	2.002			
60	M214-15101-38	goat	R	12/2	*Staphylococcus warneri*	2.133			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
61	M214-15110-16	goat	R	12/2	*Staphylococcus epidermidis*	2.248			
62	M214-15121-38	goat	R	12/2	*Staphylococcus warneri*	2.157			
63	M214-15302-10	goat	S	12/2	*Staphylococcus warneri*	2.063			
64	M214-15318-2	goat	S	12/2	*Staphylococcus warneri*	2.163			
65	M214-15319-3	goat	S	12/2	*Staphylococcus caprae*	2.037			
66	M214-15331-27	goat	R	12/2	*Staphylococcus warneri*	2.178			
67	M214-15530-7	goat	S	12/2	*Staphylococcus warneri*	2.054			
68	M214-15530-8	goat	S	12/2	*Staphylococcus chromogenes*	2.113			
69	M214-15532-11	goat		12/2	*Staphylococcus caprae*	2.048			
70	M214-15543-33	goat	S	12/2	*Staphylococcus warneri*	1.954			
71	M214-15508-3	goat		12/2	*Staphylococcus simulans*	2.14			
72	M214-15512-10	goat	S	12/2	*Staphylococcus warneri*	2.141			
73	M214-15518-22	goat	S	12/2	*Staphylococcus caprae*	2.108			
74	M214-15526-39	goat	S	12/2	*Staphylococcus caprae*	2.038			
75	M214-15543-34	goat	S	12/2	*Staphylococcus caprae*	1.909			
76	M214-15546-40	goat	S	12/2	*Staphylococcus warneri*	2.134			
77	M214-15279-3	goat	S	12/2	*Staphylococcus caprae*	2.001			
78	M214-15284-14	goat	S	12/2	*Staphylococcus pasteuri*	2.178			
79	M214-15287-20	goat	R	12/2	*Staphylococcus warneri*	2.198			
80	M214-15287-19	goat	R	12/2	*Staphylococcus warneri*	2.114			
81	M214-15288-22	goat	R	12/2	*Staphylococcus warneri*	2.241			
1	M214-15290-26	goat	S	12/2	*Staphylococcus warneri*	1.975			
S	M214-15291-27	goat	S	12/2	*Staphylococcus warneri*	1.972			
3	M214-15291-28	goat	R	12/2	*Staphylococcus warneri*	2.044			
4	M214-15298-2	goat	R	12/2	*Staphylococcus warneri*	1.952			
5	M214-15302-9	goat	S	12/2	*Staphylococcus warneri*	2.037			
6	M214-15302-10	goat	S	12/2	*Staphylococcus warneri*	1.946			
7	M214-15303-12	goat	S	12/2	*Staphylococcus warneri*	2.057			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
8	M214-15304-13	goat	S	12/2	*Staphylococcus warneri*	2.007			
9	M214-15304-14	goat	S	12/2	*Staphylococcus warneri*	2.098			
10	M214-15306-18	goat	R	12/2	*Staphylococcus warneri*	2.18			
11	M214-15306-19	goat		12/2	*Staphylococcus warneri*	2.086			
12	M214-15308-22	goat	R	12/2	*Staphylococcus warneri*	2.134			
13	M214-15312-30	goat	S	12/2	*Staphylococcus warneri*	1.803			
14	M214-15313-31	goat	R	12/2	*Staphylococcus warneri*	2.194			
15	M214-15320-5	goat	R	12/2	*Staphylococcus warneri*	2.303			
16	M214-15320-6	goat	R	12/2	*Staphylococcus warneri*	2.137			
17	M214-15322-10	goat	S	12/2	*Staphylococcus warneri*	2.054			
18	M214-15323-12	goat	S	12/2	*Staphylococcus simulans*	2.323			
19	M214-15324-13	goat	S	12/2	*Staphylococcus warneri*	2.276			
20	M214-15324-14	goat	R	12/2	*Staphylococcus warneri*	2.122			
21	M214-15325-15	goat	R	12/2	*Staphylococcus warneri*	2.194			
22	M214-15327-19	goat	S	12/2	*Staphylococcus warneri*	2.211			
23	M214-15327-20	goat	S	12/2	*Staphylococcus warneri*	2.123			
24	M214-15328-22	goat	R	12/2	*Staphylococcus pasteuri*	1.851			
24	M214-15328-22	goat	R	12/2	*Staphylococcus epidermidis*	1.83			
25	M214-15321-27	goat		12/2	*Staphylococcus caprae*	2.179			
26	M214-15534-16	goat	S	12/2	**no peaks found**	<0	04/03	not reliable identification	1.257
27	M214-16105-8	goat	R	12/2	*Staphylococcus epidermidis*	2.22			
28	M214-16107-11	goat	R	12/2	*Staphylococcus epidermidis*	2.189			
29	M214-16111-20	goat	R	12/2	*Staphylococcus warneri*	2.027			
30	M214-16112-21	goat	R	12/2	*Staphylococcus epidermidis*	2.169			
31	M214-16112-22	goat	R	12/2	*Staphylococcus epidermidis*	2.217			
32	M214-16114-25	goat	R	12/2	*Staphylococcus warneri*	2.172			
33	M214-16116-29	goat	R	12/2	*Staphylococcus epidermidis*	2.281			
34	M214-16118-34	goat	R	12/2	*Staphylococcus epidermidis*	2.124			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
35	M214-16120-37	goat	R	12/2	*Staphylococcus warneri*	2.129			
36	M214-16125-1	goat	R	12/2	*Staphylococcus epidermidis*	2.212			
37	M214-16128-1	goat	S	12/2	*Staphylococcus epidermidis*	2.093			
38	M214-16128-2	goat	S	12/2	*Staphylococcus epidermidis*	2.249			
39	M214-16129-1	goat	R	12/2	*Staphylococcus epidermidis*	2.237			
40	M214-16130-1	goat	S	12/2	*Staphylococcus caprae*	2.088			
41	M214-16103-3	goat	S	12/2	*Staphylococcus caprae*	2.055			
42	M214-16110-17	goat	S	12/2	*Staphylococcus caprae*	2.154			
43	M214-16114-26	goat	S	12/2	*Staphylococcus caprae*	2.104			
44	M214-16117-31	goat	S	12/2	*Staphylococcus caprae*	2.121			
45	M214-16123-1	goat	S	12/2	*Staphylococcus caprae*	2.172			
46	M214-16125-2	goat	S	12/2	*Staphylococcus caprae*	1.846			
47	M214-16130-2	goat	R	12/2	*Staphylococcus epidermidis*	2.196			
48	M214-16206-16	goat	S	12/2	*Staphylococcus warneri*	2.091			
49	M214-16296-1	goat	S	12/2	*Staphylococcus warneri*	2.112			
50	M214-16301-1	goat	S	12/2	*Staphylococcus warneri*	2.149			
51	M214-16303-2	goat	S	12/2	*Staphylococcus warneri*	2.129			
52	M214-16305-2	goat	S	12/2	*Micrococcus luteus*	2.179			
53	M214-16312-2	goat	S	12/2	*Staphylococcus warneri*	2.157			
54	M214-16313-2	goat	S	12/2	*Staphylococcus warneri*	2.144			
55	M214-16319-3	goat	S	12/2	**not reliable identification**	1.657	04/03	*Staphylococcus caprae*	1.992
56	M214-16320-1	goat	S	12/2	*Staphylococcus warneri*	2.019			
57	M214-16320-2	goat	S	12/2	*Staphylococcus warneri*	2.036			
58	M214-16322-1	goat	S	12/2	*Staphylococcus caprae*	2.048			
59	M214-16322-2	goat	R	12/2	*Staphylococcus epidermidis*	2.108			
60	M214-16323-4	goat	S	12/2	*Staphylococcus warneri*	2.257			
61	M214-16324-2	goat	S	12/2	*Staphylococcus warneri*	2.162			
62	M214-16325-4	goat	R	12/2	*Staphylococcus epidermidis*	1.906			

Nr.	ID number	Source	Penicillin resistance (S=sensible, R=resistant)	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score	Date	RESULTS: identification by MALDI-TOF MS using EX method (best match)	Score
63	M214-16326-1	goat	S	12/2	*Staphylococcus warneri*	1.809			
64	M214-16326-2	goat	S	12/2	*Staphylococcus warneri*	1.928			
65	M214-16687-1	goat	R	12/2	*Rothia amarae*	1.813			
66	M214-17999-1	goat	S	12/2	*Staphylococcus epidermidis*	2.099			
67	M214-18001-6	goat	S	12/2	*Staphylococcus epidermidis*	2.243			
68	M214-18004-11	goat	S	12/2	*Staphylococcus epidermidis*	2.06			
69	M214-18005-13	goat	S	19/2	*Staphylococcus epidermidis*	1.994			
70	M214-18007-17	goat	S	19/2	*Staphylococcus epidermidis*	1.886	20/2	*Staphylococcus epidermidis*	2.093
71	M214-18010-23	goat	R	19/2	*Staphylococcus warneri*	1.914	20/2	*Staphylococcus warneri*	2.154
72	M214-18011-26	goat	R	19/2	*Staphylococcus warneri*	1.982	20/2	*Staphylococcus warneri*	2.36
73	M214-18016-35	goat	S	19/2	*Staphylococcus epidermidis*	2.09			
74	m214-18017-2	goat	R	19/2	*Staphylococcus warneri*	1.94	20/2	*Staphylococcus warneri*	2.336
75	M214-18018-1	goat	S	19/2	*Staphylococcus epidermidis*	2.044			
76	M214-18019-2	goat	S	19/2	*Staphylococcus simulans*	1.812	20/2	*Staphylococcus simulans*	1.96
77	M214-18020-1	goat	S	19/2	**not reliable identification**	1.569	20/2	**not reliable identification**	1.471
78	M214-18021-1	goat	S	19/2	*Staphylococcus epidermidis*	1.956	20/2	*Staphylococcus epidermidis*	2.228
79	M214-18022-1	goat	R	19/2	*Staphylococcus warneri*	1.915	20/2	*Staphylococcus warneri*	2.228
80	M214-18055-1	goat	S	19/2	*Staphylococcus warneri*	1.747	20/2	*Staphylococcus warneri*	2.086
81	M214-18059-10	goat	R	11/2	*Staphylococcus warneri*	1.972			
1	M214-18060-11	goat	S	11/2	*Staphylococcus warneri*	2.058			
2	M214-18060-12	goat	S	11/2	*Staphylococcus warneri*	1.975			
3	M214-18061-13	goat	S	11/2	*Staphylococcus warneri*	2.141			
4	M214-18064-20	goat	R	11/2	*Staphylococcus warneri*	1.97			
5	M214-18066-24	goat	S	11/2	*Staphylococcus warneri*	2.165			

Appendix 20: Identification of strains by using MALDI-TOF MS and classified as the *Streptococcus* by TINE Mastitis lab

Nr.	ID number		Source	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score
1	M214	16386-4	Cow	10/2	*Aerococcus viridans*	2.028
2	M214	16391-3	Cow	10/2	*Lactococcus lactis*	2.328
3	M214	16397-4	Cow	10/2	*Streptococcus uberis*	2.303
5	M214	16348-3	Cow	10/2	*Streptococcus uberis*	2.118
6	M214	16378-2	Cow	10/2	**not reliable identification**	1.439
7	M214	16405-4	Cow	10/2	*Arcanobacterium pluranimalium*	1.945
8	M214	16448-1	Cow	10/2	*Aerococcus viridans*	1.983
9	M214	16348-4	Cow	10/2	*Streptococcus uberis*	2.338
10	M214	16356-1	Cow	10/2	*Aerococcus viridans*	1.94
11	M214	16361-3	Cow	10/2	**not reliable identification**	1.694
12	M214	16361-4	Cow	10/2	*Lactococcus lactis*	2.274
13	M214	16420-1	Cow	10/2	*Aerococcus viridans*	1.855
14	M214	16436-1	Cow	10/2	*Streptococcus uberis*	2.303
15	M214	16436-2	Cow	10/2	*Lactococcus lactis*	2.325
16	M214	16439-2	Cow	10/2	*Aerococcus viridans*	1.851
17	M214	16462-2	Cow	10/2	*Aerococcus viridans*	1.971
18	M214	16472-4	Cow	10/2	*Lactococcus lactis*	2.199
19	M214	16473-4	Cow	10/2	*Streptococcus uberis*	2.182
20	M214	16476-2	Cow	10/2	*Streptococcus uberis*	2.266
21	M214	16476-3	Cow	10/2	*Streptococcus uberis*	2.276
22	M214	16476-4	Cow	10/2	*Enterococcus faecalis*	2.399
23	M214	16491-3	Cow	10/2	*Streptococcus uberis*	2.097
24	M214	16489-2	Cow	10/2	*Lactococcus lactis*	2.34
25	M214	16489-3	Cow	10/2	*Lactococcus lactis*	2.403
26	M214	16490-2	Cow	10/2	*Enterococcus faecalis*	2.287
27	M214	16492-3	Cow	10/2	*Streptococcus uberis*	2.036
28	M214	16502-2	Cow	10/2	*Lactococcus lactis*	2.336
29	M214	16493-4	Cow	10/2	*Enterococcus faecalis*	2.349
30	M214	16497-1	Cow	10/2	*Enterococcus faecalis*	2.423

Nr.	ID number		Source	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score
31	M214	16497-4	Cow	10/2	*Enterococcus faecalis*	2.296
32	M214	16483-4	Cow	10/2	*Streptococcus uberis*	1.957
33	M214	16572-4	Cow	10/2	*Streptococcus uberis*	1.779
34	M214	16599-32	Cow	10/2	*Streptococcus uberis*	2.074
35	M214	16600-35	Cow	10/2	*Lactococcus garvieae*	1.986
36	M214	16602-2	Cow	10/2	*Lactococcus lactis*	2.323
37	M214	16606-20	Cow	10/2	*Streptococcus parauberis*	2.066
38	M214	16608-26	Cow	10/2	*Aerococcus viridans*	1.784
39	M214	16608-27	Cow	10/2	**not reliable identification**	1.603
40	M214	16614-12	Cow	10/2	*Enterococcus faecalis*	2.273
41	M214	16620-14	Cow	10/2	*Streptococcus parauberis*	2.042
42	M214	16626-1	Cow	10/2	*Streptococcus lutetiensis*	2.01
43	M214	16627-8	Cow	10/2	**not reliable identification**	1.648
44	M214	16631-1	Cow	10/2	*Streptococcus uberis*	2.134
45	M214	16631-3	Cow	10/2	*Streptococcus uberis*	2.342
46	M214	16632-1	Cow	10/2	*Enterococcus faecium*	2.487
47	M214	16632-3	Cow	10/2	*Enterococcus faecium*	2.3
48	M214	16636-4	Cow	10/2	*Lactococcus lactis*	2.003
49	M214	16637-2	Cow	10/2	*Streptococcus uberis*	1.959
50	M214	16637-3	Cow	10/2	*Lactococcus lactis*	2.297
51	M214	16640-3	Cow	10/2	*Streptococcus uberis*	2.13
52	M214	16652-1	Cow	10/2	*Lactococcus lactis*	2.326
53	M214	16653-1	Cow	10/2	*Aerococcus viridans*	1.804
54	M214	16654-3	Cow	10/2	*Lactococcus lactis*	2.247
55	M214	16656-3	Cow	10/2	*Streptococcus uberis*	2.274
56	M214	16658-2	Cow	10/2	*Streptococcus uberis*	2.15
57	M214	16660-1	Cow	10/2	*Lactococcus lactis*	2.339
58	M214	16660-2	Cow	10/2	*Lactococcus lactis*	2.396
59	M214	16660-3	Cow	10/2	*Streptococcus uberis*	2.283
60	M214	16662-4	Cow	10/2	*Streptococcus uberis*	2.258
61	M214	16672-1	Cow	10/2	*Enterococcus faecalis*	1.899
62	M214	16674-1	Cow	10/2	*Aerococcus viridans*	1.702

Nr.	ID number		Source	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score
63	M214	16674-2	Cow	10/2	*Aerococcus viridans*	1.748
64	M214	16675-4	Cow	10/2	*Lactococcus lactis*	2.34
65	M214	16681-4	Cow	10/2	*Enterococcus faecalis*	2.184
66	M214	16529-1	Cow	10/2	*Lactococcus garvieae*	1.74
67	M214	16514-4	Cow	10/2	**not reliable identification**	1.666
68	M214	16528-2	Cow	10/2	*Enterococcus faecium*	2.19
69	M214	16528-3	Cow	10/2	*Enterococcus faecalis*	2.272
70	M214	16529-2	Cow	10/2	*Lactococcus garvieae*	2.074
71	M214	16529-3	Cow	10/2	*Streptococcus uberis*	2.209
72	M214	16529-4	Cow	10/2	*Streptococcus uberis*	2.317
73	M214	16547-4	Cow	10/2	*Streptococcus uberis*	2.207
74	M214	16531-3	Cow	10/2	*Streptococcus uberis*	2.343
75	M214	16536-1	Cow	10/2	*Enterococcus faecalis*	2.094
76	M214	16538-3	Cow	10/2	*Streptococcus uberis*	2.162
77	M214	16555-2	Cow	10/2	*Enterococcus faecalis*	2.002
78	M214	16551-3	Cow	10/2	*Streptococcus uberis*	2.348
79	M214	16553-3	Cow	10/2	*Streptococcus parauberis*	1.993
80	M214	16563-3	Cow	10/2	*Streptococcus uberis*	2.278
81	M214	16555-4	Cow	10/2	*Streptococcus uberis*	2.237
1	M214	16559-3	Cow	11/2	*Streptococcus parauberis*	2.144
2	M214	16563-2	Cow	11/2	**not reliable identification**	1.61
3	M214	16569-4	Cow	11/2	*Lactococcus lactis*	2.4
4	M214	16563-4	Cow	11/2	**not reliable identification**	1.576
5	M214	16565-2	Cow	11/2	*Enterococcus faecalis*	2.326
6	M214	16569-1	Cow	11/2	*Lactococcus lactis*	2.237
7	M214	16584-2	Cow	11/2	*Enterococcus faecalis*	2.402
8	M214	16576-1	Cow	11/2	*Streptococcus uberis*	2.324
9	M214	16576-2	Cow	11/2	*Streptococcus lutetiensis*	2.013
10	M214	16581-2	Cow	11/2	*Lactococcus lactis*	2.218
11	M214	16585-3	Cow	11/2	*Lactococcus lactis*	2.23
12	M214	16587-3	Cow	11/2	*Lactococcus lactis*	2.121
13	M214	16589-3	Cow	11/2	*Enterococcus faecalis*	2.337

Nr.	ID number		Source	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score
14	M214	16455-2	Cow	11/2	*Streptococcus dysgalactiae*	2.121
15	M214	16459-1	Cow	11/2	*Streptococcus dysgalactiae*	2.243
16	M214	16459-4	Cow	11/2	*Streptococcus dysgalactiae*	2.207
17	M214	16461-2	Cow	11/2	*Streptococcus dysgalactiae*	2.371
18	M214	16467-1	Cow	11/2	*Streptococcus dysgalactiae*	2.271
19	M214	16393-1	Cow	11/2	*Streptococcus dysgalactiae*	2.133
20	M214	16405-4	Cow	11/2	*Arcanobacterium pluranimalium*	1.843
21	M214	16432-1	Cow	11/2	**not reliable identification**	1.075
22	M214	16672-3-1	Cow	11/2	**not reliable identification**	1.64
23	M214	16611-37	Cow	11/2	*Streptococcus dysgalactiae*	2.207
24	M214	16611-40	Cow	11/2	*Streptococcus dysgalactiae*	2.217
25	M214	16613-5	Cow	11/2	*Streptococcus dysgalactiae*	2.221
26	M214	11627-6	Cow	11/2	*Streptococcus dysgalactiae*	2.24
27	M214	16648-2	Cow	11/2	*Streptococcus pluranimalium*	1.772
28	M214	16602-3	Cow	11/2	*Streptococcus dysgalactiae*	2.196
29	M214	16606-18	Cow	11/2	*Streptococcus dysgalactiae*	2.109
30	M214	16606-19	Cow	11/2	*Streptococcus dysgalactiae*	2.196
31	M214	16607-23	Cow	11/2	*Streptococcus dysgalactiae*	2.218
32	M214	16479-3	Cow	11/2	*Streptococcus dysgalactiae*	2.224
33	M214	16502-4	Cow	11/2	*Streptococcus dysgalactiae*	2.124
34	M214	16549-1	Cow	11/2	*Streptococcus dysgalactiae*	2.252
35	M214	16549-3	Cow	11/2	*Streptococcus dysgalactiae*	2.127
36	M214	16549-4	Cow	11/2	*Streptococcus dysgalactiae*	2.065
37	M214	16562-4	Cow	11/2	*Streptococcus dysgalactiae*	2.262
38	M214	16467-3	Cow	11/2	*Streptococcus dysgalactiae*	2.209
39	M214	16467-4	Cow	11/2	*Streptococcus dysgalactiae*	1.928
40	M214	16475-2	Cow	11/2	*Streptococcus dysgalactiae*	2.261
41	M214	16475-4	Cow	11/2	*Streptococcus dysgalactiae*	2.068
42	M214	16480-3	Cow	11/2	*Streptococcus dysgalactiae*	2.148
43	M214	16480-4	Cow	11/2	*Streptococcus dysgalactiae*	2.153
44	M214	16508-3	Cow	11/2	*Streptococcus dysgalactiae*	2.07

Nr.	ID number		Source	Date	RESULTS: identification by MALDI-TOF MS using DT method (best match)	Score
45	M214	16514-3	Cow	11/2	*Streptococcus dysgalactiae*	2.167
46	M214	16526-4	Cow	11/2	*Streptococcus dysgalactiae*	2.108
47	M214	16531-1	Cow	11/2	*Streptococcus dysgalactiae*	2.147
48	M214	16490-3	Cow	11/2	*Streptococcus dysgalactiae*	2.195
49	M214	16493-1	Cow	11/2	*Streptococcus dysgalactiae*	2.315
50	M214	16495-3	Cow	11/2	*Streptococcus dysgalactiae*	2.075
51	M214	16497-3	Cow	11/2	**not reliable identification**	1.367
52	M214	16508-1	Cow	11/2	*Streptococcus dysgalactiae*	2.059

Appendix 21: Determination of Beta-lactamase resistance of 40 strains selected along Staphylococcus group by MALDI-TOF MS

Number	ID number	Resistant (R)/ Sensible (S) to Penicillin G (diffusion disk method)	Peaks found by MALDI-TOF MS (Biotyper, Bruker) Matrix used: 2,5-DHB	Resistance / Sensible pattern detected by MALDI-TOF MS
1	14409-15	S	No peaks identified, poor quality of the spot	
2	14410-18	R	No peaks identified, poor quality of the spot	
3	14408-14	R	No peaks identified, poor quality of the spot	
4	14555-32	R	No peaks identified, poor quality of the spot	
5	14411-19	R	No peaks identified, poor quality of the spot	
6	15016-27	R	No peaks identified, poor quality of the spot	
7	15100-35	S	No peaks identified, poor quality of the spot	
8	15015-25	S	No peaks identified, poor quality of the spot	
9	15102-40	R	No peaks identified, poor quality of the spot	
10	14494-12	R	No peaks identified, poor quality of the spot	
11	14911-18	R	No peaks identified, poor quality of the spot	
12	14845-12	S	No peaks identified, poor quality of the spot	
13	15125-6	S	No peaks identified, poor quality of the spot	
14	13857-6	S	No peaks identified, poor quality of the spot	
15	14523-7	S	No peaks identified, poor quality of the spot	
16	14526-14	S	No peaks identified, poor quality of the spot	
17	14884-3	R	No peaks identified, poor quality of the spot	
18	14886-8	R	No peaks identified, poor quality of the spot	
19	15271-1	R	No peaks identified, poor quality of the spot	
20	15272-1	R	No peaks identified, poor quality of the spot	
21	15272-2	R	No peaks identified, poor quality of the spot	
22	13864-19	R	1) 357.073 Da 2) 378.925 Da 3) 402.724 Da	**Sensible pattern**

Number	ID number	Resistant (R)/ Sensible (S) to Penicillin G (diffusion disk method)	Peaks found by MALDI-TOF MS (Biotyper, Bruker) Matrix used: 2,5-DHB	Resistance / Sensible pattern detected by MALDI-TOF MS
23	14404-6	R	No peaks identified, poor quality of the spot	
24	14486-36	S	No peaks identified, poor quality of the spot	
25	15057-30	R	No peaks identified, poor quality of the spot	
26	14549-20	S	1) 357.420 Da 2) 379.356 Da 3) 402.175 Da	**Sensible pattern**
27	15071-17	S	No peaks identified, poor quality of the spot	
28	15092-19	S	No peaks identified, poor quality of the spot	
29	15084-4	S	No peaks identified, poor quality of the spot	
30	13706-16	S	No peaks identified, poor quality of the spot	
31	14536-33	S	No peaks identified, poor quality of the spot	
32	14481-26	S	No peaks identified, poor quality of the spot	
33	13857-5	R	1) 374.822 Da 2) 397.423 Da 3) 418.935 Da	**Resistant pattern**
34	14547-15	R	1) 374.822 Da 2) 397.423 Da 3) 418.747 Da	**Resistant pattern**
35	14533-27	S	No peaks, poor quality of the spot	
36	13833-2	S	No peaks, poor quality of the spot	
37	14480-23	R	1) 374.822 Da 2) 397.000 Da 3) no peak at 419 Da 4) no peak at 379 Da 5) 402.909 Da 6) 356.839 Da	**Resistance pattern detected** Probably strain intermediate- resistant
38	15038-31	R	No peaks identified, poor quality of the spot	
39	14489-2	S	No peaks identified, poor quality of the spot	
40	14534-30	S	No peaks identified, poor quality of the spot	