An overview of applied CoRFiLaC research to support traditional cheeses.

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Mission

- Developing research of the historic Sicilian cheeses and their production, in order to evidence the importance of tradition, biodiversity and respect of nature in a civilized society.

- Offering opportunity of job and specialization to many technicians and researchers.
Tools

• Extension Service
• Center for Elaboration of Data, “CED”
• PDO Certification
• Laboratories
• Cheese Pilot Plant and Aging Center
• Mediterranean Institute of Culinary Art of Sicily, “Micas”
• Caciotecca Regionale
• Marketing
Tools

• **Extension service**
  
  • It gathers producer’s needs and understands their future requirements.
  
  • The Extension service involves in its experimental protocols over 300 dairy farms, producing more than 60% of Sicilian milk for productive and reproductive data analysis.
  
  • The data, processed in real time in collaboration with CED, are communicated to farmers offering them scientific and technical support for management decisions.
Tools

- **CED**

It receives and elaborates data from:

- productive and reproductive events of dairy cows,
- analytical data from the laboratories,
- and the results from the experimental protocols.
Tools

• Laboratories
  • Laboratory for cattle feed analysis
  • Laboratories for Milk and Dairy Products
  • Laboratory for Antioxidants Analysis
  • Flavour Laboratory
  • Sensory Analysis Laboratory
  • Biochemistry of Protein Laboratory
  • Microbiology Laboratory
Tools

- **Laboratories**
- Laboratory for cattle feed analysis

created in order to provide information on Sicilian and southern feeds and forage that grows in the Mediterranean area.
Tools

- **Laboratories**

- Laboratory for milk and dairy products

  - monitors milk and cheese quality, animal metabolic diseases and reproduction efficiency through milk urea, ketons, progesterone tests, plus all those tests necessary to certify PDO cheeses.
The scientific knowledge acquired in the 1990's on typical Sicilian dairy productions allowed us to evaluate the key-factors of producing and aging of typical Sicilian cheeses, especially Ragusano and Pecorino Siciliano. P.D.O. recognition in 1996. CoRFiLaC was awarded from "MIPAF" the authorization to certify Ragusano and Pecorino Siciliano with the P.D.O. label in 2000.
Literature has reported that several milk components have anti-carcinogenic properties. Conjugated linoleic acid “CLA”, has been demonstrated to have anti-carcinogenic effects (*in vivo* and *in vitro*) anti-atherogenic effects, effects on the alteration of the distribution of the nutrients, on lipid metabolism, on the treatment of type II diabetes by reducing hyperglycemia, on the immune response and on the mineralization of the bone.

This lab studies potential healthy qualities of dairy products.
Tools

• **Laboratories**

• Flavour Laboratory

Gas Chromatography Olfactometry (GCO)
GC Mass
Retronasal Aroma Simulator (RAS)
Smart nose
**Tools**

- **Laboratories**
- **Sensory Analysis Laboratory**

  - Panelists have been highly trained.
  - Experimental cheeses as well those aiming for the PDO recognition are tested and a sensory profile is traced.
Tools

- **Laboratories**
  - Protein Biochemistry Laboratory
    - analyzes caseins and whey proteins factors influencing cheese quality and characteristics
    - studies typical molecules of traditional Sicilian cheeses deriving from proteolytic processes during cheese ripening.
Tools

- **Laboratories**
- **Microbiology Laboratory**

- studies the microflora of historic Sicilian cheeses, and the importance of wooden equipment for cheese production,
- supports the milk quality program of the Extension Service,
- supports the cheese pilot plant’s activity with microbiological tests,
- uses the Scanning Electronic Microscope, “SEM”.
Tools

- **Cheese pilot plant and aging center**

CoRFiLaC’s cheese makers learned to produce quality cheeses that were similar or equal to the quality of the local cheeses. This gives credit to research results, and facilitates transmitting these results to the real world.
Tools

- Mediterranean Institute of Culinary Art of Sicily, “Micas”

To witness the wine and gastronomic heritage of the Mediterranean basin, searching for tradition and using it as a carrier of history, culture and customs.
CoRFiLaC, a link between consumers and producers

CONSUMPTION

Consumer

Marketing MICAS

Information, Education, Promotion

Quality Guarantee

CONSUMPTION

Producer

Extension service PDO

Request for Consulting and Service/Information about Production and Management

PRODUCTION

Laboratories CED
Cheese plant/Aging center

RESEARCH

Consumer directed

Producer directed

Service, Consulting, Information, Education
Overview
Producer directed Research -
Measurement of feed particles by image analysis

Having the proper particle size distribution of forages is important for dairy nutrition

Usually forage particle size analysis is done by sequential sieving

Development of a method to measure single feed particles by “image analysis” using Matlab®
Consumer directed Research

The real challenge is to demonstrate scientifically that traditionally produced cheeses are “different”.

**Biodiversity factors**
- Healthy properties
- Food safety and traditional production systems
- Aromatic and sensorial profile of traditional cheeses
Consumer directed Research –

*Lactic acid bacteria from a natural biofilm of Tina, a wooden vat, potential contributors to Ragusano cheese fermentation*

Objectives:
- To determine the difference of the biofilm among stainless steel, plastic, and wooden tina.
- To verify the release of bacterial cells from the biofilm into the milk.
- To characterize the natural biofilm of the wooden tina.

- Sterile swabs inside the tina
- Filled with sterile (UHT) milk
  - @ 34°C (50L each)
- Milk left into the tina until 20’

Analysis of milk and wooden tina
Consumer directed Research –

**Lactic acid bacteria from a natural biofilm of Tina, a wooden vat, potential contributors to Ragusano cheese fermentation**

The porous structure of the tina allows the settlement of a niche of bacterial communities which are released into milk during the Ragusano cheese making process.

- *Lcc. lactis* subsp. *Lactis*
- *Ec. durans*
- *Str. macedonicus*
- *Ec. Faecium*
- *Stc. thermophilus*
and goes through the surface inside the external wood vessels
CLSM confirms that bacteria also colonize the internal wood layers.
Consumer directed Research – Microstructure of traditional Sicilian Cheeses by scanning electron microscope (SEM)

Cheese microstructure: Globular casein micelles that join together into clusters and chains

To examine the internal microstructure of the main traditional Sicilian cheeses, by scanning electron microscope (SEM)
-9 (5 pressed and 4 pasta filata) typical Sicilian cheeses were analysed

Relative proportions of casein, calcium, moisture and fat.
Consumer directed Research – *Microstructure of traditional Sicilian Cheeses*, and quantitative analysis by scanning SEM images.

**Fibrous microstructure**
- *Pasta Filata cheeses*

**Spounge-like microstructure**
- *Pressed cheeses*

- **Ragusano DOP 9 Months**
  - Moisture: 34.63%
  - Porosity: 0.2377
  - Pores: 828

- **Fiore Sicano 1 Month**
  - Moisture: 40.92%
  - Porosity: 0.3525
  - Pores: 1004
Consumer directed Research – Measurement of Gas Holes and Mechanical Openness in Cheese by Image Analysis

Gas production in cheeses:
- Typical characteristic defect

Measurement of gas production and quality control in cheese manufacturing

Development of an image analysis method using Matlab® to measure gas hole areas in cheese slices

Calculation of the black pixel area as a percentage of the total surface area
Consumer directed Research –
Microbiological safety of Ragusano PDO cheese through traditional farmhouse manufacturing

Factors affecting the microbiological safety:
- Raw milk pathogens

Natural antibacterial activities of raw milk:
- Fast acidification of the curd
- High temperatures and exposure time of the curd
- Salt content

Cheese manufacturing conditions:
Geographic discrimination between Pecorino Siciliano cheeses by electronic nose and sensory analyses

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Results

Cheeses separated by geographic area mainly on PC1, with SE and NE cheeses separating on PC2.

Important attributes in NE and SE cheeses were overall aroma and butyric aroma intensities, saltiness, spiciness and hardness.

Important attributes in W cheeses were moistness, mushroom, floral and fruity aromas.
Characterization of bacterial ecosystem in Pecorino Siciliano Cheese produced in different areas of Sicily

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INTRODUCTION

Pecorino Siciliano is an artisanal pressed cheese made using raw ewe’s milk and traditional equipments. The specificity of Protected Denomination of Origin assigned to Pecorino Siciliano (CEE Decree n. 1107, 12/06/1996) implied that its unique sensorial characteristics are related to the production areas, the race and the nutrition of the ewes and the traditional cheese manufacturing practices (Gazzetta Ufficiale. N° 1263, Luglio 1996).

OBJECTIVES

The aim of the present study was to evaluate the influence of different production areas in Sicily, on the dynamics of the microbial communities of artisanal cheese by Temporal Temperature Gel Electrophoresis (TTGE).

MATERIAL AND METHODS

Seven farms were selected, by microbiological and chemical composition of cheese samples, between 21 farms allocated in seven different areas of Sicily (Fig. 1): Iblean (I), Etnea (E), South Center (SC), North Centre (NC), Western (W), Western Centre (WC) and Peloritan (P); for each farm, four cheese-making were carried out. Milk and curd at 0 days were sampled just for first and third trial while milk ripened for two, four and eight months were obtained from each cheese-making. All cheese samples were analysed in two layers: internal and rind. PCR-TTGE was performed on all experimental samples: the DNA extraction from different samples and the amplification of V3 region of the 16S (rRNA gene were carried out, as described by Licitra et al. (2007). For TTGE analysis, a DCode universal mutation detection system was used to separate the V3 region PCR product. Migration was performed at 41 V for 16h with a temperature gradient of 63°C to 70°C (rate of 0.4°C 1h-1) for bacterial species with low Guanine an Citosine content (GC). After staining with ethidium bromide the gels were photographed on a UV transillumination table and analyzed using Gel Compare software (BioNumerics -Applied Maths, Version 4.6). The bands were identified by comparison with a TTGE species database developed by Parsyre et al. (2007). TTGE is a genetic molecular method based on direct analysis of DNA in the environment and on the separation of DNA molecules that differ by singles based allowing to analyse diversity within bacterial communities (Mayers et al. 1987).

RESULTS AND DISCUSSION

TTGE profiles derived from PCR amplicons of cheese samples manufactured at farms of different areas were showed in figure 2. It was selected one cheese-making for each farm and the list of main microorganisms used as reference was illustrated in Table 1. The predominance of Lc. lactis subsp. cremoris, Lb. delbrueki subsp. lactis and S. termophilus was observed. The presence of a bande relative to S. galbouls subsp. macdonitus was detected too and Staphylococcus equorum was observed to be peculiar in the rind layers of cheese samples. An high GC bacteria as proponibacteria and corinebacteria were identified too.

Some differences were due to farms location were detected, as displayed even by comparison of milk samples TTGE profiles (Fig.3). The band relative to St. equorum, in the milk samples withdrawn from Peloritan area farms (farm P), was observed to be predominant in all cheese samples too. E. faecium predominat in the West area samples (farm W) and with less extent in the others areas, was observed to be absent in the North Centre farm.

Only in the Centre areas (farms NC and SC) a constant presence of Lb. fermentum was detected. Lc. lactis resulted to be dominant in East area farms (I, E and SC) while it decreased in the Centre area farms. Lb. delbrueki subsp. lactis was strongly detected in West area farms (WC and W) and in Centre area farms (NC and NC) while in East area farms was observed to be weakly represented by two bands in the internal layers of cheese samples at 2 and 4 months of the Etnea area (farm E).

CONCLUSIONS

This study showed the dominance of Lc. lactis, St. termophilus, Lb. delbrueki and St. equorum in the traditional artisanal Pecorino Siciliano cheese. Some differences in the bacterial ecosystem, among all farms, were found as the dominance of Lb. fermentum in the Centre area, E. faecium in the West area and high variability of Propionibacteria in the East area. The bacterial distribution in milk samples based on farm location, was approximately maintained in cheese samples profile. This relationship between microorganism detected in milk samples and that one detected in cheese samples suggested that the quality of starter raw milk, probably affected by geographic area, was thought to be directly related to variability development in bacterial ecosystem of cheese.

REFERENCES

International Dairy Products
Molecular characterization of Algerian Cheese Bouhezza by PCR-TTGE

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INTRODUCTION

For all the population of the world, the interest of identification and characterization of traditional products became more and more important. Characterization of Bouhezza traditional cheese, which manufactured in the East of Algeria, has started on some aspects since a few years (Aissaoui Zitoun and Zitoun, 2006). Bouhezza can be made from goat, ewe or cow’s raw and/or fermented milk “lben” and its production was limited at rural area and only for a self consumption. It is obtained by spontaneous coagulation and utilizes a goat skin container for draining and ripening during more than three weeks. The aim of this work is to approach the microbiology of the cheese and it evolution during cheese-making and ripening specially with the PCR-Temporal Temperature Gel Electrophoresis method.

MATERIALS AND METHODS

1. Cheese-making based on Traditional Diagram
1.1. Crude Matter and Materials
- **lben** = Spontaneous fermented raw milk, churned and partially skimmed.
- Cow’s raw milk.
- Chekkoua = permeable container made with goat skin treated in a mixture of salt + juniper and then washed with soapy water and rinsed.

1.2. Adopted Diagram for experimental cheesemaking
- (1) Starting the manufacture in *chekoua* by salted *lben* (25 pL)/
- (2) Continuous addition (all 3 days) of salted *lben* for six weeks;
- (3) Finishing and correcting additions by whole raw milk, for acid and salted tastes, during the last weeks. Once the cheese is elaborated, it is spiced on with red hot pepper.

2. Analysis

**Lben, raw cow’s milk and Bouhezza cheese** are studied. Microbiological evolutions are studied at 7, 15, 21, 41, 56, and 70 days on two experimental cheese-making (C1 and C2) in similar conditions. Like in traditional consumption, red pepper is added to a separated sample of the final cheese (70 days) for a rapid comparison.

**RESULTS and DISCUSSION**

**Fig.1.** TTGE profiles of Bouhezza cheese made from two cheese-making (C1 and C2) at different ripening time: 7 days, 15d, 21d, 41d, 56d and 70d without pepper (NP) and with pepper (WP).

**Fig.2.** Evolution of microbiological characteristic of Bouhezza cheese (70 days).

- Total aerobic flora is between 106 to 107 cfu/g; its essentially formed by the indigenous lactic acid bacteria: lactobacillus and lactococcus (106 to 107 cfu/g).
- Yeasts and moulds are present on all stage of cheese-making/ripening (104 to 105 cfu/g).

**Cheese-making C1**
- The predominance of all samples is *L. lactis*, which presence was confirmed by specific PCR tests.
- *Lb. paracasei* and *Lb. delbruekii* was detected too. They are used as starter in different cheeses.
- The presence of some bacterial species sounds depending of ripening days. For example: the band corresponding to *Leucconostoc cremoris* was detected at about 21 days of ripening; *Lactobacillus plantarum* was observed to be *Lb. helveticus* was detected at about 21 days of ripening; *S. thermophilus* was observed to be *S. gallolyticus* subsp. *macedonicus*, *Lactococcus lactis* subsp. *cremoris*, *S. thermophilus* or *B. cereus*. The bands corresponding to *Lb. helveticus* and *Lb. delbruekii* was detected too. They are used as starter in different cheeses.

**Cheese-making C2**
- The presence of some bacterial species sounds depending of ripening days. For example: the band corresponding to *Leucconostoc cremoris* prevails in cheese samples at few ripening days (until 21 days), while *Lb. helveticus* was detected at about 21 days of ripening; *S. thermophilus* subsp. *macedonicus* was observed to be slightly present till 41 days to disappear later while the *Lb. delbruekii subsp. lactis* was evident after 41 days.
- The bands corresponding to *S. equorum, Lb. delbruekii* and *S. thermophilus* were detected in cheeses after 41 days probably due to addition of whole raw milk.
- The presence of a few high-GC-content species, like coryneform bacteria was observed.
- Other species shows sporadic presence along the 70 days of ripening (*Lb. paracasei, B. cereus, Corynebacterium flavescens, Lb. buchneri and Bifidobacterium longum*). The evolution of these species must be confirmed.
- The indigenous LAB of *lben* are the main flora occurring in the refining of the cheese. The raw milk brings from its part a complement of other species which can grow in the conditions of the cheese and participate too in the refining.

**REFERENCES**

- Manu et al., 2000; Cocuret et al., 2003; Lb. plantarum is detected in almost samples.
- Lb. helveticus was detected too. They are used as starter in different cheeses.
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- The indigenous LAB of *lben* are the main flora occurring in the refining of the cheese. The raw milk brings from its part a complement of other species which can grow in the conditions of the cheese and participate too in the refining.
Aroma profile characterization of traditional Algerian Bouhezza cheese

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INTRODUCTION
For all the population of the world, the interest of identification and characterization of traditional products became more and more important. Characterization of Bouhezza traditional cheese, which manufactured in the East of the Algeria, has started on some aspects since a few years (Aissaoui Zitoun et al., 2006). This cheese can be produced by goat, sheep or cow milk. It can be also made from mixed milks in different proportions. Bouhezza cheese manufacturing requires the preparation of both a natural container "chekoua" and a spontaneous fermented milk, rather than skinned and little acid "bjen". The chekoua is a bag obtained from goat or sheep skin, treated with salt and juniper berry. It has also an important role as separator of serum and solid phase (ultrafilter). Manufacturing process is carried out by adding "bjen" and raw milk in order to correct both acidity and saltiness. On the average cheese making process is completed after 70 days when pepper is added. The aim of this preliminary study was to characterize the volatile organic compounds (VOCs) of Bouhezza cheese produced in different farms.

MATERIALS AND METHODS

The traditional Bouhezza samples obtained from five different farms (F2, F3, F4, F5, F6) were analyzed by:

Smart Nose (SN) system which allowed the direct analysis by MS of volatile organic compounds (VOCs). The ion fragments obtained by SN were then processed by a multivariate statistical PCA.

Dynamic headspace purge and trap apparatus (Tekmar 8900) in combination with a gas chromatographyolfactometry/mass selective detector (GC/MS/O) (modified Hewlett Packard 6890 gas chromatograph by Datu Inc., Geneva, NY) to analyze odour active compounds. Statistical software was used to elaborate GCO results (JMP® 8.0.1).

RESULTS and DISCUSSION

Table 1 - Volatile compounds in Bouhezza cheese by P&T-GC/MS/O.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Class</th>
<th>Descriptor</th>
<th>LRIa</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
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<td>954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neodecalin-1-carboxylic acid</td>
<td>terepene</td>
<td>red pepper</td>
<td>1202</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>geraniol</td>
<td>terepene</td>
<td>wine/floral</td>
<td>1278</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>isohesperidin</td>
<td>terepene</td>
<td>floral/orange</td>
<td>1130</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

CA applied to SN results showed a good separation (PC1 77.93%; PC2 13.05%) among Bouhezza cheeses indicating the uniqueness of the homemade products (figure 1). Olfactometry results (Martin et al., 1998; Acree & Barnard 1994) confirmed a high variability for the aroma profiles (table 1). F2 sample had the richest profile (23 VOCs) among all farms, followed by F5 (19 VOCs), F3 (18 VOCs), F4 (15 VOCs) and F6 (13 VOCs). All samples were mainly characterized both by aldehydes and ester compounds. Among aldehydes they were octanal, nonanal, (E,E)-2,4-decadienal, (E)-2-nonenal that gave, respectively, orange, apple, hay, and green notes. There were identified the following free fatty acids: ethyl octanoate, ethyl hexanoate, ethyl butanoate and propyl-1-methyl-acetate. These released respectively, wine, floral, and fruity notes on cheese aroma profiles.

Statistical analysis were performed using a nominal logistic model on the presence of VOCs compounds. Data showed that probability of VOCs presence is marginaly dependent from farms, thus farm traditional method of production did not significantly affect cheese VOCs presence (Wald Statistic χ² = 4.55; p<0.05). The high variability of Bouhezza cheese was clearly detected by Smart Nose because the high resolution power of the instrument in detecting the single aromatic ion mass, these results clearly indicated the peculiarity of the single farm producing cheese. On the other side GCO analyses showed different profiles with some odor active compounds in common of the Bouhezza samples analyzed. Both instruments, at different levels and with different statistical results, confirmed that the farm traditional cheese-making process, the milk and the different spices used during manufacturing contribute to the uniqueness of this product. More farms and cheese samples should be investigated to prove the linkage of the Bouhezza cheese to the territory of production in order to demonstrate the uniqueness of the product.

CONCLUSION


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REFERENCES

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Objective
The aim of this work was to study the Chekoua bacterial microflora by Confocal Laser Scanning Microscopy, Scanning Electron Microscopy and by PCR-temporal temperature gel electrophoresis.

Background
Chekoua is the goatskin bag used for draining and ripening of the traditional Bouhezza cheese. Before use, the goatskin is first treated with salt and juniper berry, tied and rinsed. The obtained container is filled up with Lbren and stored overnight and then rinsed. After cheese making, the Chekoua can be rinsed and dried for another cheese making.

Material and Methods
Sampling
Two replicates of fresh skin (FS) were used, prepared, sampled and analyzed before (FS1, FS2) and after contact with Lbren (FSL1, FSL2).

SEM
The samples were dehydrated and dried (Lortal et al., 2009) in a graduated ethanol series (75, 85, 95 and 100%) and dried by the critical point method in CO2. The dried samples were mounted on SEM stubs, coated with gold-palladium and placed on the SEM equipment. The samples were analyzed by the scanning electron microscopy Jeol JSM 5600LV (Jeol, Tokyo, Japan), operating at an accelerating voltage of 15 kV, a working distance of 12 - 18 mm and a magnification of 5000 - 10000X.

TTGE
The DNA extraction from different samples and the amplification of V3 region of the 16S rRNA gene by the use of universal primers, were carried out as described by Licitra et al. (2007). A DCode universal mutation detection system was used to separate the V3 region PCR product. Migration was performed at 41V for 16h with a temperature gradient of 63°C to 70°C (rate of 0.4°C/h) for bacteria of low GC content. After staining with ethidium bromide the gels were photographed on a UV transillumination table and analyzed using Gel Compare software. The bands were identified by comparison to a TTGE specie database developed by Parayre et al. (2007).

Microbiology
Detection of pathogens was performed on biofilm samples freshly collected, by using BAXIX System (DuPont Qualicon, UK). Enrichment for Salmonella was carried out using modified tryptone soya broth (Oxoid) at 36°C for 24 h; 10 µL were then transferred in 500 µL BHI (Oxoid) for a further incubation for 3h at 37°C. Enrichment for Listeria was done in Listeria selective enrichment broth base (Oxoid), at 30°C for 3h. A volume of 0.1 mL of enriched broth was transferred in 9.9 mL of MoPS-BLEB (Oxoid) and incubated at 37°C for 18 h. Enrichment for Escherichia coli O157:H7 was done with E/C broth (Reduced Bile Salt) added with novobiocin (Oxoid), at 36°C for 24h. Lysis was performed following the BAXIX System instructions.

Conclusions
The biofilm ecosystem of the final Chekoua was composed by bacteria and yeasts. The coexistence of those groups might be the result of their commensalistic interaction, which is fundamental for the cheese safety. The combination of such conditions inhibits many spoilage bacteria and filamentous fungi, thereby substantially increasing shelf life and safety of Bouhezza cheese.

Results
Goat skin surface resulted lacking in bacteria. Samples observed before Lbren addition (FS1, FS2) were less populated (Fig. 2A, 1B) than the samples examined after Lbren adjunct (FSL1, FSL2) (Fig. 2C, 1D).

Just isolated bacteria entrapped in the network of the epidermic tissue were detected in FS1, FS2 (Fig. 2A, 1D).

FL were instead observed only in skin biofilm profiles.

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As displayed from comparison of all molecular profiles in cluster analysis (Fig. 6), all milk samples (before and after contact) resulted similar between them and revealed some differences with skin profiles, above all in the high-GC region. Indeed the presence of a few bands corresponding to Bacillus subtilis, Lactobacillus and Leuconostoc was observed to be slightly present in the molecular profiles of milk before contact with goat skin, but its band was very intense in one of the two fresh skins, to disappear in Lbren at once after contact with skin.

Microbiological analysis showed no pathogens and an increasing of mesophilic LAB and yeast in Lbren after contact.

References

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Vista lato ingresso

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