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Microbial Diversity of Traditionally Processed Cheese from Northeastern Region of Transylvania (Romania)

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Abstract

The composition and production technology of the cheese are extremely diverse. There are a wide variety of microbial species on their surface, with a much smaller number inside of the product. The microbiota of the cheese may be composed of beneficial microorganisms, spoilage and foodborne pathogens. Identification and characterization of the microorganisms present in these products are important nutrition, food safety and technological aspects. During our work we evaluated the prevalence of allochthonous bacteria and microscopic fungi in traditionally processed cheeses from northeastern region of Transylvania, with classical microbiological culture methods. Based on the results the microbiota of the analysed cheeses was highly diversified. The identified bacteria with the highest prevalence from different selective media, were as follows: *Escherichia coli*, *Enterococcus durans*, *Enterococcus faecalis*, *Shigella flexnerii*, *Proteus vulgaris*, *Stenotrophomonas maltophilia*, *Staphylococcus equorum subsp. equorum*, *Staphylococcus equorum subsp. linens*, *Halomonas alkaliphila*, *Kocuria rhizophila*, *Hafnia paralvei*, *Bacillus licheniformis* and *Klebsiella michiganensis*.

Keywords: microbial diversity, traditionally processed cheeses, starter culture, allochthonous bacteria

1. Introduction

Cheese belongs to dairy products representing an important part of human balance diet for hundreds of years. It contains the essential macronutrients and micronutrients. Different factors as environmental conditions, geographical region, processing technologies and the local customs determine the development and release of the huge variety of cheese. One prevention method of milk spoilage over the centuries has been the production of cheese, meanwhile the nutritional benefits are conserving [1].

Cheese is the result of a fermentation process driven by lactic acid bacteria. Different bacteria, yeast and molds constitute the complex microbial ecosystem of this product. Throughout the ripening, the microbes release proteinases and lipases that are involved in the protein and fat hydrolysis, resulting amino acids and fatty acids. These compounds represent important flavour precursors for cheeses [2].

The cheese microbiota is involved in the development of the appearance texture, flavour and aroma. Both starter culture organisms and allochthonous microorganisms are part of the specific microbial ecosystem.

The quality and the processing of the milk, the environmental conditions (temperature, humidity, salt), the applied technology influence the quantity and the diversity of the specific species of microorganisms. Also, this fact is affected by the microbial conditions in the manufactures [3].

Cheese can be considered a privileged product with beneficial or negative impact on health, influenced by the nutritional composition, salt and bioactive compound content. The negative effect is related to the presence of pathogenic bacteria such as *Listeria* and *Escherichia coli* [1].

2. Factors controlling microbial growth in cheeses

In various types of cheese, the growth and development of microbes are influenced by physical and chemical parameters (water activity, pH, ripening temperature, redox potential), chemical composition (salt content, presence of nitrates) and by the metabolites as organic acids and the protein like compounds released by starter cultures named bacteriocins [2, 4].

Water activity (a_w) of cheese range between 0.917–0.988. Vast majority of non-starter bacteria, spoilage and pathogens are favoured by this condition. The optimum a_w of starter bacteria is under these values. Osmophilic yeasts support a_w less than 0.60 [4]. The a_w is related to sodium chloride (NaCl) and the produced metabolites (lactate, acetate, and propionate) content. These compounds decrease the vapour pressure of water as the result of their dissolution in the moisture of the cheese. Higher the concentration of these metabolites, the reduced the a_w is. Without vacuum or sealed packaging, the moisture content is reduced, due to evaporation, causing a gradient in the a_w of the cheese. This gradient commonly is proportional with the cheese size and is lower in the external part. At low a_w the lag phase of bacterial growth is prolonged [2].

The salt content decreases the a_w value of the cheese and consequently is an antimicrobial agent. For example, for the brined-cured cheeses at the beginning of the ripening process, the salt concentration is much higher on the cheese surface than in the inside the block. For this reason, salt tolerant microorganisms can grow on the surface of this type of cheeses. Surface-ripened cheeses microbiota include *Brevibacterium linens* and *Debaryomyces hansenii* growing at 15% NaCl with a_w ~0.916 [4]. Low salt content was associated with the development of coryneforms, micrococci, and staphylococci. The growth of these microorganisms is supported by 10–15% NaCl. 10% NaCl doesn't affect the development of *P. camemberti*. 20% NaCl is tolerated by several strains of *P. roqueforti*, whereas *Geotrichum candidum* is susceptible to salt [2]. Concentration of 15 g/L NaCl is used in manufacturing of Protected Designation of Origin (PDO) Serpa cheese [5]. NaCl can have a negative impact on cheese, affecting the enzymatic and microbial activities. This lead to the change of biochemistry processes as lactose metabolism, hydrolysis of fats and casein, the flavour compound development and the cheese pH formation. Paracasein aggregation or hydration is influenced by the salt -, calcium content and pH. These determinate the water binding properties of the casein matrix, the textural, rheological and cooking aspects and the ability to synerese [6].

Most bacteria grow best at neutral pHs. In acidic conditions ($\text{pH} < 4$) only several bacteria are able to grow, for example *Lactobacillus* spp. Molds and yeasts prefer pHs lower than 4.5. Viability of bacteria is affected by metabolites. Various weak organic acids (as sorbic and propionic acid) are causing bacterial lysis. These

compounds cross the bacterial cell membrane releasing protons inside the cell and leading to acidification. In cheese environment, the main occurring organic acids are acetic, lactic and propionic acids. Propionic acid exerts antimicrobial effect against molds [4]. The slight acidic range in curd (pH = 4.5–5.3) contributes significantly to the growth and development of bacteria in cheese [7]. Microbial growth is determined also, by the ripening temperature. The optimum temperatures for the frequently used lactic acid bacteria, belonging to mesophilic and thermophilic bacterial groups, is 35°C and 55°C respectively.

Maturation temperature should play a role in preventing the growth of undesirable microbes as secondary flora, spoilage and pathogenic microorganisms, thus avoiding the losses [4].

The food hygiene is taken very seriously in the dairy industry. The quality of dairy products depends on raw milk quality and the processing conditions. Before ripening the number of microorganisms showed higher values in artisanal soft cheese from Germany named kochkäse, made from raw milk in contrast with cheese from pasteurised milk. After maturation, the amount of microorganisms was identical in the two samples. There is a close correlation between the temperature and ripening time. The ripening time is decreased at 35°C with the rising microorganism population. The maturation temperature at 15°C favour the lactic acid bacteria growth [8].

Redox potential (E_{redox}) values in cheese is approximately equal with $E_{\text{redox}} = -250$ mV, during the maturation process is reduced, due to unknown mechanisms. The redox potential is different inside and outside of the cheese that specifies the microbiota.

It was shown that the cheese surface is characterized by aerobic bacteria namely *Brevibacterium* spp., *Bacillus* spp. and *Micrococcus* spp. The predominant microbes inside the cheese are anaerobic or facultative aerobic microorganisms [4].

For the prevention of bacterial gas formation by coliforms and *Clostridium tyrobutyricum*, different chemicals are added to milk. In case of cheeses as Gouda and Edam cheeses potassium or sodium nitrate is added in amount of 20 g/100 L raw milk). In the milk or curd, the xanthine oxidoreductase catalyses the formation of nitrites from nitrates, that inhibit the aforementioned progress. Also carcinogenic compound as nitrosamine may resulted from the reaction of nitrites with aromatic amino acids [2].

Environmental factors influence the growth and development of lactic acid bacteria in cheese. The environmental factors and the various amount of NaCl, glucose and lactose affect the growth and biofilm formation of bacterial strains originated from Serbian traditionally processed cheese (*Enterococcus hirae* KGPMF9, *Lactococcus lactis* subsp. *lactis* KGPMF23, *Lactobacillus fermentum* KGPMF29, *Streptococcus uberis* KGPMF2). The limiting values for the factors were 6.5% of NaCl and 4°C. Except for *E. hirae* KGPMF9. *S. uberis* KGPMF2 different concentration of the carbohydrates and temperature of 37°C induced the planktonic growth and biofilm formation of the analysed bacteria. In case of biofilm formation basic media in contrast to acidic media was limiting factor [9].

3. Starter bacteria

In cheese processing lactic acid bacteria are used as starter cultures, contributing to organoleptic characteristics and preservation of the product. These strains are added in a different form, grown in milk or mediums. Also, in France or Italy the whey incubated the day before use is applied as starter culture. Starters comes from the fact that these bacteria start the lactic acid production. These belong to

mesophilic and thermophilic bacteria [2, 10]. Mesophilic bacteria include lactococci (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*,) and often leuconostocs (*Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis*). Thermophilic cultures include *Streptococcus thermophilus* and different species of *Lactobacillus* as *Lb. delbrueckii* and *Lb. helveticus* [10].

Cheddar cheese, soft cheeses, and most Dutch cheeses are produced with mesophilic starters. Starter culture have to meet different expectations as lactic acid production, reduction of pH, to be resistant to bacteriophages, inhibition of pathogenic and spoilage bacteria, contribute to the flavour, texture formation and have health benefit. Depending on the type of cheese the starter culture contributes to the development of nature of the cheese due to lipolysis, proteolysis and autolysis of the cells [10, 11].

Beside the lactic acid bacteria *Debaryomyces hansenii* and *Yarrowia lipolytica* yeasts are promising cheese starter cultures affecting the cheese maturation [12]. An example of molds as secondary starter culture are *Penicillium roqueforti* and *Penicillium camemberti* used in the production of blue- veined cheese. They tolerate the high and medium salinity conditions and possess high proteolytic activity [13]. There are traditional processed cheeses without starter culture, for example Spanish varieties. In these case at the beginning the high proportion of microbiota came from the milks' microbiota as lactococci.

4. The characteristic cheese microbiota

Dairy products are perishable foods providing a good medium for microbial growth. Foodborne pathogens as *Staphylococcus aureus* in raw milk can be originated from infected lactating animals, undesirable milking conditions that lead to milk contamination with animal faeces and other materials. Incorrectly handled equipment, cleaning, sanitizing equipment, water or air bleeds can provoke a multitude of contamination with various microorganisms [14].

Superior varieties of cheeses as Emmental, Gruyère, Comté, Parmigiano Reggiano, Reblochon and Roquefort are made from raw milk. The microbial safety of these products is ensured by the heat treatment at a higher temperature > 50°C, with duration of 1 h. The challenge of this type of cheeses represents the pathogen bacteria from raw milk that can grow during the process [15].

The different types of cheeses are characterized by diverse microbiota. The raw milk cheeses (soft and hard, short or long matured, starter added or nonstarter added cheeses, and ripened in brine or unbrined cheeses) own their distinct microflora.

Gram-negative bacteria and salt-tolerant bacteria belonging to different genus, including *Halomonas*, *Vibrio*, and *Hafnia* spp., and salinity tolerant bacteria, including *Arthobacter*, *Micrococcus*, *Brevibacterium* and *Brachybacterium* spp., have been characteristic bacteria of raw milk cheese surface microbiota [16].

The environmental conditions of the dairy farm have an impact on the microflora of the raw milk and therefore influence manufacturing. The main spore formers in milk are species of *Clostridium*, *Bacillus* and *Geobacillus* genus. These bacteria are the follows: *C. sporogenes*, *C. butyricum*, *C. tyrobutyricum*, *B. cereus*, *B. sporothermodurans*, *G. stearothermophilus*. For the occurrence of defects in cheese as off-flavors, late blowing is responsible *C. tyrobutyricum*.

Bacteria species of *Enterococcus* are widespread in dairy products affecting negatively or positively the flavor development as the result of hydrolytic processes, proteolysis and peptidolysis. Their origin is related to raw material or to starter culture composition. In southern European artisanal cheeses, *Enterococci*'s number

is higher than 10^7 CFU g⁻¹. This bacteria is an opportunistic nosocomial pathogen. Also, *Enterococci* could play a role in antibiotic resistance transfer and hospital-acquired infections. Consequently, the occurrence of this in dairy products is suspicious [2, 17]. In Gouda cheese the *Ec. malodoratus*, as its name suggest lead to the development of bad flavours [15]. *Streptococcus* species also can be isolated from milk [17].

Fox et al. [15] reported that in cheese the non-lactic acid bacteria are bacteria belonging to genera *Agrococcus*, *Arthrobacter*, *Brevibacterium*, *Brachybacterium*, *Corynebacterium*, *Microbacterium*, *Propionibacterium*, *Pediococcus*, *Micrococcus*, *Kocuria*, *Kytococcus*, *Staphylococcus*.

Aged cheese microbiota includes non-starter lactic acid bacteria. The predominantly found bacteria are facultatively heterofermentative as *Lactobacillus casei* and *L. paracasei*. It occurs occasionally *Pediococcus* spp. and obligately heterofermentative *Lactobacillus* spp., such as, *L. brevis* and *L. fermentum*.

Non-starter lactic acid bacteria origin is the raw material and/or the processing environment. A little part of the lactobacilli are resistant to the thermal treatment, pasteurization, for example in Emmental cheese obtained from raw milk can survive the 52°C temperature.

Majority of non-starter lactic acid bacteria can be characterized as: facultative anaerobes, salt-and acid-tolerant and able to grow in cheese. The involvement of these bacteria in the production of cheese flavour is unclear [2].

The number of *Micrococcus* and *Staphylococcus* is higher than 10^6 CFU g⁻¹ in different types of cheese. It was found on the surface of soft smear-ripened, semi hard and hard cheeses. *Staphylococcus saprophyticus*, *S. equorum*, *S. vitulus*, and *S. xylosus* were described as main staphylococci occurred in cheese. *Kocuria*, *Nesterenkonia*, *Kytococcus*, *Dermacoccus*, and *Micrococcus* were found in cheese. *Kocuria rosea*, *K. varians*, *Dermacoccus sedantarius*, *Micrococcus lylae*, and *M. luteus* isolated from the surface of cheese. The function of *Micrococcus* and *Staphylococcus* in cheese is unclear, but many of them are related to the production of proteinases and lipases [2].

Coryneform bacteria, especially *Brevibacterium linens* is involved in the red or orange colour formation of the surface in smear-ripened cheeses. Therefore, intentionally is applied on the surface of cheeses after brining. It was proved that *Arthrobacter*, *Agrococcus*, *Brachybacterium*, *Corynebacterium*, and *Microbacterium* spp. have an important role. Origin of these corynebacteria is the brine and shelving. It is believed that staphylococci, coryneforms and micrococci, can be originated from skin due to manual handling, comprising the microflora of the skin [2].

Swiss-type cheese as Emmental and Comte' contain propionic acid bacteria, *Propionibacterium freudenreichii*. Their pivotal role is the metabolization of lactate produced by the starter bacteria to propionate, acetate, and CO₂ [2].

Cheese can be a reservoir and carrier of probiotic bacteria for human. Due to the low redox potential, this product is a protective environment for probiotics. Their resistance to changes in pH represents an advantage in the acidic environment of gastrointestinal track. Probiotic bacteria applied in cheese production include *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. johnsonii*, *L. reuteri*, *L. rhamnosus*, *Bifidobacterium bifidum*, *B. animalis*, *B. brevis*, *B. longum*, *B. infantis* [7].

Escherichia coli and coliforms are hygienic indicator bacteria. There are limit values for cheeses as well as for raw-milk cheeses.

The level of indicator microorganisms is less than 10 CFU/g and not more than 100 CFU/g, these representing the limit values in many countries.

In those instances, where cheeses had high levels of indicator bacteria, most studies attributed these to the use of poor-quality raw milk which contained high levels of indicator flora or to unsanitary conditions, or both.

The occurrence of coliforms in dairy products reveal low grade raw milk, poor hygienic conditions under production process. Coliforms could be avoided by respecting personal hygiene and sanitation process.

Human pathogens including *Salmonella* spp., *Streptococcus* spp., *L. monocytogenes*, *Helicobacter pylori*, *Campylobacter* spp., *Escherichia coli*, *Coxiella burnetii*, *Mycobacterium* spp., *Brucella* spp., tick-borne encephalitis virus (TBEV), *Staphylococcus aureus*, *Arcanobacter pyogenes*, *Toxoplasma gondii*, *Bacillus cereus*, *Leptospira*, *Clostridium* spp., and *Yersinia enterocolitica* can be present in raw milk cheeses. The inadequate fermentation conditions contribute to the growth and development of these pathogens [16].

At the origin of unwanted yeasts and molds appearance in cheeses could stand environmental factors, improper processing conditions or infested starter culture. Several yeast could affect negatively (sometimes positively) the cheese. These microbes can be involved in taste and flavour development, affecting the quality attributes also, causing spoilage and release unpleasant aroma compounds. Clinically relevant yeast was detected in different type of cheese as *Candida albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* [3].

The advanced hygiene and quality control contributed to the decrease of gas production by yeasts during cheese manufacturing [2].

A diverse group of yeasts represent a high proportion of surface microflora of different type of smear- and mold-ripened cheeses, including Comte', Tilsit, Limburger, Blue, and Camembert. These microorganisms level can reach 10^6 – 10^7 CFU/g and are characterized by tolerance to low pH and high salt concentrations.

The most prevalent yeast species isolated from cheese were *Galactomyces candidus*, *C. parapsilosis*, *C. sake*, *C. batistae*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Pichia kudriavzevii* [2, 3, 7].

The most common molds in cheese include *Penicillium roqueforti* in Blue cheeses (e.g., Stilton, Roquefort, and Gorgonzola) and *Penicillium camemberti* in surface mold-ripened cheeses (e.g., Camembert and Brie) [2, 3, 7]. Molds isolated from various retail cheese were: *Penicillium verrucosum*, *P. paneum*, *P. brevicompactum*, *P. gladioli*, *Aspergillus niger*, *A. flavus*, *A. phoenicis*, *Cladosporium sphaerospermum*, *C. cladosporioides*, *Eurotium rubrum* [3]. The presence of *P. commune*, which is closely related to *P. camemberti*, can cause discoloration of cheese surfaces and the development of off-flavours [15].

5. Microbiota and characteristics of traditionally processed cheeses

Artisanal cheeses represent a particular category of cheeses that is appreciated worldwide. Particular properties of products as the individual sensory aspects are related to local environment, cultural and historical features [8].

One of the challenges of artisanal cheese producers is to keep the microbial diversity of the product and exploit their beneficial effects. The traditional process, including the wooden equipment, microorganisms are the main factor in food safety issues [18].

In raw milk, over 400 species of lactic acid bacteria, Gram-positive and negative, catalase-positive bacteria, yeast and mould have been found. In cheese cores the diversity is not very large, typical lactic acid bacteria are the predominant microorganisms, whereas the surface of cheeses is rich in several species of bacteria, moulds and yeast.

Diversity within the cheeses results from considerable variation in distribution and growth of the same bacteria in the various cheese. Raw milk cheeses compared to the others are characterized by an intense and rich flavour. This results from the native microflora of the raw material and is not a character of cheeses made from treated milk [18].

The artisanal cheeses possess a heterogeneous microbiota characterized by a typical population dynamic. The unique microflora is associated with geographical indication for that region [19].

Perhaps natural milk cultures are used for Italian traditional cheeses including Asiago, Pecorino Siciliano, Canestrato Pugliese, Castelmagno and Fossa. The origin of these cultures is the raw milk. The processing comprises a heat treatment of the raw milk for 15–30 min on 60–65°C, fast cooling and back slopping technique. Thermophilic species of that type of cultures are *Streptococcus thermophilus*, *Streptococcus macedonicus*, *Enterococcus*, and *Lactobacillus* spp. The whey based cultures contain aciduric and thermophilic strains. Among the cultivable bacteria *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus fermentum*, and *Str. thermophilus* were identified [20].

Central and Southern Italian cheese is “Pecorino” with PDO status, processed traditionally. The raw milk and natural microflora contribute to the development of typical organoleptic attributes. The main representants are *Enterococcus faecium*, *E. faecalis*, *Lactobacillus plantarum*, *L. brevis*, *Lactococcus lactis* subsp. *cremoris* [19].

In traditional cheese production crucial role have the autochthonous starter cultures, the main steps of manufacturing are more affected by it, but the final product are less. This refers only to that cheeses that are processed in the same geographical area and with settled circumstances. Numerous parameters have effect on the specific characteristics of cheese produced in different locations. This is case of PDO Pecorino Siciliano that is manufactured in different places of Sicily. There are some common parameters that must be applied during production as raw ewe’s milk, lamb rennet paste, wooden equipment and no added starter.

The inconstancy in the raw milk quality and the variable autochthonous microbiota determine the alteration of the attributes as organoleptic characteristics of artisanal cheese manufactured in different locations. With the application of lactic acid bacteria, the characteristics of PDO Pecorino Siciliano cheese production could be stabilized [21].

Brazilian traditional processed cheeses differ from the commercial product in their unique characteristics. These are produced with tradition, the essence of production is transferred from one generation to the next. These type of cheese are characterized by unique organoleptic properties, typical aroma, flavour, and colour due to the typical endogenous microbiota. This microbiota includes lactic acid bacteria, yeasts, fungi that source is the raw material and the production environment. Among the lactic acid bacteria isolated from artisanal cheese are mainly *Lactobacillus plantarum* and other strains as *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactococcus lactis*, *Lactobacillus rhamnosus*, *Pediococcus pentosaceus*, *Lactobacillus curvatus*, *Lactobacillus paraplantarum*, *Lactococcus garvieae*, and *Pediococcus acidilactici* [22].

The traditionally processed cheeses from raw milk are very popular and preferred by the local population, therefore is a great need for the microbiological safety of this product. For this reason, there are characterized lactic acid bacteria with multi effect traits as anti-listerial effect isolated from artisanal cheeses. *Enterococcus faecalis* (1–37, 2–49, 2–388 and 1–400, *Lactobacillus brevis* 2–392, *Lactobacillus plantarum* 1–399 a4) have antibacterial effect against *L. monocytogenes* growth during the low temperature storage soft cheese and decrease the ripening time of semi-hard cheeses aged at ambient temperature [23].

Based on Margalho et al. [24] research from the 220 lactic acid bacteria originated from artisanal Brasil cheese twenty-two isolates had probiotic traits and role in biopreservation. These strains are characterized by resistance to gastric acids as low pH values, bile salts, and also possess good adhesion properties, mostly to enterocyte-like cells (Caco-2). One of the lactic acid bacteria, namely *Lactobacillus plantarum* (1QB77) was able to produce bacteriocin and exhibited inhibition effect against enterotoxigenic *S. aureus* and *L. monocytogenes*. It was able to survive the processing conditions also.

Valuable source of microorganisms with specific characteristics are the artisanal cheese due to their high biodiversity influenced by the local ecosystem.

The Russian artisanal Buryatia cottage cheese is characterized by typical taste and flavour determined by the numerous lactic acid bacteria. From the 62 isolated and assayed lactic acid bacteria of Russian cheese belonged to different 6 genera, 14 species/subspecies. The full-length 16S rRNA sequencing revealed a high diversity of bacteria including: *Lc. lactis*, *S. thermophilus*, *Lactococcus raffinolactis*, *Acetobacter cibinongensis*, *Lactobacillus helveticus*, *Klebsiella pneumonia*, *Acinetobacter johnsonii* and *Klebsiella oxytoca* [25].

Northern Moroccan artisanal cheese is characteristic of rural regions. Due to the raw material, the goat's milk, this cheese possesses unique organoleptic properties. Galiou et al. [26] revealed that the microbiota of 28 Northern Moroccan artisanal cheeses included *Enterococcus* spp. (249 isolates), *Lactococcus lactis* (36), *Lactobacillus plantarum* (7) and *L. paracasei* (2). Besides, there were still present coliforms, yeasts, microscopic fungi and in some sample *Listeria monocytogenes*.

The Spanish Manchego is traditionally processed product, obtained with enzymatic coagulation. It is a matured, uncooked and pressed cheese, characterized by high fat content. The 248 strains of predominant lactic acid bacteria obtained from manufacture and ripening process of two dairies included species as *L. plantarum*, *L. brevis*, *L. paracasei* subsp. *paracasei*, *L. fermentum*, *L. pentosus*, *L. acidophilus* and *L. curvatus*. In samples from both dairies, the species *L. plantarum*, *L. brevis* and *L. paracasei* subsp. *paracasei* dominated during ripening [27].

Portuguese Serpa cheese is designated as Protected Designation of Origin (PDO) cheese that is characterized also, by particular appreciated aroma and flavour. The organoleptic attributes have resulted from the not pasteurized raw ovine milk components and the addition of extracts of *Cynara cardunculus* L.. Based on literature data, the most relevant bacteria species of Serpa cheese are *Lactobacillus* spp. (*L. paracasei*, *L. plantarum*, *L. brevis*, *L. pentosus*, *L. curvatus*), *Leuconostoc mesenteroides*, *Lactococcus lactis*, coliforms, *Enterococcus faecalis*, *E. faecium*, *E. hirae*, *Hafnia alvei*, *Galactomyces* spp., *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Candida zeylanoides*, *Pichia fermentans*, *Cryptococcus oeirensis*, *Yarrowia lypolytica* [28].

Northern French PDO Maroilles cheese is a soft type cheese with washed rind. The duration of maturation are 4–6 weeks according to the size, while the rinds are washed weakly with salt water. It is characterized by a surface microbiota *Geotrichum candidum*, *Kluyveromyces lactis*, *Brevibacterium linens* and *B. aurantiacum*. The industrially and traditionally processed cheeses differ in microorganism's composition, total mesophilic flora, rate of lactic acid bacteria and *Enterobacteriaceae*. The occurrence of *Enterobacteriaceae* in traditionally processed Maroilles reveals unhygienic conditions during manufacturing process [1].

North-western Iranian Koopeh is obtained from raw sheep or cow milk in rural households, and the ripening is under the soil for four months. This cheese is semi-hard type, characterized by high fat content, by buttery and pungent flavour. It has beneficial effects as antioxidant and antimicrobial activities attributed to antioxidant peptides and aromatic herbs content (*Allium ampeloprasum*, *Cuminum*

cyminum, *Nigella sativa*, *Thymes vulgaris*) [29]. The non-starter lactic acid bacteria are the dominant microorganisms of the ripening steps.

The artisanal Mexican cheese, named Chiapas cream cheese is semi-ripened type. Organoleptic characteristics include the creamy and acidic taste. The predominant acidifying bacteria are the endogenous lactic acid bacteria adapted to the local climatic, tropical conditions. The bacterial strains originated from this type of cheese are characterized by high antioxidant capacity and proteolytic activity. It was showed that these bacteria could to produce bioactive compounds with role in functional foods, resulted from hydrolysis of proteins and also contain angiotensin-converting enzyme [30].

The PDO Parmigiano Reggiano traditional cheese is obtained from raw cow's milk and added whey starters obtained with back sloping. It is a long ripened type of cheese, produced in defined geographical areas (Po river's valley). The biochemical characteristics of cheese matrix are the result of the adventitious bacterial dynamics.

In cheese post brining process, that can take a 24 month different lactic acid bacteria were detected applying cultural and non-cultural methods: *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, *Pediococcus acidilactici*, *Levilactobacillus brevis*, *Lacticaseibacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii ssp. lactis*, *Lentilactobacillus buchneri*, *Schleiferilactobacillus harbiniensis*, *Lentilactobacillus kefir*, *Lactococcus lactis*, *Streptococcus suis*, *S. thermophilus* and *Lentilactobacillus parabuchneri* [31].

Merchán et al. [32] reported that the 149 yeast from Extremadura region traditional cheese, identified by ISSR-PCR technique included *D. hansenii*, *D. vindobonensis*, *K. marxianus*, *K. lactis*, *C. cabralensis*, *C. pararugosa*, *C. zeylanoides*, *Y. lipolytica*, *P. fermentans*, *P. cactophila*, *P. kudriazevii* and *P. jadinii*. *K. lactis* and *P. fermentans* were characterized by tolerance to the most restrictive stomach and bile salts stress conditions. *P. fermentans* was characterized by antimicrobial, antioxidant, hydrophobicity and auto-aggregation ability. *Kluyveromyces* spp. strains posed antimicrobial activity. The studied traditional cheese also contained strains with probiotic features as different strains of *P. fermentans*, *P. kudriazevii*, *K. marxianus*, *D. hansenii*, *Y. lipolytica*.

The turkish artisanal cheese Tulum is a semi-hard cheese, ripened in goat skin and characterized by a unique flavour and microbial diversity. The texture is porous thus permeable to air and water. The microorganisms identified by 16 S rRNA sequencing from this cheese during ripening included: *Enterococcus faecium*, *E. hirae*, *E. durans*, *E. faecalis*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus brevis*, *Lactococcus lactis*, *Streptococcus gallolyticus*, *Weissella paramesenteroides*, *Eubacterium rangiferina*. During ripening the level of Lactococci and Streptococci decreased. At the end of the maturation the DGGE profile of the bacterial community formed from *Streptococcus gallolyticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus lutetiensis*, *Streptococcus* spp., and *Enterococcus hirae*. At the end of the maturation, the most prevalent species were *Enterococcus* spp. and *Streptococcus* spp. [33].

The MALDI-TOF MS method resulted that greek artisanal cheese non-starter lactic acid bacterial community consisted of *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Pediococcus pentosaceus* and *Enterococcus faecium*. The first two were the most predominant species. This type of cheese is obtained from raw sheep and goat milk without added starter cultures [34].

The PDO Robiola di Roccaverano soft Italian cheese is processed from raw goat milk with natural starters. From fresh and matured cheese samples the following microorganisms were identified *Lactococcus lactis* subsp. *cremoris*, *Lactococcus*

lactis subsp. *lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, *Lactobacillus brevis*, *Leuconostoc citreum*) and microscopic fungi (*Geotrichum candidum*, *Kluyveromyces marxianus*, *Saturnispora silvae*, *Yarrowia lipolytica*, *Kluyveromyces lactis*, *Trichosporon coremiiforme* [35].

Kashkaval is an artisanal pasta filata cheese, obtained from sheep's raw milk without added starters, but now days appeared the industrialized form of this cheese using pasteurised milk with an added commercial starter. Microbiology studies of this raw milk cheese revealed that the dominant bacteria were the mesophilic non-starter lactobacilli and enterococci. In the pasteurized milk cheese *Lactococcus lactis* starter were dominant [36].

Nicastrese goat's cheese is a Southern Italian traditional product obtain from raw goat milk coagulated with lamb rennet, without any added starter cultures. The local aromatic Mediterranean plants impart unique sensorial characteristics to the milk. The typical flavour of this type of cheese is resulted from the macro-nutrient transformations during the maturation time. The autochthonous microbiota is responsible for the biochemistry of ripening leading to unique sensorial features. The following bacteria were detected from Nicastrese goat's cheese samples: *Lactococcus lactis* subsp. *lactis*, *Lactobacillus plantarum*, *Enterococcus faecalis*, *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Enterococcus faecium*, *Lactococcus garvieae*, *Enterococcus durans*, *Enterococcus hirae* and *Streptococcus thermophilus* [37].

The southern Mexican Poro de Tabasco cheese is an artisanal product obtained from raw whole cow's milk and with natural whey starters. The unique character is attributed to salting, pressing process and the use of a paraffin coating. It is a hard fresh type of cheese with full-fat content. Dominant bacteria strains of this type of cheese include *Lactobacillus* spp., *Lactococcus* spp., and *Streptococcus* spp.. The microbial level it was reduced during the processing. The final products were free of *E. coli*, *Salmonella* spp., and *L. monocytogenes*. Meanwhile, *S. aureus* and its toxins were detected in few samples. This could be eliminated with heat treatment and good manufacturing practices [38].

11 artisanal Irish cheeses (soft, hard, and semi hard) microbiome analysis by high throughput DNA sequencing revealed the presence of *Lactococcus*, *Leuconostoc*, and *Lactobacillus*. *Corynebacterium*, *Facklamia*, *Flavobacterium*, and *Cronobacter* were found in rind cheeses. The lactococci proportion were higher in naturally ripened rinds compared to smear and washed rinds [39].

A traditional fried cottage cheese contained strains of *Galactomyces geotrichum* molds responsible for the production of polyunsaturated fatty acids. This microscopic fungi is part of natural microflora and might have a role in omega-3 lipid production in foods and cheese [40].

The predominant bacteria of Fresh ovine cheese were represented by *Lactococcus lactis* strains added as starter culture. The characteristic of this type of cheese is given by the saffron (*Crocus sativus* L.) applied as additive. The total and lactococci bacteria level were high after storage of the product. Due to saffron content the cheese possesses antibacterial activity toward coliforms and enterococci [41].

The Calabrian (Italy) artisanal cheese lactic acid bacteria possess antibacterial effect. This group of bacteria contributed to the growth reduction of *L. monocytogenes* in vitro conditions [42].

The component of the microbiota of cheeses is influenced by the manufacture conditions as the used cheese making vessels. Generally, in artisanal cheese production, the wood is a representing material, that is a natural reservoir for microorganisms. It was showed that in these circumstances the lactic acid bacteria level is high.

In the maturation process the shelves made from wood also are responsible for appearance of coryneform bacteria, moulds, and yeasts [17].

Gram-negative bacteria strains belonging to different families were detected on the surface of matured soft cheese. These were: *Enterobacteriaceae*, *Moraxellaceae*, *Pseudoalteromonadaceae*, *Pseudomonadaceae*, *Sphingobacteriaceae* and *Vibrionaceae* spp. The Gram-negative bacteria were attributed to undesirable and defective texture and flavour. This defects resulted from extracellular proteolytic and lipolytic enzymes activities of bacteria. An example is the occurrence of *Pseudomonas* spp. that influence negatively the organoleptic characteristics of cheese due to release of ethyl esters and alcohols. It is supposed that the Gram-negative bacteria belonging to natural microbiome affecting positively the product technological and sensorial features. A strain of *Proteus* spp. closely related to *P. hauseri* and strains of *Psychrobacter* spp. have been contributed to flavour development of smear type cheeses. In French traditional cheeses were detected Gram-negative bacterial strains with double effect. There were bacteria strains characterised by no tolerance to acidic conditions in simulated gastro-intestinal stress, to human serum and showed no adherence to Caco-2 cell and were no virulence. In contrast to *Hafnia paralvei* 920, *Proteus* spp. (close to *P. hauseri*) UCMA 3780, *Providencia heimbachae* GR4, and *Morganella morganii* 3A2A that were toxic in *in vitro* conditions possessing virulence factors [43].

6. Occurrence of allochthonous microorganisms in traditionally processed cheese

The detection of allochthonous bacteria and microscopic fungi in raw milk cheese raise food safety issues because those products can harbour diverse spoilage and pathogenic microbes. We evaluated the microbial quality of 13 traditionally processed cheese from northeastern region of Transylvania. The processed cheese are made from unpasteurized cow and sheep milk. It is considered that the milk quality is affected by grazed grass, the special feeding strategy in that region.

The detection of the various pathogen bacteria was carried out by cultivation methods on different selective media: for the detection and enumeration of *Escherichia coli* TBX Chromo Agar was used, ChromoBio[®] Coliform agar was applied for the detection of coliforms, for the detection of *Salmonella* spp. Brilliance[™] Salmonella Agar Base was used, for the detection of *Shigella* spp. Salmonella Shigella (SS) Modified Agar was applied, for the detection of *Campylobacter jejuni* Campylobacter Blood-Free Selective Agar Base Modified CCDA was used, for *Yersinia enterocolitica* detection the Yersinia Selective Agar Base was used, for *Listeria monocytogenes* detection Listeria mono Differential Agar Base was used, for *Bacillus cereus* detection ChromoBio[®] Cereus Base was used, for *C. perfringens* Clostridial Differential Broth was used, for the detection of *Pseudomonas aeruginosa* Pseudomonas Isolation Agar Base was applied. The detection of microscopic fungi was carried out by spread plate method on ChromoBio[®] Candida and Czapek Dox Agar [44, 45].

Isolation of the most representative bacteria with high count and characteristic colony morphology was followed by identification of strains on species-level by 16 rDNA sequencing method. Genomic DNA isolation of isolated bacterial strains from the selective mediums was realized with AccuPrep[®] Genomic DNA Extraction Kit from Bioneer, according to the manufacturer's protocol. A part of the bacterial 16S rDNA gene was amplified with universal primers 27f and 1492r (5' AGAGTTTGGATCMTGGCTCAG 3', 5' TACGGYTACCTTGTTACGACTT 3').

The PCR products were purified using *GeneJET PCR Purification Kit* (Thermo Scientific, K070) and sequencing was carried out [46].

Our results of the microbiological quality of the assayed artisanal cheese showed diverse values. The highest total count of coliforms, *Escherichia coli* and *Salmonella* spp. was detected in the cheese 7 sample (**Table 1**). The mean number of *Campylobacter jejuni* was 10^3 CFU/g and 10^2 – 10^3 CFU/g in the case of *Yersinia enterocolitica*. The highest level was detected in the case of cheese 10 sample $8 \cdot 10^4$ CFU/g *C. jejuni* and $4.1 \cdot 10^4$ CFU/g *Y. enterocolitica*. The highest number of *Listeria monocytogenes* was detected in cheese 7 sample $7.5 \cdot 10^5$ CFU/g, in case of *Staphylococcus aureus* the highest count was $1.9 \cdot 10^7$ CFU/g in sample cheese 6. The highest level of *Bacillus cereus* was $2 \cdot 10^5$ CFU/g in sample cheese 7 (**Table 1**). The *Clostridium perfringens* was detected in two samples cheese 3, cheese 8 samples.

The count of microscopic filamentous fungi in the assayed samples obtained by us varied 10^2 – 10^3 CFU/g. The highest load was found in cheese 4 sample $5 \cdot 10^4$ CFU/g. The maximum value detected in the case of yeasts was $4.5 \cdot 10^5$ CFU/g in cheese 9 sample (**Table 2**). The developed filamentous fungi belonged to *Aspergillus* and *Penicillium* genera (**Figure 1**).

The identified 27 bacterial strains isolated on various selective media according to 16S rDNA sequence analysis include: *Stenotrophomonas maltophilia*, *Escherichia coli*, *Staphylococcus equorum* subsp. *equorum*, *Staphylococcus equorum* subsp. *linens*, *Klebsiella michiganensis*, *Halomonas alkaliphila*, *Proteus vulgaris*, *Shigella flexneri*, *Enterococcus durans*, *Enterococcus faecalis*, *Bacillus licheniformis*, *Kocuria rhizophila*, *Hafnia paralvei* (**Figure 2**).

The occurrence of *Stenotrophomonas maltophilia* in cheese samples originated from different regions of France was reported by Coton et al. 2012 [47]. This multidrug-resistant bacteria causing nosocomial infections, can be found in different environments as aquatic or humid, in natural soil or plant rhizosphere [48, 49]. In immunocompromised patients this bacteria was associated with pneumonia and bacteremia. Also can cause fatal hemorrhagic pneumonia. It has been found that the *StmPr1* gene is involved in pathogenicity [48]. Biofilm formation (the presence of genes *rpfF* and *smf-1* genes) was considered that play role in virulence [50].

In dairy products the presence of *Escherichia coli* indicates fecal contamination. It was detected in Brazilian raw milk that could be appear in Minas Frescal-type cheeses produced from raw milk. The risk could be eliminated with the heat treatment of milk [51]. Metz et al. 2020 [52] reported that *E. coli* and coliforms (detected in raw milk) number, rise during the early steps of processing. At the end of the cheese making, including fermentation and ripening, their level decline by numerous logs or the final product is free of this bacteria. Contaminated raw milk of domestic animals and dairy products could be the reservoir of verotoxin-producing *Escherichia coli* (VTEC). This bacteria can cause severe infections also. Outbreaks of *Escherichia coli* O157:H7 was related to contaminated raw milk. It was reported that *E. coli* O157 was growth in initial phase of preparation of Fontina PDO cheese. The heat treatment of curd cooking not affected the bacteria. Only during ripening decreased the level of *E. coli*. It was considered that the a_w reduction and the lactic acid could contribute to the reduction of *E. coli* O157 level [53]. In Cacioricotta goat cheese *E. coli* O157:H7 could survive after 90 days of ripening. This fact gives reason for that the low level contaminated raw milk represents a threat to consumers [54].

The plasmid-mediated colistin resistance gene in *E. coli* from hard cheese represents a high risk factor because cheese is consumed without heat treatment [55]. During manufacturer process of raw milk cheese only the low temperature conditions of maturation affected the growth negatively of shigatoxigenic *E. coli* and *L. monocytogenes* [56].

Cheese sample	<i>Escherichia coli</i>	Coliforms	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Campylobacter jejuni</i>	<i>Yersinia enterocolitica</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>
Cheese 1	$2.7 \cdot 10^2$	$2.4 \cdot 10^4$	1·10	<10	$7.6 \cdot 10^3$	$5.4 \cdot 10^2$	$1.9 \cdot 10^4$	$2.9 \cdot 10^3$	<10
Cheese 2	<10	$9 \cdot 10^3$	6·10	<10	<10	4·10	$2 \cdot 10^5$	$9.9 \cdot 10^6$	$1.5 \cdot 10^5$
Cheese 3	$9 \cdot 10$	$8 \cdot 10^2$	$1.3 \cdot 10^2$	<10	$2.5 \cdot 10^3$	<10	$9.1 \cdot 10^3$	$1.15 \cdot 10^3$	<10
Cheese 4	<10	<10	<10	<10	$1.3 \cdot 10^3$	$1 \cdot 10^2$	$3.4 \cdot 10^4$	$5.1 \cdot 10^5$	<10
Cheese 5	<10	<10	<10	<10	<10	<10	$1 \cdot 10^3$	$5 \cdot 10^4$	$1 \cdot 10^3$
Cheese 6	$1.2 \cdot 10^3$	<10	$4.6 \cdot 10^2$	<10	$5 \cdot 10^3$	$7.2 \cdot 10^3$	$4 \cdot 10^3$	$1.9 \cdot 10^7$	$2 \cdot 10^4$
Cheese 7	$6.8 \cdot 10^3$	$8 \cdot 10^4$	$3.2 \cdot 10^4$	<10	$2.6 \cdot 10^3$	$1 \cdot 10^2$	$7.5 \cdot 10^5$	$1.22 \cdot 10^5$	$2 \cdot 10^5$
Cheese 8	<10	<10	<10	<10	$1.8 \cdot 10^3$	$1.57 \cdot 10^3$	$7.3 \cdot 10^4$	$1.55 \cdot 10^7$	<10
Cheese 9	<10	$2.6 \cdot 10^4$	<10	<10	7·10	$2.79 \cdot 10^2$	$8.1 \cdot 10^3$	$1 \cdot 10^3$	$4 \cdot 10^5$
Cheese 10	<10	$2 \cdot 10^2$	<10	<10	$8 \cdot 10^4$	$4.1 \cdot 10^4$	$2.51 \cdot 10^3$	$3 \cdot 10^2$	<10
Cheese 11	$1 \cdot 10^2$	$2 \cdot 10^2$	<10	<10	$1 \cdot 10^4$	<10	$5.6 \cdot 10^3$	$1 \cdot 10^3$	$9.1 \cdot 10^4$
Cheese 12	$1 \cdot 10^3$	$1.7 \cdot 10^3$	<10	<10	3·10	<10	$9.6 \cdot 10^3$	$4.2 \cdot 10^3$	<10
Cheese 13	<10	<10	<10	<10	<10	<10	$3.8 \cdot 10^2$	$7 \cdot 10^2$	$2 \cdot 10^3$

Table 1.
Microbial load of cheese samples (CFU/g).

Cheese sample	Molds	Candida spp.
Cheese 1	4.3·10 ²	<10
Cheese 2	7·10 ²	<10
Cheese 3	3.5·10 ³	<10
Cheese 4	5·10 ⁴	<10
Cheese 5	9·10 ³	<10
Cheese 6	2.4·10 ⁴	3.5·10 ³
Cheese 7	1.2·10 ²	1.3·10 ²
Cheese 8	9·10 ²	7.3·10 ²
Cheese 9	7·10 ²	4.5·10 ⁵
Cheese 10	7·10 ²	<10
Cheese 11	5·10	3.19·10 ⁵
Cheese 12	0	3.75·10 ³
Cheese 13	0	2.89·10 ³

Table 2.
Occurrence of microscopic fungi in the analyzed cheese samples (CFU/g).

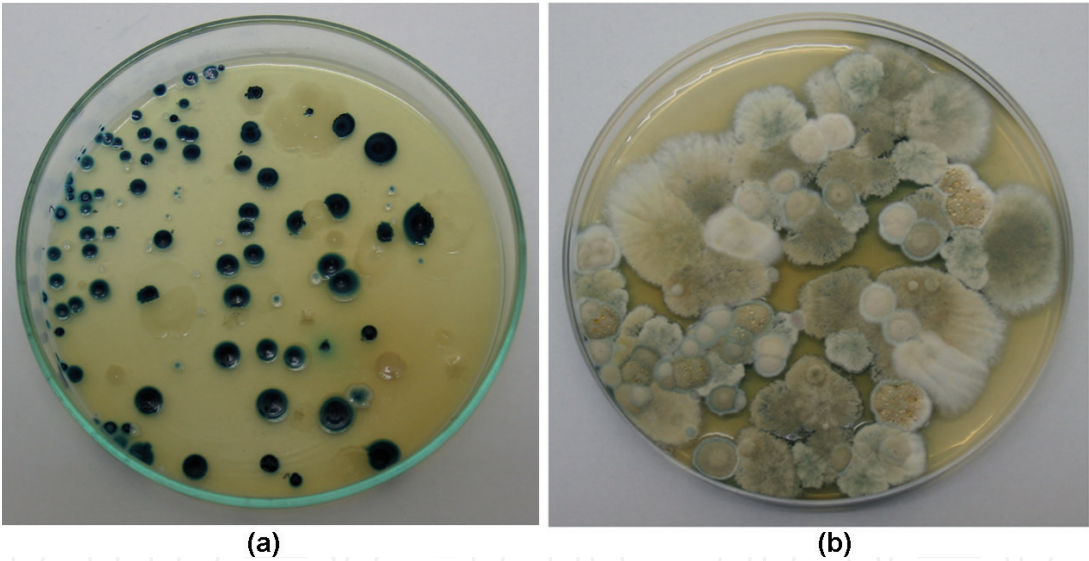


Figure 1.
Colonies of aerobic spore-forming bacteria from cheese 6 sample (A) and microscopic fungi from cheese 10 sample (B) on selective agar plates.

One of the predominant bacteria from staphylococci is *S. equorum* in cheeses owing antilisterial activity. *S. equorum* and *S. equorum* subsp. *linens* was mentioned as potential new starter culture [57]. *S. equorum* also characterized by high salinity tolerance (up to 25%) was the predominant species of the long maturation processes.

In traditional fermented foods staphylococci were the dominant species. These bacteria are involved in the development of sensorial features. *Staphylococcus carnosus*, *S. xylosus* and *S. equorum* with the production of low molecular-weight compounds, including aldehydes, amines, amino acids esters and free fatty acids take part in flavor development. This is possible due to various biochemical transformations as reduction of nitrates to nitrite and then to nitrous oxide, proteolysis and lipolysis [58]. With DNA–DNA hybridization method it was revealed that

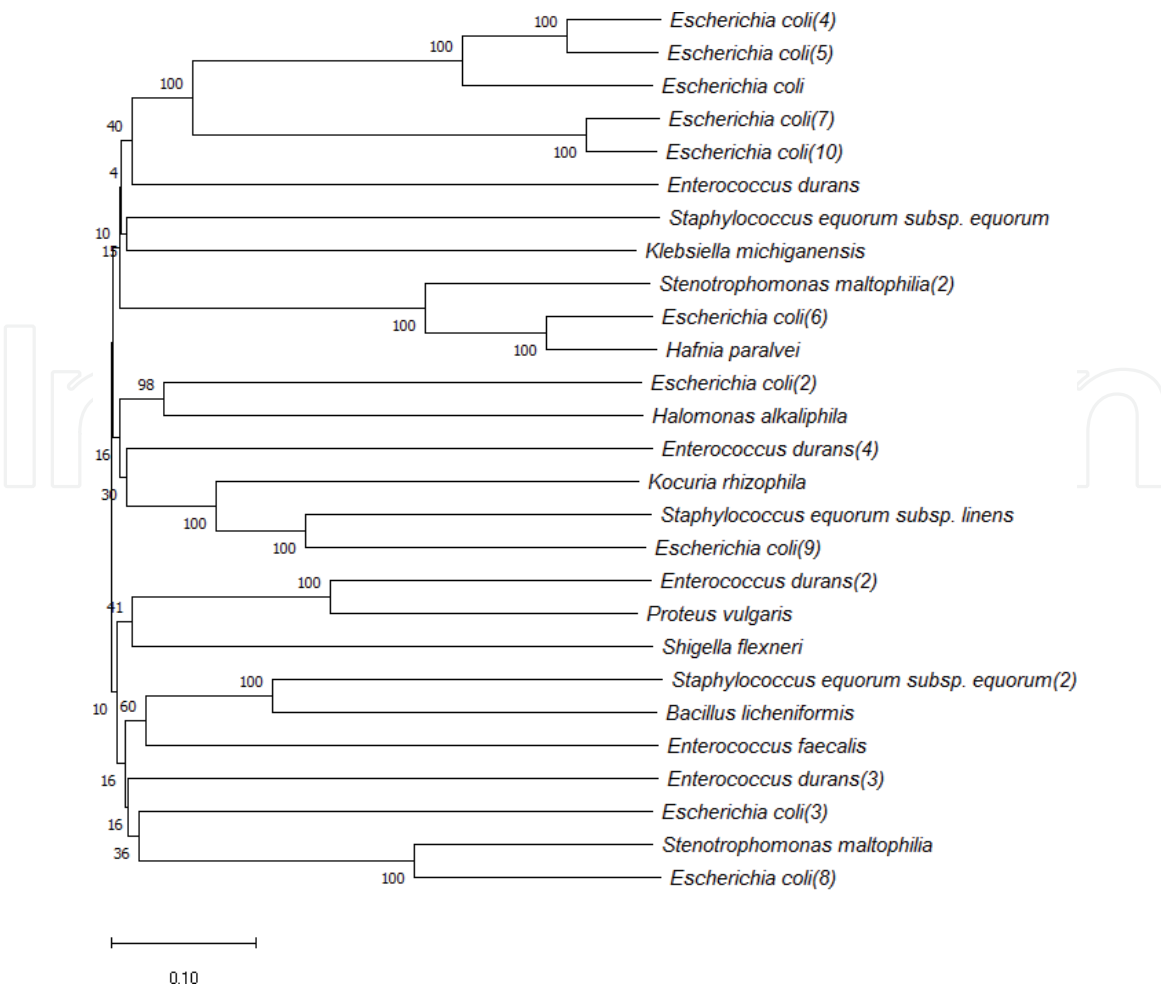


Figure 2.
Phylogenetic tree based on similarities of 16S rDNA partial sequences of bacterial isolates using the neighbor-joining method.

Staphylococcus equorum subsp. *linens* subsp. nov. and *Staphylococcus equorum* subsp. *linens* was part of the dominant microflora of surface ripened Swiss mountain raw milk cheese. Characteristics of these two strains include the ability to grow between 6 and 40°C and in salinity conditions 13% NaCl [59].

Based on literature data *Klebsiella michiganensis* originated from traditionally processed cheese is considered an emerging pathogen, causing nosocomial and bloodstream infections. It was isolated from hospital samples. Different virulence factors were identified in this bacteria as capsule locus type, plasmid replicon types, cell adherence and iron permitting acquisition. *K. michiganensis* is a multidrug resistant bacteria harboring (KPC)-3 carbapenemase gene, carbapenemase gene *bla*OXA-181 and other 11 genes encoding different antibiotic resistance. The carbapenemase gene encoding plasmid is originated from *Klebsiella pneumoniae* [60, 61]. Mitra et al. [62] reported that *K. michiganensis* strain S8 possess cadmium accumulating capacity contributing to enhance the productivity of rice.

The other identified bacteria from cheese was *Halomonas alkaliphila*. According to Sun et al. [63] is a halophilic proteobacteria with role in nitrogen cycle of marine environments, converting inorganic nitrogen compounds.

It was reported that *Proteus vulgaris* potentially could be applied to enhance the flavor based on the production of widest varieties and the largest quantities of volatile compounds on the surface of a smear-ripened cheese [64]. In surface-ripened cheeses proteobacteria as *Proteus* are responsible for the development of purple pigmentation of cheese rinds [65].

Shigella flexneri is known as a human pathogen infecting the colonic epithelial cells. With reduction of intercellular tension is able to spread between cells [66].

Jamet et al. [67] reported that enterococci take part of the dominant microflora of raw milk cheeses ranging from 10^2 to 10^8 CFU/g. There are contradictory aspects regarding the *Enterococcus* lactic acid bacterial strains. It was considered that these strains of bacteria are capable of causing diseases. Studies are showing that *Enterococcus* bacterial strains are promising starter cultures owing relevant technological and probiotic characteristics. *E. durans* LM01C01 with beneficial characteristics in cream cheeses lowered the *S. aureus* contamination level [68]. *Enterococcus durans* LAB18s possess probiotic potential, antimicrobial, antioxidant characteristics and selenium bioaccumulation ability. The culture supernatant of aforementioned bacteria exhibited inhibitory effect against *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Corynebacterium fimi*. The intracellular extract of *E. durans* LAB18s showed antibacterial effect against *L. monocytogenes*, *E. coli*, *B. cereus*, *Staphylococcus aureus*, *S. enteritidis*, *Ps. aeruginosa*, *Ae. hydrophila* and *Corynebacterium fimi* [69].

Enterococcus durans SJRP29 originated from cheese is thought to be an advantageous safe strain with practical use due to the beneficial characteristics (tolerance to 3% NaCl, good adhesion and without bile salt hydrolase activity or mucin degradation) [70]. Enterococci are involved in antibiotic resistance gene transfer among non-pathogenic and pathogenic bacteria. It has been reported that the predominant antibiotic resistant enterococci in French cheeses were *E. faecalis*, *E. faecium* and *E. durans*. Antibiotic resistance was showed against tetracycline, minocycline, erythromycin, kanamycin and chloramphenicol [67]. *L. monocytogenes* and *E. faecalis* were true cases of horizontal gene transfer in Minas Frescal cheese [71]. In another study it was concluded that antibiotic resistance and virulence factors can be transferred with high rate during cheese and sausage fermentation to enterococci [72].

B. licheniformis due to high resistance of environmental factors and heat treatments is involved in the biofilm formation in milk processing plants, but this can be avoiding with the pH and milk residues control [73]. *B. licheniformis* is a food spoilage and poisoning bacteria causing serious diseases as enteric disease, septicemia, peritonitis, ophthalmitis [74]. Also these spore forming bacteria are promising probiotic strains. It was reported that *Bacillus* strains could survive the harsh environment of Requeijão cremoso Brazilian cheese [75].

Kocuria as a non-pathogenic bacteria is part of skin microbiota. Several studies reported that it was associated with peritonitis and urinary tract infection. Recently it has been reported that *Kocuria rhizophila* is causing catheter-related bacteremia with endocarditis [76].

The genus *Hafnia* belonging to Enterobacteriaceae comprise bacterial strain predominant in nature, gastrointestinal tracts of mammals and also in food products. *Hafnia* sp. and *Enterococcus faecalis* was associated with cholecystitis [77].

7. Conclusions

The microflora of traditionally processed cheese comprise of diverse and heterogeneous group of microbes. These microorganisms have specific role in product formation providing the unique characteristics of the cheese. Also, it could appear microorganisms that are emerging pathogens. For these reason in traditional manufacture process have to pay attention to personal hygiene and sanitation process. The support of autochthonous bacteria in fermented products have to be a priority to contribute to the preservation of the organoleptic characteristics typical of that geographical region.

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References

- [1] Nacef M, Lelièvre-Desmas M, Drider D, Flahaut C, Chollet S. Artisanal and industrial Maroilles cheeses: Are they different? Comparison using sensory, physico-chemical and microbiological Approaches. *International Dairy Journal*. 2019; **89**:42-52
- [2] Cogan TM, Microbiology of cheeses, 2011. In: *Encyclopedia of Dairy Sciences* (Second Edition), Fuquay JW (ed.), Academic Press, pp. 625-631
- [3] Banjara N, Suhr MJ, Hallen-Adams HE. Diversity of yeast and mold species from a variety of cheese types. *Current Microbiology*. 2015; **70**(6):792-800
- [4] Pintado M, Gomes da Cruz A, Rodrigues de Sá PBZ. Cheese Microbiology, 2015. In: *Dairy Microbiology and Biochemistry Recent Developments*, Özer BH, Akdemir-Evrendilek G (eds.), CRC Press, Boca Raton, pp. 113-116
- [5] Araújo-Rodrigues H, Tavaría FK, dos Santos MTPG, Alvarenga N, Pintado MM. A review on microbiological and technological aspects of Serpa PDO cheese: An ovine raw milk cheese. *International Dairy Journal*. 2020; **100**, 104561
- [6] Guinee TP. Salt in cheese, 2007. In: *Cheese problems solved*, McSweeney PLH (ed.), CRC Press, Cambridge, pp. 80-99
- [7] Hayaloglu AA. Cheese: Microbiology of cheese. 2016, Reference Module in Food Science.
- [8] Schlei, K.P., Angioletti, B.L., de Carvalho, L.F., Bertoli, S.L., Reiter, M.G.R., de Souza, C.K., Influence of temperature on microbial growth during processing of kochkäse cheese made from raw and pasteurised milk, *International Dairy Journal*, 2020, **109**, 104786
- [9] Muruzović MŽ, Mladenović KG, Čomić LR. In vitro evaluation of resistance to environmental stress by planktonic and biofilm form of lactic acid bacteria isolated from traditionally made cheese from Serbia. *Food Bioscience*. 2018; **23**: 54-59
- [10] Altieri C, Ciuffreda E, Di Maggio B, Sinigaglia M. Lactic acid bacteria as starter cultures. *Starter Cultures in Food Production*. 2017; 1-15
- [11] Parente E, Cogan TM, Powell IB. Starter Cultures: General Aspects. *Cheese*. 2017; 201-226
- [12] Buzzini P, Di Mauro S, Turchetti B. Yeasts as starter cultures. *Starter Cultures in Food Production*. 2017; 16-49
- [13] Dantigny P., Bevilacqua A. Fungal starters. *Starter Cultures in Food Production*. 2017; 50-63
- [14] Lourenco A, Fraga-Corral M, De Colli L, Moloney M, Danaher M, Jordan K. Determination of the presence of pathogens and anthelmintic drugs in raw milk and raw milk cheeses from small scale producers in Ireland. *LWT - Food Science and Technology*. 2020; **130**, 109347
- [15] Fox PF, Guinee TP, Cogan TM, McSweeney PLH. *Fundamentals of Cheese Science*, 2nd Edition, Springer, New York. 2017; pp:113, 373-381
- [16] O'Sullivan O, Cotter PD. Microbiota of Raw Milk and Raw Milk Cheeses, 2017. In: *Cheese. Chemistry, Physics & Microbiology*, vol.1., General Aspects, 4th Edition, McSweeney PLH, Fox PF, Cotter PD, Everett DW (ed.), Academic Press, London, pp. 301-310

- [17] Gagnon M, Hamelin L, Fréchette A, Dufour S, Roy D. Effect of recycled manure solids as bedding on bulk tank milk and implications for cheese microbiological quality. *J. Dairy Sci.* 2020; **103**:128-140
- [18] Montel M-C, Buchin S, Mallet A, Delbes-Paus C, Vuitton DA, Desmasures N, Berthier F. Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology.* 2014; **177**: 136-154
- [19] Centi V, Matteucci F, Lepidi A, Del Gallo M, Ercole C. Microbiological and biochemical aspects of inland Pecorino Abruzzese cheese. *Heliyon.* 2017; **3**, e00258.
- [20] Gobetti M, Neviani E, Fox P. *The Cheeses of Italy: Science and Technology.* 2018. Springer, Cham, pp: 68-70
- [21] Gaglio R, Franciosi E, Todaro A, Guarcello R, Alfeo V, Randazzo CL, Settanni L, Todaro M. Addition of selected starter/non-starter lactic acid bacterial inoculums to stabilise PDO Pecorino Siciliano cheese production. *Food Research International.* 2020; **136**, 109335
- [22] Margalho LP, Feliciano MD, Silva CE, Abreu JS, Piran MVF, Sant'Ana AS. Brazilian artisanal cheeses are rich and diverse sources of nonstarter lactic acid bacteria regarding technological, biopreservative, and safety properties—Insights through multivariate analysis. *J. Dairy Sci.* 2020; **103**:7908-7926
- [23] Campagnollo FB, Margalho LP, Kamimura BA, Feliciano MD, Freire L, Lopes LS, Alvarenga VO, Cadavez VAP, Gonzales-Barron U, Schaffner DW. Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional Brazilian cheeses. *Food Microbiology.* 2018; **73**:288-297
- [24] Margalho LP, Jorge GP, Noleto DAP, Silva CE, Abreu JS, Piran MVF, Brocchi M, Sant'Ana AS. Bio preservation and probiotic potential of a large set of lactic acid bacteria isolated from Brazilian artisanal cheeses: From screening to *in product* approach. *Microbiological Research.* 2021; **242**, 126622
- [25] Jin H, Mo L, Pan L, Hou Q, Li C, Darima I, Yu J. Using PacBio sequencing to investigate the bacterial microbiota of traditional Buryatian cottage cheese and comparison with Italian and Kazakhstan artisanal cheeses. *J. Dairy Sci.* 2018; **101**:6885-6896
- [26] Galiou OE, Zantar S, Bakkali M, Laglaoui A, Centeno JA, Carballo J. Chemical and microbiological characteristics of traditional home made fresh goat cheeses from Northern Morocco. *Small Ruminant Research.* 2015; **129**: 108-113
- [27] Sánchez I, Seseña S, Poveda JM, Cabezas L, Palop L. Genetic diversity, dynamics, and activity of *Lactobacillus* community involved in traditional processing of artisanal Manchego cheese. *International Journal of Food Microbiology.* 2006; **107**:265-273
- [28] Araújo-Rodrigues H, Tavaría FK, dos Santos MTPG, Alvarenga N, Pintado MM. A review on microbiological and technological aspects of Serpa PDO cheese: An ovine raw milk cheese. *International Dairy Journal.* 2020; **100**, 104561
- [29] Banihashemi SA, Nikoo M, Ghasempour Z, Ehsani A. Bioactive peptides fractions from traditional Iranian Koopeh cheese; lactic fermentation products. *Biocatalysis and Agricultural Biotechnology.* 2020; **29**, 101798

- [30] Gonzalez-Gonzalez CR, Machado J, Correia S, McCartney AL, Elmore JS, Jauregi P. Highly proteolytic bacteria from semi-ripened Chiapas cheese elicit angiotensin-I converting enzyme inhibition and antioxidant activity. *LWT - Food Science and Technology*. 2019; **111**:449-456
- [31] Levante A, Bertani G, Bottari B, Bernini V, Lazzi C, Gatti M, Neviani E. How new molecular approaches have contributed to shedding light on microbial dynamics in Parmigiano Reggiano cheese. *Current Opinion in Food Science*. 2021; **38**:131-140
- [32] Merchán AV, Benito MJ, Galván AI, de Herrera SR-MS. Identification and selection of yeast with functional properties for future application in soft paste cheese. *LWT - Food Science and Technology*. 2020; **124**, 109173
- [33] Dermici T, Akin N, Atik DS, Özkan ER, Dertli E, Akyol İ. Lactic acid bacteria diversity and dynamics during ripening of traditional Turkish goatskin Tulum cheese produced in Mut region assessed by culturing and PCR-DGGE. *LWT - Food Science and Technology*. 2021; **138**, 110701
- [34] Gantzias C, Lappa IK, Aerts M, Georgalaki M, Manolopoulou E, Papadimitrou K, De Brandt E, Tsakalidou E, Vandamme P. MALDI-TOF MS profiling of non-starter lactic acid bacteria from artisanal cheeses of the Greek island of Naxos. *International Journal of Food Microbiology*. 2020; **323**, 108586
- [35] Biolcati F, Andrighetto C, Bottero MT, Dalmaso A. Microbial characterization of an artisanal production of Robiola di Roccaverano cheese. *Journal of Dairy Science*. 2020; **103** (5): 4056-4067
- [36] Pappa EC, Kondyli E, Samelis J. Microbiological and biochemical characteristics of Kashkaval cheese produced using pasteurised or raw milk. *International Dairy Journal*. 2019; **89**, 60-67
- [37] Pino A, Liotta L, Randazzo CL, Todaro A, Mazzaglia A, De Nardo F, Chiofalo V, Caggia C. Polyphasic approach to study physico-chemical, microbiological and sensorial characteristics of artisanal Nicastrese goat's cheese. *Food Microbiology*. 2018; **70**: 143-154
- [38] De la Rosa-Alcaraz M de IÁ, Ortiz-Estrada ÁM, Heredia-Castro PY, Hernández-Mendoza A, Reyes-Díaz R, Vallejo-Cordoba B, González-Córdova AF. Poro de Tabasco cheese: Chemical composition and microbiological quality during its artisanal manufacturing process. *J. Dairy Sci*. 2020; **103** (4): 3025-3037
- [39] Jonnala BRY, McSweeney PLH, Sheehan JJ, Cotter PD, Sequencing of the cheese microbiome and its relevance to industry. *Frontiers in Microbiology*. 2018; **9**, 1020
- [40] Grygier A, Myszka K, Juzwa W, Bialas W, Rudzińska M. *Galactomyces geotrichum* mold isolated from a traditional fried cottage cheese produced omega-3 fatty acids. *International Journal of Food Microbiology*. 2020; **319**, 108503
- [41] Aktypis A, Christodoulou ED, Manolopoulou E, Georgala A, Daferera D, Polysiou M. Fresh ovine cheese supplemented with saffron (*Crocus sativus* L.): Impact on microbiological, physicochemical, antioxidant, colour and sensory characteristics during storage. *Small Ruminant Research*. 2018; **167**:32-38
- [42] Panebianco F, Giarratana F, Caridi A, Sidari S, De Bruno A, Giuffrida A. Lactic acid bacteria isolated from traditional Italian dairy products: activity against *Listeria monocytogenes* and modelling of microbial competition

in soft cheese. LWT - Food Science and Technology. 2021; **137**, 110446

[43] Imran M, Desmasure N, Coton M, Coton E, Le Flèche-Matéos A, Irlinger F, Delbès-Paus C, Stahl V, Montel M-C, Vernoux J-P. Safety assessment of Gram-negative bacteria associated with traditional French cheeses. Food Microbiology. 2019; **79**: 1-10

[44] Dunca S, Ailiesei O, Nimițan E, Ștefan M. Microbiologie aplicată, Editura Tehnopress, Iași, pp. 246-253.

[45] Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union 322/13 7.12.2007.

[46] Laslo É, Mara Gy, Funkenhauzer B, Dobri E, Salamon R.V., Lányi Sz, Ábrahám B. Isolation and screening of lactic acid bacteria from naturally fermented sources with high biotechnological potential. Studia UBB Chemia. 2015; **4**: 95-106.

[47] Coton, M, Delbès-Paus C, Irlinger F, Desmasure N, Le Flèche A, Stahl V, Montel M-C, Coton E. Diversity and assessment of potential risk factors of Gram-negative isolates associated with French cheeses. Food Microbiology. 2012; **29**: 88-98

[48] Imoto W, Kaneko Y, Yamada K, Kuwabara G, Yamairi K, Wataru Shibata W, Oshima K, Niki M, Nakaie K, Watanabe T, Asai K, Kawaguchi T, Kakeya H. A mouse model of rapidly progressive fatal haemorrhagic pneumonia caused by *Stenotrophomonas maltophilia*. Journal of Global Antimicrobial Resistance. 2020; **23**:450-455

[49] Ma J, Feng J, Shan Y, Zhao Y, Qiao H, Xie L, Lin X, Wang C, Chuai X. Characteristic antimicrobial resistance of clinically isolated *Stenotrophomonas*

maltophilia CYZ via complete genome sequence. Journal of Global Antimicrobial Resistance. 2020, **23**:186-193

[50] Azimi A, Aslanimehr M, Yaseri M, Shadkam M, Douraghi M. Distribution of *smf-1*, *rmlA*, *spgM* and *rpff* genes among *Stenotrophomonas maltophilia* isolates in relation to biofilm-forming capacity. Journal of Global Antimicrobial Resistance. 2020; **23**:321-326

[51] Ribeiro Júnior JC, Silva FF, Lima JBA, E. H. Ossugui EH, Teider Junior PI, Campos ACLP, Navarro A, Tamanini R, Ribeiro J, Alfieri AA, Beloti V. Molecular characterization and antimicrobial resistance of pathogenic *Escherichia coli* isolated from raw milk and Minas Frescal cheeses in Brazil. J. Dairy Sci. 2019; **102**:10850-10854

[52] Metz M, Sheehan J, Feng PCH. Use of indicator bacteria for monitoring sanitary quality of raw milk cheeses – A literature review. Food Microbiology. 2020; **85**, 103283

[53] Bellio A, Bianchi DM, Vitale N, Vernetti L, Gallina S, Decastelli L. Behavior of *Escherichia coli* O157:H7 during the manufacture and ripening of Fontina Protected Designation of Origin cheese. J. Dairy Sci. 2018; **101**:4962-4970

[54] Ioanna F, Quaglia NC, Storelli MM, Castiglia D, Goffredo E, Storelli A, De Rosa M, Normanno G, Caputi Jambrenghi A, Dambrosio A. Survival of *Escherichia coli* O157:H7 during the manufacture and ripening of Caciocotta goat cheese. Food Microbiology. 2018; **70**:200-205

[55] Ombarak RA, Awasthi SP, Hatanaka N, Yamasaki S. Detection of plasmid mediated colistin resistance *mcr-1* gene in ESBL producing *Escherichia coli* isolated from raw milk hard cheese in Egypt. International Dairy Journal. 2021; **117**,104986.

- [56] Alshaibani D, Machado RAM, Calder BL, Perry JJ. Survival of *Listeria monocytogenes* and shigatoxigenic *Escherichia coli* during the production and aging of farmstead-style cheese. *International Dairy Journal*. 2020; **110**, 104801
- [57] Blaiotta G, Ercolini D, Mauriello G, Salzano G, Villani F. Rapid and Reliable Identification of *Staphylococcus equorum* by a Species-Specific PCR Assay Targeting the *sodA* Gene. *System. Appl. Microbiol.*, 2004; **27**, 696-702
- [58] Jeong D-W, Han S, Lee J-H. Safety and technological characterization of *Staphylococcus equorum* isolates from jeotgal, a Korean high-salt-fermented seafood, for starter development. *International Journal of Food Microbiology*. 2014; **188**:108-115
- [59] Place RB, Hiestand D, Gallmann HR, Teuber M. *Staphylococcus equorum* subsp. *linens*, subsp. nov., A Starter Culture Component for Surface Ripened Semi-Hard Cheeses. *System. Appl. Microbiol.* 2003; **26**:30-37
- [60] Seiffert SN, Wüthrich D, Gerth Y, A. Egli A, Kohler P, Nolte O. First clinical case of KPC-3-producing *Klebsiella michiganensis* in Europe. *New Microbes and New Infections*. 2019; **29**: 100516
- [61] King TL, Schmidt S, Thakur S, Fedorka-Cray P, Keelara S, Harden L, Essack SY. Resistome of a carbapenemase-producing novel ST232 *Klebsiella michiganensis* isolate from urban hospital effluent in South Africa. *Journal of Global Antimicrobial Resistance*. 2021
- [62] Mitra S., Pramanika K, Ghosh PK, Sorena T, Sarkar A, Dey RS, Pandeye S, Maiti TK. Characterization of Cd-resistant *Klebsiella michiganensis* MCC3089 and its potential for rice seedling growth promotion under Cd stress. *Microbiological Research*, 2018; **210**:12-25
- [63] Sun X, Chen B, Li O, Liu N, Xia B, Zhu L, Qu K. Toxicities of polystyrene nano- and microplastics toward marine bacterium *Halomonas alkaliphila*, *Science of the Total Environment*, 2018; **642**:1378-1385
- [64] Deetae P, Saint-Eve A, Spinnler HE, Helinck S. Critical effect of oxygen on aroma compound production by *Proteus vulgaris*. *Food Chemistry*. 2011; **126**:134-139
- [65] Kamelamela N, Zalesne M, Morimoto J, Robbat A, Benjamin E. Wolfe, Indigo- and indirubin-producing strains of *Proteus* and *Psychrobacter* are associated with purple rind defect in a surface-ripened cheese. *Food Microbiology*. 2018; **76**: 543-552
- [66] Duncan-Lowey JK, Wiscovitch AL, Wood TE, Goldberg MB, Russo BC. *Shigella flexneri* Disruption of Cellular Tension Promotes Intercellular Spread. *Cell reports*. 2020; **33**, 108409
- [67] Jamet E, Akary E, Poisson M-A, Chamba J-F, Bertrand X, Serror P. Prevalence and characterization of antibiotic resistant *Enterococcus faecalis* in French cheeses. *Food Microbiology*, 2012; **31**:191-198
- [68] Estilarte ML, Tymczyszyn EE, Serradell M de los Á, Carasi P. Freeze-drying of *Enterococcus durans*: Effect on their probiotics and biopreservative properties. *LWT - Food Science and Technology*, 2021; **137**, 110496
- [69] Pieniz S, Andreazza R, Anghinoni T, Camargo F, Brandelli A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. *Food Control*. 2014; **37**: 251-256
- [70] Amaral DMF, Silva LF, Casarotti SN, Nascimento LCS, Penna ALB. *Enterococcus faecium* and *Enterococcus durans* isolated from cheese:

Survival in the presence of medications under simulated gastrointestinal conditions and adhesion properties. J. Dairy Sci. 2017; **100**:933-949

[77] Osuka H, Hitomi S, Koganemaru H, Kaneko T. A case of bacteremia caused by *Hafnia paralvei*, J Infect Chemother. 2011; **17**:855-857.

[71] Haubert L, dos Santos Cruzen CE, Fiorentini AM, da Silva WP. Tetracycline resistance transfer from foodborne *Listeria monocytogenes* to *Enterococcus faecalis* in Minas Frescal cheese. International Dairy Journal. 2018; **87**:11-15

[72] Cocconcelli PS, Cattivellia D, Gazzola S. Gene transfer of vancomycin and tetracycline resistances among *Enterococcus faecalis* during cheese and sausage fermentations. International Journal of Food Microbiology. 2003; **88**:315– 323

[73] Dat NM, Hamanaka D, Tanaka F, Uchino T. Control of milk pH reduces biofilm formation of *Bacillus licheniformis* and *Lactobacillus paracasei* on stainless steel. Food Control. 2012; **23**:215-220

[74] I.C. Fernández-No, M. Guarddon, K. Böhme, A. Cepeda, P. Calo-Mata, J. Barros-Velázquez*, Detection and quantification of spoilage and pathogenic *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis* by real-time PCR, Food Microbiology 28 (2011) 605-610

[75] Soares MB, Almada CN, Almada CN, Rafael CR, Martinez RCR, Pereira EPR, Balthazar CF, Adriano G. Cruz AG, Ranadheera CS, Sant'Ana AS. Behavior of different *Bacillus* strains with claimed probiotic properties throughout processed cheese (“requeijão cremoso”) manufacturing and storage. International Journal of Food Microbiology. 2019; **307**, 108288

[76] Pierron A, Zayet S, Toko L, Royer PY, Garnier P, Gendrin V. Catheter-related bacteremia with endocarditis caused by *Kocuria rhizophila*, Médecine et maladies infectieuses, 2020, DOI: 10.1016/j.medmal.2020.09.007.