Microbial ecology of artisanal products from the Piedmonst region (North West Italy) and antimicrobial activity of the autochthonous populations

Microbial ecology of artisanal products from North West of Italy and antimicrobial activity of the autochthonous populations

Barbara Dal Belloa, Kalliopi Rantsioua, Alberto Belliob, Giuseppe Zeppaa, Roberto Ambrosolia, Tiziana Civerab, Luca Cocolina, Corresponding author contact information, E-mail the corresponding author

Abstract

A total of about 1000 autochthonous strains were isolated from fresh and fermented artisanal products (cheese and meat) of the Piedmont area (Northwest of Italy) and screened for their antimicrobial activity. Among them, 98 bacteriocin-producing strains were detected and identified by molecular methods and genetic determinants encoding the antimicrobial proteins were targeted by PCR. The bacteriocins produced by isolates showed antimicrobial activity towards different spoilage and pathogenic microorganisms such as Listeria monocytogenes, Staphylococcus aureus, Clostridium tyrobutyricum and Brochothrix thermosphacta. Identification of the bacteriocin producing strains resulted in a large number of Lactococcus and Enterococcus strains with a broad spectrum of activity towards more that one spoilage and/or pathogenic microorganisms tested. In several strains it was possible to detect a combination of different genes responsible for the bacteriocin activity. These newly isolated bacteriocin-producing strains represent potential starters or co-starters to be used in food stabilization using the approach of the biopreservation.

Keywords

Autochthonous strains; Antimicrobial activity; Bacteriocins; Biopreservation

1. Introduction

Piedmont is a region (Northwest of Italy) of gastronomic traditions and production of typical quality products. Ministry of Agriculture has appointed 369 goods as typical artisanal products (Piedmont region regulation number 173/98 and 350/99), 12 as PDO (Protected Denomination of Origin) products and 5 as PGI (Protected Geographical Indication) (EC Regulation 510/2006), mainly represented by cheese and meat products. The quality of the local ingredients and raw materials, the specific conditions of the processing and ripening and the composition of the microbial population confer to the final products suitable sensory properties. In particular, complex sensory characteristics of dairy and meat products are linked to the presence of specific ecotypes of bacteria (Briggiler-Marcó et al., 2007; Broadbent, Brotherson, Johnson, & Oberg, 2002; Vernile et al., 2008). Furthermore, studies reported the considerable contribution of lactic acid bacteria (LAB), as great part of the autochthonous microbial community of typical cheeses (Coueret et al., 2004, Giraffa, 2003, Hugas et al., 2003 and Svec et al., 2005) and sausages (Klingberg et al., 2005 and Talon et al., 2007). In this last case, also the coagulase-negative cocci (CNC) are involved (Murru et al., 2005 and Rantsiou and Cocolin, 2006).

Numerous researches demonstrated that the autochthonous microflora present in several traditional products, other than improving the final technological and sensory characteristics, possess inhibition activity towards spoilage and pathogenic bacteria, and this has been reported for both dairy (Abdelbasset and Djamila, 2008, Cocolin et al., 2007, Diop et al., 2007 and Lasagno et al., 2002) and meat products (Ravytz et al., 2008 and Simonová et al., 2006).

This antibacterial activity may often be due to the production of organic acids (lactic and acetic acids), with a consequent reduction in the pH, or to the production of a number of antimicrobial substances such as hydrogen peroxide, diacetyl and bacteriocins (Ennahar et al., 2000, González et al., 2007 and Lasagno et al.,

2002). In recent years more attention has been dedicated to the study of bacteriocins and their mode of action for possible application in food processes. Specifically, bacteriocins produced by Lactobacillus, Lactococcus and Enterococcus strains (Cleveland et al., 2001, Cotter et al., 2005, Deegan et al., 2006, Foulquié Moreno et al., 2006, Giraffa, 2003 and Sobrino-Lòpez and Martìn-Belloso, 2008) were in depth investigated. Bacteriocins are peptides ribosomally synthesized by bacteria and have the capability to interfere with the growth of many foodborne spoilage and pathogenic bacteria (Diop et al., 2007).

Nowadays the consumers demand for more natural products, with a reduction of chemically synthesized preservatives. In this context, a new approach to food stabilization, called biopreservation, based on the antagonism displayed by one microorganism towards another, was established, linking LAB, other protective cultures and bioprotection afforded by natural products. According to Stiles (1996), biopreservation refers to extended storage life and enhanced safety of food using natural or controlled microflora and (or) associated antibacterial products.

The aims of this study were: i) to study the microbial ecology of some fresh and fermented artisanal products (cheese and meat) of the Piedmont region and ii) to investigate the potential role of the representative microflora as bioprotection agents, determining their antimicrobial spectrum of activity towards different food borne spoilage and pathogenic microorganisms. Bacteriocin-producing strains were identified by molecular methods and genetic determinants encoding the antimicrobial proteins were targeted by PCR.

2. Materials and methods

2.1. Determination of microbial ecology by culture-dependent methods

Artisanal cheeses (13 fresh and 37 ripened) and meat products (8 fresh and 43 fermented) were supplied by producers located in the Piedmont area. The samples were analyzed by using traditional microbiological methods to determine the predominant populations in the food products. Ten grams of each sample were diluted in 40 mL of sterile Ringer® solution (Oxoid, Italy) and homogenized in a blender (Stomacher® 400 Circulator, International PBI S.p.a, Italy) for 1 min. For microbial enumeration appropriate decimal dilutions were prepared and plated, in triplicate, on different media: lactic acid bacteria (LAB) on de Man Rogosa Sharp agar (MRS, Oxoid) and M17 agar (Oxoid) incubated at 30 °C for 48 h; enterococci on Kanamycin Aesculin Azide agar (KAA, Fluka, Italy), supplemented with kanamycin solution (20 μg/mL, Oxoid) incubated at 37 °C for 48 h; coagulase-negative cocci (CNC) on Mannitol Salt agar (MSA, Oxoid) incubated at 30 °C for 48 h; yeasts and moulds on malt extract agar (Oxoid) supplemented with tetracycline solution (1 μg/mL, Sigma, Milan, Italy) incubated at 30 °C for 48–72 h. After the incubation time, colonies were counted and means and standard deviations were calculated.

2.2. Detection of antimicrobial activity

Several foodborne spoilage and pathogenic bacteria were considered in this study as indicator strains. Gram positive bacteria included: Listeria monocytogenes NCTC 10527, Staphylococcus aureus ATCC® 6538™, Clostridium tyrobutyricum DSM 2637 and Brochothrix thermosphacta ATCC® 11509™, while Gram negative bacteria were Escherichia coli NCTC 12097 and Salmonella enteritidis from the laboratory collection of the DIVAPRA, previously identified by molecular methods. In order to screen a high number of colonies for antimicrobial activity, the count plates (with a maximum of 300 colonies) of MRS, M17, KAA and MSA were replicated in Brain Hearth Infusion (BHI) agar (Oxoid), by using the replica plate method. A number of plates corresponding to the indicator strains included in this study were produced. After 24–48 h of incubation at

30 °C, the visible colonies grown on the plates were covered with a layer of soft BHI agar (8 g/L agar), containing about 105–106 cells/mL of the indicator strain. The plates were kept at 30 °C for 24 h in aerobic condition except for C. tyrobutyricum DSM 2637, that was incubated at 37 °C for 24 h in anaerobic condition using the anaerogen system (Oxoid). After the incubation period, the colonies producing zone of growth inhibition in the indicator lawn were transferred in BHI broth and incubated for 24 h at 30 °C. Putative lactobacilli, lactococci, enterococci and micro/staphylococci were subsequently purified and their antimicrobial activity was confirmed by the agar-well-diffusion assay (AWDA) as described by Toba, Samant, and Itoh (1991). For the AWDA an initial volume of overnight cultures were filtered sterilized through a 0.2 μm filter (Millipore, Italy) and the pH was adjusted to 6.5–7 with 1 mol/L NaOH, to eliminate the antimicrobial effect due to acid compounds. Fifty μL of neutralized culture were placed into a well of 5 mm diameter in BHI soft agar plate containing 1% inoculum of an overnight culture of each of the indicator strains considered. To confirm the proteinaceous nature of the compound, a volume (4 μL) of the proteolytic enzyme, proteinase K (25 mg/mL, Sigma) was added into the well. The plates were then incubated at optimum growth temperature of the indicator strains and examined after 24 h for inhibition zone. The presence of inhibition zones around the well containing the neutralized culture broth and the absence of the halo around the well containing the proteinase K, confirmed the production of bacteriocins or bacteriocin-like compounds by the tested strains.

2.3. DNA extraction from bacteriocin producing isolates using the Clonesaver™ FTA® cards

Genomic DNA of the bacteriocin producing isolates was extracted by using the Clonesaver™ FTA® cards (Whatman, USA). Ten μL of each fresh culture was transferred to the FTA® card and dried for at least 1 h at room temperature. Discs were punched, washed initially with 200 μL of FTA® Purification Reagent (Whatman®) and placed in PCR amplification tubes. Samples were kept for 5 min at room temperature with moderate manual mixing and subjected to a second wash with 200 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 8) for 5 min at room temperature without mixing. Each step was repeated twice. At the end of the extraction, TE buffer was discarded and the PCR reaction mixture was added.

2.4. Molecular identification of the bacteriocin-producing isolates

Strains showing antimicrobial activity were identified as described by Cocolin et al. (2004). First, the strains were subjected to a PCR reaction by using the primers P1V1GC (5′ -CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GGC GGC GTG CCT AAT ACA TGC-3′) and P2V1 (5′ -TTC CCC ACG CGT TAC TCA CC-3′), and subsequently grouped by Denaturing Gradient Gel Electrophoresis (DGGE). Strains showing the same migration pattern were grouped and representatives were subjected to amplification of the V1-V2 and V3 regions of the 16S rRNA gene by using the primers P1V1 (5′ -GCG GCG TGC CTA ATA CAT GC-3′) and P4V3 (5′ -ATC TAC GCA TTT CAC CGC TAC-3′) as reported elsewhere (Cocolin et al., 2004). PCR products were purified by QIAquick® PCR purification kit (QIAgen, Milan, Italy) and sent for sequencing to MWG Biotech (Ebersberg, Germany). The retrieved sequences were aligned in Gene Bank by using the Blast program (http://blast.ncbi.nlm.nih.gov).

2.5. DGGE analysis

The Dcode Universal Mutation Detection System™ (Bio-Rad, Milan, Italy) was used and PCR products were applied to an electrophoresis in a 0.8 mm 8% (w/v) polyacrylamide gel with a denaturant gradient from 40% to 60% in a 1X TAE buffer (2 mmol/L Tris-base, 1 mmol/L glacial acetic acid, 50 mmol/L EDTA [pH 8]). Gels were subjected to a constant voltage of 130 V for 4 h at 60 °C. After electrophoresis, they were stained for 10 min in a SYBR Green solution (Sigma) and analyzed under UV illumination by using UVI pro Platinum

Table 1
Sequences of the oligonucleotide primers used for detection of genes encoding known bacteriocins.

Gene amplified	Primer	5′-sequence-3′		References
Lactococcal bacteriocins				
nisA	Nis A	F	ATGAGTACAAAGATTTTAACTTGGATTGGT	Choi, Cheigh, Kim, & Pyun, 2000
		R	ATAAACGAATGCACTTATGATCTTACTGTT	
nisZ	Nis Z	F	AAGAATCTCTCATGAGT	Rodriguez et al., 1995
		R	CCATGTCTGAACTAACA	
lctA	Lct (481)	F	GCATCTGCACTCACTTCA	Piard, Kuipers, Rollema, Desmazeaud, & De Vos, 1993
		R	GCAAGTAAATACAAATTGCC	
lcnA	Lcn A	F	CAATCAGTAGAGTTATTAACATTTG	Martinez, Rodriguez, Medina, Hernandez, & Rodriguez, 1998
		R	GATTTAAAAAGACATTCGATAATTAT	
lcnB	Lcn B	F	GCTTGCAGTATGTTATGAGTG	van Belkum, Kok, & Venema, 1992
		R	CCTACCATCCAGGATTTTCTT	
lsbA	513	F	GCTCCAAAAAGCGCTAGATC	Villani et al., 2001
		R	GCTGGCTACGATATTGCTAG	
lacA	RM	F	ATCCTATCCGATACCGTCAG	Villani et al., 2001
		R	GTTTTCCCTGAACCATTGGG	
lclA	972	F	GCGCTCTCTTGCATAGTGAG	Martinez, Fernandez, Suarez, & Rodriguez, 1999
		R	ACTCCTCCATTAGTACCAGC	
Enterococcal bacteriocins				
as-48	AS-48	F	GAGGAGTATCATGGTTAAAGA	De Vuyst, Foulquie Moreno, & Revets, 2003
		R	ATATTGTTAAATTACCAA	
entA	ent A	F	GGTACCACTCATAGTGGAAA	De Vuyst et al., 2003
		R	CCCTGGAATTGCTCCACCTAA	
entB	ent B	F	CAAAATGTAAAAGAATTAAGTACG	De Vuyst et al., 2003
		R	AGAGTATACATTTGCTAACCC	
entP	ent P	F	GCTACGCGTTCATATGGTAAT	De Vuyst et al., 2003
		R	TCCTGCAATATTCTCTTTAGC	
entL50A	L50A	F	ATGGGAGCAATCGCAAAATTA	De Vuyst et al., 2003
		R	TTTGTTAATTGCCCATCCTTC	
entL50B	L50B	F	ATGGGAGCAATCGCAAAATTA	De Vuyst et al., 2003
		R	TAGCCATTTTTCAATTTGATC	
bacA	31	F	CCTACGTATTACGGAAATGGT	De Vuyst et al., 2003
		R	GCCATGTTGTACCCAACCATT	

Gel Software (Eppendorf, Milan, Italy). DGGE fingerprints were analyzed by the BioNumerics 4.6 software package (Applied Maths, Sint-Martens-Latem, Belgium) to normalize the gels and group the isolates.

2.6. Rep-PCR

Rep-PCR fingerprinting was performed with the single oligonucleotide primer (GTG)5 (5′ -GTGGTGGTGGTGGTG-3′) (Gevers et al., 2001 and Versalovic et al., 1994). PCR reaction, in a final volume of 25 μ L, contained 1X PCR buffer, 1.5 mmol/L MgCl2, 0.2 mmol/L dNTP, 2 µmol/L concentration of primer, 0.75 U of Taq polymerase (Applied Biosystem, Milan, Italy) and a disk containing the DNA obtained as described above. PCR amplification was carried out in a thermal cycler and the cycle used was 95 °C for 5 min as initial step, 95 °C for 30 s, annealing at 40 °C for 30 s and 65 °C for 8 min for the next 30 cycles, 65 °C for 16 min concluded the amplification.

Rep-PCR products were electrophoresed in a 2% agarose gel for 4 h at a constant voltage of 120 V in 1X TBE buffer (10 mmol/L Tris-borate, 1 mmol/L EDTA, pH 8.0) and externally stained by using ethidium bromide (0.5 µg/mL, Sigma). A 1 Kb DNA ladder (Sigma) was used as a molecular size marker. The rep-PCR profiles were visualized under ultraviolet light, followed by digital image capturing using a CCD UVI pro Platinum 1.1 (Eppendorf). The resulting fingerprints were analyzed by the BioNumerics 4.6 software package (Applied Maths). The similarity among digitized profiles was calculated using the Pearson correlation, and an average linkage (UPGMA) dendrogram was derived from the profiles.

2.7. Targeting the bacteriocin encoding gene by PCR amplification

PCR was used to amplify the genes of two known nisins (A and Z), lactococcins (A, B, 513, 972) and lacticins (RM, 481) in lactococci, and enterocins (A, P, B, L50A, L50B, AS-48, 31) in enterococci, using the primers listed in Table 1. The reaction was performed in a final volume of 25 µL containing 1X PCR buffer, 1.5 mmol/L MgCl2, 0.2 mmol/L dNTP, 0.2 µmol/L of each bacteriocin primer and 1 U of Taq polymerase (Applied Biosystem) and the DNA as described above. The cycles used were 95 °C for 5 min as initial step,

95 °C for 1 min, annealing at 42 °C (for the primers of Nis A and Nis Z), 56 °C (for the primers Lct (481), 513, AS-48, ent A, ent P, ent B, L50A, L50B, RM, 972, Lcn A, Lcn B) or 52 °C (for enterocin 31) for 1 min, and 72 °C for 1 min for the next 30 cycles, 72 °C for 5 min concluded the amplification. Fragments were visualized on 2% agarose gels added of ethidium bromide using the 100 bp ladder (Sigma, Italy) as the molecular weight.

3. Results and discussion

In this study artisanal cheeses and meat products of Piedmont area were analyzed to determine the predominant microbial populations and to investigate their potential antimicrobial activity towards different foodborne spoilage and pathogenic microorganisms.

The highest microbial presence in the cheese and meat products considered was constituted by LAB (lactobacilli and lactococci), followed by enterococci, CNC and yeasts. Various studies have been published on the microbial characterization of traditional Italian cheeses (Dolci et al., 2008a, Dolci et al., 2008b, Fortina et al., 2003 and Randazzo et al., 2005) and traditional sausages (Cocolin et al., 2004, Mauriello et al., 2004 and Parente et al., 2001) underlining the importance of heterogeneous microflora to define typical characteristics of the products.

Considering the fresh cheeses, LAB species (lactobacilli and lactococci) dominated the microbial ecology with counts of about 9 log10 Colony Forming Units (CFU)/g for the majority of the fresh samples, while ripened cheeses demonstrated values from 7 to 9 log10 CFU/g. In fresh cheeses enterococci showed viable counts between 7 and 9 log10 CFU/g, while in ripened cheeses counts were most frequently determined to be between 5 and 7 and 7 to 9 log10 CFU/g. The counts of the yeasts for the majority of fresh cheeses followed to the enterococci trend, reaching values not higher than 9 log10 CFU/g. More heterogeneity was shown in the counts of the moulds. In the fresh cheeses they spanned from less than 3 to 9 log10 CFU/g, while in ripened cheeses they were determined to be no more than 5 log10 CFU/g. In all the samples of both fresh and ripened cheeses the counts of CNC resulted to be from 3 to 9 log10 CFU/g.

Also in meat products the predominant microbiota was represented by LAB (lactobacilli and lactococci). In fresh sausages, LAB counts were between 3 and 9 log10 CFU/g, while in fermented product, lactobacilli, with values of about 9 log10 CFU/g, dominated. Enterococci generally showed low values from less than 3 to 5 log10 CFU/g in most samples. Only two samples of fermented sausages had counts with more than 5 log10 CFU/g. More heterogeneity was observed for the counts of the yeasts and CNC showing variable values from less than 3 to more than 7 log10 CFU/g in both fresh and fermented meat products. Two samples of fermented meats showed counts of about 9 log10 CFU/g for the CNC populations. In general the moulds showed low counts.

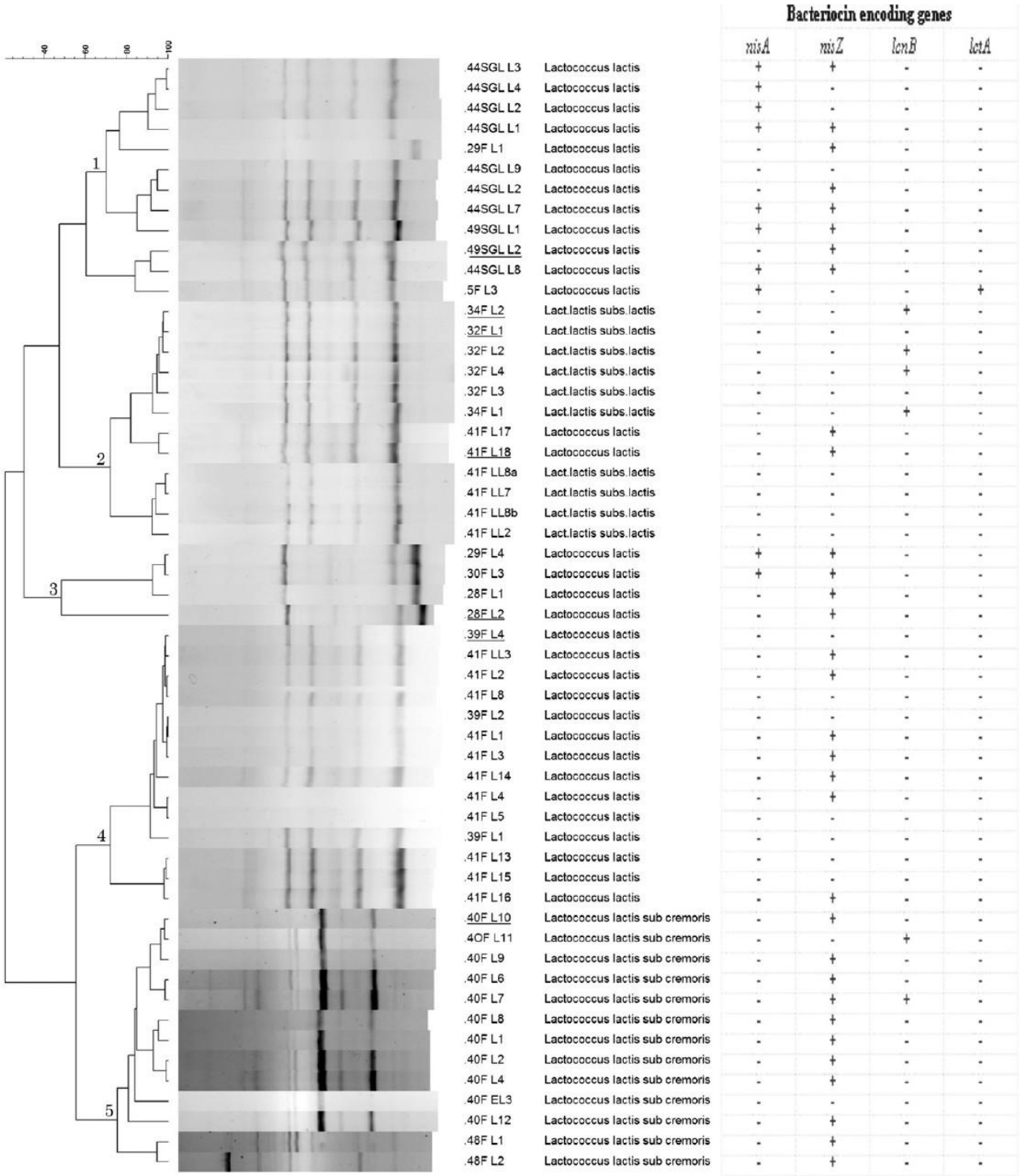

Fig. 1. Dendrogram generated after cluster analysis of the digitized Rep-PCR fingerprints of *Lactococcus lactis* bacteriocin-producing strains. Reference strains identified by 16S rRNA sequencing are underlined. The clusters are indicated by numbers. Presence (+) and absence (−) of the bacteriocin encoding genes for each strain are also indicated.

The high presence of LAB is fundamental because they are the biological basis for the production of a great number of fermented foods (Lasagno et al., 2002). An important contribution of these bacteria in fresh and fermented foods is also to preserve the nutritional qualities of the raw material and inhibit the growth of spoilage and pathogenic bacteria (Mattila-Sandholm, Mättö, & Saarela, 1999). Various studies demonstrated that several genera of LAB, naturally present in food ecosystems, have the capability to produce bacteriocins (Cleveland et al., 2001 and Cocolin et al., 2007).

After the first screening, a total of about 1000 colonies, possibly possessing antimicrobial activity, were observed in the replica plates, prepared from the sampling plates and overlaid with the six foodborne

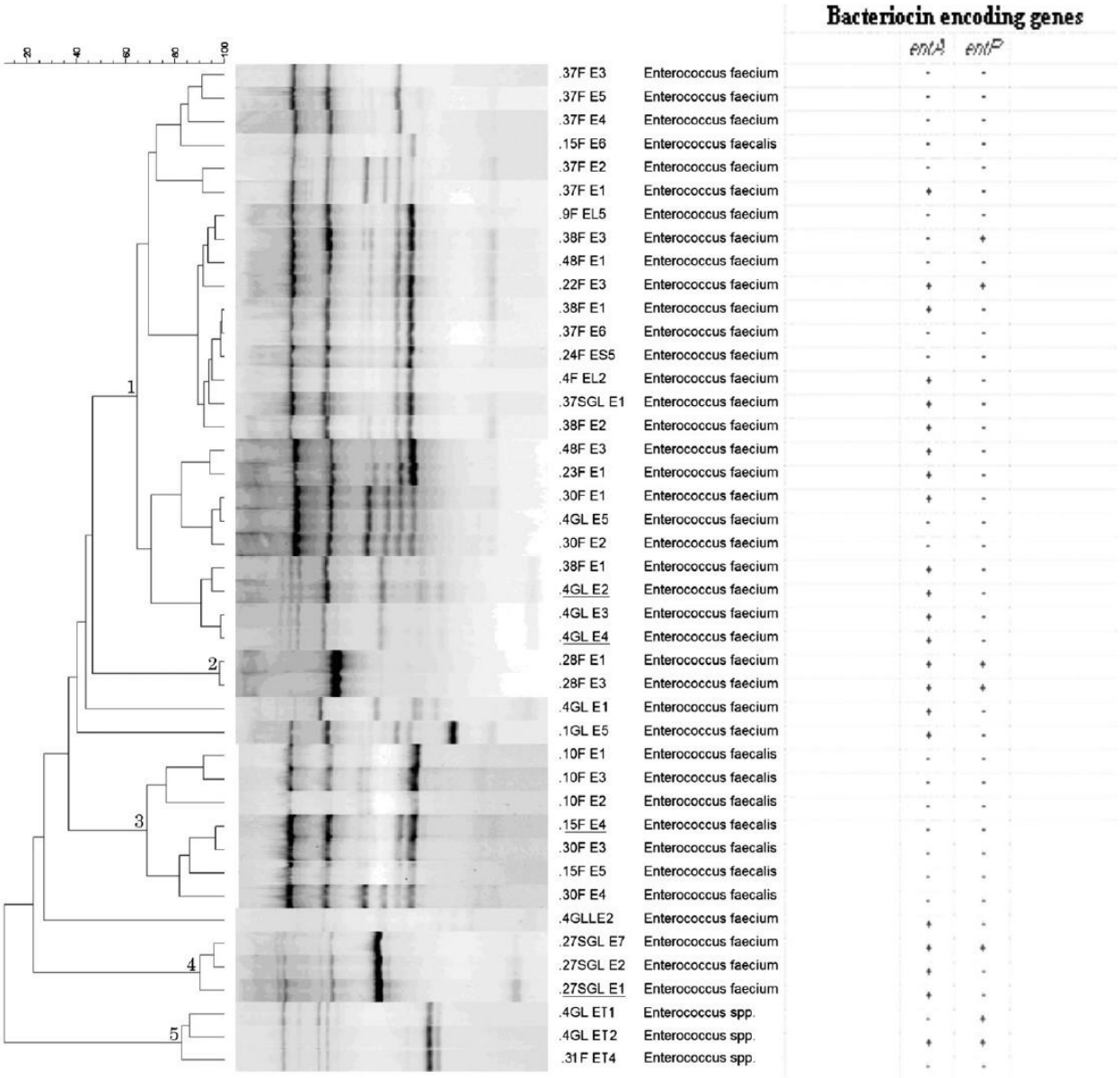

Fig. 2. Dendrogram generated after cluster analysis of the digitized Rep-PCR fingerprints of *Enterococcus* bacteriocin-producing strains. Reference strains identified by 16S rRNA sequencing are underlined. The clusters are indicated by numbers. Presence (+) and absence (−) of the bacteriocin encoding genes for each strain are also indicated.

spoilage and pathogenic bacteria tested. These colonies were all isolated and subjected to a first screening by using the AWDA test against the microorganism towards which originally they showed the antimicrobial activity in the replica plates. A total of 159 colonies were confirmed positive producing an halo of inhibition towards single indicator strains, such as C. tyrobutyricum DSM 2637 (15 colonies), Staph. aureus ATCC 6538 (42 colonies), B. thermosphacta ATCC 11509 (46 colonies), L. monocytogenes NCTC 10527 (33 colonies), E. coli NCTC 12097 (3 colonies) and S. enteritidis (20 colonies). In order to identify the nature of the antimicrobial compound, a second AWDA was carried out with neutralized and proteinase K treated supernatants. The results obtained highlighted that 19 colonies produced halo for acidity action, 98 colonies were bacteriocin producers, while 42 did not confirm the antimicrobial activity. It is interesting to notice that among the 98 bacteriocin producing isolates, 93 colonies lost their potential after proteinase K treatment, whereas 5 colonies from cheese samples (1 isolate active against Staph. aureus ATCC 6538 and 4 against L. monocytogenes NCTC 10527) showed an halo of inhibition also after proteinase K treatment. In order to investigate a possible inhibition due to the production of hydrogen peroxide, the supernatants of these cultures were treated with catalase as described previously (Cocolin et al., 2007), however no loss of

activity was observed (data not shown). These 5 colonies were also subjected to the identification of the bacteriocin genes by PCR, as for all the other isolates. With respect to the data of the first screening, no colony confirmed inhibitory activity toward the Gram negative bacteria. Among the 98 colonies that possessed antimicrobial activity due to the production of a bacteriocin-like compound, 56 were inhibitory towards more that one indicator strain. This evidence has been previously reported by other authors (Aymerich et al., 1996, Casaus et al., 1997 and Eijsink et al., 1998) and highlights the potential as bioprotective agents of the LAB strains. Inhibitory activity towards the indicator strains was further confirmed by using at least two other strains of the same species obtained from the collection of the DIVAPRA (data not shown).

The remaining 42 colonies showed antimicrobial activity against only one indicator strains (data not shown).

After PCR amplification and DGGE analysis, the 98 bacteriocin producing isolates were grouped in six different migration profiles. Representative strains of each group were subjected to sequencing of the 16S rRNA gene. The identifications obtained were as follows: Lactococcus lactis (32 strains), Lact. lactis subsp. lactis (10 strains), Lact. lactis subsp. cremoris (13 strains), Enterococcus faecium (32 strains), Ent. faecalis (8 strains) and Enterococcus spp. (3 strains).

The bacteriocin-producing isolates were subjected to the molecular characterization to define the intra-species biodiversity. Rep-PCR using the (GTG)5 primer was successfully used for identification of different LAB (Gevers et al., 2001 and Svec et al., 2005) including lactococci (Huys et al., 2006, Prodělalová et al., 2005, Quadghiri et al., 2005 and Zamfir et al., 2006). The dendrogram of the Lact. lactis strains presented 5 clusters with relatively low homology (Fig. 1). This indicates an overall high biodiversity among the different strains of this species. Cluster 2 grouped mainly Lact. lactis subsp. lactis isolates and cluster 5 put together strains identified as Lact. lactis subsp. cremoris. It is possible to notice a clear division between Lact. lactis subsp. cremoris and all other Lact. lactis strains. The 43 Enterococcus isolates (Fig. 2) were grouped in five clusters and the strains were clearly distinguished based on their identification. Cluster analysis revealed a clear distinction between Enterococcus strains. In particular, three clusters (1, 2 and 4) contained Ent. faecium, cluster 3 grouped Ent. faecalis strains, while cluster 5 grouped strains of Enterococcus spp. It is interesting to notice that isolate 15F E6, identified as Enterococcus faecalis by PCR-DGGE, grouped together Ent. faecium isolates, underlining a probable mistake in the identification. In addition, three Ent. faecium strains (4 GL E1, 1 GL E5 and 4GLL E2) did not group with other strains.

Interestingly, samples 29F and 41F, for Lact. lactis and 4 GL and 30F for Enterococcus spp. respectively, presented multiple bacteriocin producing populations, since strains isolated from these samples were positioned in different clusters in the respective dendrograms. For these samples, the advantage in terms of food safety is evident. Moreover, at least in some cases, strains that based on the molecular characterization by RAPD resulted to be very similar, carry different bacteriocin encoding genes.

Table 2
Bacteriocins encoding genes harboured in *Lactococcus lactis* isolates from different artisanal products of Piedmont region. The source of isolation, the inhibitory activity, the type of bacteriocins produced and the Rep-PCR cluster are also reported.

Strain code	Identification	Cluster n.	Bacteriocin/s encoding genes	Source of isolation	Inhibitory activity[a]
29F L4	*Lactococcus lactis*	3	*nis A, Z*	Fresh cheese (*Caprino presamico*)	*S, B, L*
30F L3	*Lactococcus lactis*	3	*nis A, Z*	Ripened cheese (*Toma*)	*S, L*
28F L1	*Lactococcus lactis*	3	*nis Z*	Ripened cheese (*Toma*)	*S, L*
28F L2	*Lactococcus lactis*	3	*nis Z*	Ripened cheese (*Toma*)	*S, L*
44SGL L9	*Lactococcus lactis*	1	Not identified	Fermented meat (*Salame crudo*)	*S*
44SGL L2	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S*
44SGL L7	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S*
49 SGL L1	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S*
41F L17	*Lactococcus lactis*	2	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L18	*Lactococcus lactis*	2	*nis Z*	Ripened cheese (*Toma*)	*S, L*
44SGL L3	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S*
44SGL L4	*Lactococcus lactis*	1	*nis A*	Fermented meat (*Salame crudo*)	*S*
44SGL L2	*Lactococcus lactis*	1	*nis A*	Fermented meat (*Salame crudo*)	*S*
44SGL L1	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S*
29F L1	*Lactococcus lactis*	1	*nis Z*	Fresh cheese (*Caprino presamico*)	*S, L*
49SGL L2	*Lactococcus lactis*	1	*nis Z*	Fermented meat (*Salame crudo*)	*S*
44SGL L8	*Lactococcus lactis*	1	*nis A, Z*	Fermented meat (*Salame crudo*)	*S, L*
5F L3	*Lactococcus lactis*	1	*nis A, lctA* (481)	Fresh cheese (*Toumin dal mel*)	*B*
39F L4	*Lactococcus lactis*	4	not identified	Ripened cheese (*Tometta*)	*S, L*
41F LL3[b]	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L2	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L8	*Lactococcus lactis*	4	not identified	Ripened cheese (*Toma*)	*S, L*
39F L2	*Lactococcus lactis*	4	not identified	Ripened cheese (*Tometta*)	*S, L*
41F L1	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L3	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L14	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L4	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F L5	*Lactococcus lactis*	4	not identified	Ripened cheese (*Toma*)	*S, L*
39F L1	*Lactococcus lactis*	4	not identified	Ripened cheese (*Tometta*)	*S, L*
41F L13	*Lactococcus lactis*	4	not identified	Ripened cheese (*Toma*)	*S, L*
41F L15	*Lactococcus lactis*	4	not identified	Ripened cheese (*Toma*)	*S, L*
41F L16	*Lactococcus lactis*	4	*nis Z*	Ripened cheese (*Toma*)	*S, L*
41F LL8a[b]	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*S, L*
41F LL7[b]	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*S, L*
41F LL8b[b]	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*S, L*
41F LL2	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*S, L*
34F L2	*Lactococcus lactis* subsp. *lactis*	2	*lcnB*	Fresh cheese (*Mattonella vaccina*)	*B*
32F L1	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*B*
32F L2	*Lactococcus lactis* subsp. *lactis*	2	*lcnB*	Ripened cheese (*Toma*)	*B*
32F L4	*Lactococcus lactis* subsp. *lactis*	2	*lcnB*	Ripened cheese (*Toma*)	*B*
32F L3	*Lactococcus lactis* subsp. *lactis*	2	not identified	Ripened cheese (*Toma*)	*B*
34F L1	*Lactococcus lactis* subsp. *lactis*	2	*lcnB*	Fresh cheese (*Mattonella vaccina*)	*B*
40F L10	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L11	*Lactococcus lactis* subsp. *cremoris*	5	*lcnB*	Ripened cheese (*Tometta*)	*S, L*
40F L9	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L6	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L7	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z, lcnB*	Ripened cheese (*Tometta*)	*S, L*
40F L8	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L1	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L2	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F L4	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
40F EL3[b]	*Lactococcus lactis* subsp. *cremoris*	5	not identified	Ripened cheese (*Tometta*)	*S, L*
40F L12	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Tometta*)	*S, L*
48F L1	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Toma*)	*S, B, L*
48F L2	*Lactococcus lactis* subsp. *cremoris*	5	*nis Z*	Ripened cheese (*Toma*)	*S, B, L*

[a] (S) *Staph. aureus* ATCC® 6538™; (B) *B. thermosphacta* ATCC® 11509™; (L) *L. monocytogenes* NCTC 10527; (C) *C. tyrobutyricum* DSM 2637.
[b] colony for which the proteinase K treatment did not eliminate the antimicrobial activity.

The results obtained by targeting the bacteriocin genes in the lactococcal isolates are presented in Table 2, where also their origin and spectrum of activity are presented. The presence of the gene/s responsible for the bacteriocin production was revealed in almost all the isolates tested. Among 55 Lactococcus isolates, 40 produced at least one nisin (A or Z), lacticin (481) and lactococcin B (LcnB). In particular, the combination of nisA and Z were detected in 8 Lact. lactis, the presence of the gene nisZ alone was observed for 23 isolates, whereas the bacteriocin structural gene of nisA alone was detected in 2 isolates. The combination of the genes lctA and nisA was found in one Lact. lactis as for one Lact. lactis subsp. cremoris we detected the combination of nisZ and lcnB. The structural genes for the bacteriocins lcnA, 513, lacA and lclA were never detected. For 15 strains of Lactococcus isolates (8 Lact. lactis, 6 Lact. lactis subsp. lactis and 1 Lact. lactis subsp. cremoris) the primers used in the amplifications did not give any PCR product. Lastly, within the 5 strains for which the proteinase K treatment did not eliminate the antimicrobial activity, identified as Lact. lactis (1 isolate), Lact. lactis subsp. lactis (3 isolates) and Lact. lactis subsp. cremoris (1 isolate), only the Lact. lactis showed a positive amplification with the primers for nisin Z (Table 2).

Among the 32 Ent. faecium isolates, 22 were positive for at least one bacteriocin. EntA and entP structural genes were detected in most of the isolates. In particular, 17 strains showed a positive result for one single entA, 1 strain for a single entP, while the combination of entA and entP was found for 4 strains. The gene for ent B, L50A, L50B and AS-48 never occured among Ent. faecium strains (Table 3). The 3 Enterococcus spp. showed the presence of a single entP structural gene, 1 strain contained the combination of enterocins A and P and for 1 strain no positive amplification for the structural gene was obtained with the primers used. Surprisingly no gene was detected in all Ent. faecalis strains (8 isolates).

Table 3
Bacteriocins encoding genes harboured in *Enterococcus* isolates from different artisanal products of Piedmont region. The source of isolation, the inhibitory activity, the type of bacteriocins produced and the Rep-PCR cluster are also reported.

Strain code	Identification	Cluster n.	Bacteriocin/s encoding genes	Source of isolation	Inhibitory activity[a]
37F E3	*Enterococcus faecium*	1	not identified	Ripened cheese (*Tometta*)	*B*
37F E5	*E.nterococcus faecium*	1	not identified	Ripened cheese (*Tometta*)	*B*
37F E4	*Enterococcus faecium*	1	not identified	Ripened cheese (*Tometta*)	*B*
37F E2	*Enterococcus faecium*	1	not identified	Ripened cheese (*Tometta*)	*B*
37F E1	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Tometta*)	*B*
9F EL5	*Enterococcus faecium*	1	not identified	Fresh cheese (*Nostrale di vacca*)	*C, L*
38F E3	*Enterococcus faecium*	1	*entP*	Ripened cheese (*Toma*)	*L*
48F E1	*Enterococcus faecium*	1	not identified	Ripened cheese (*Toma*)	*L*
22F E3	*Enterococcus faecium*	1	*entA, entP*	Ripened cheese (*Tometta di capra*)	*C, L*
38F E1	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*L*
37F E6	*Enterococcus faecium*	1	not identified	Ripened cheese (*Tometta*)	*L*
24F ES5	*Enterococcus faecium*	1	not identified	Ripened cheese (*Caciotta di pecora*)	*C, L*
4F EL2	*Enterococcus faecium*	1	*entA*	Fresh cheese (*Nostrale di vacca*)	*C, L*
37SGL E1	*Enterococcus faecium*	1	*entA*	Fresh meat (*Salsiccia*)	*L*
38F E2	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*B, L*
48F E3	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*B, L*
23F E1	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*S, B, L*
30F E1	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*B, L*
4GL E5	*Enterococcus faecium*	1	not identified	Fermented meat (*Salame crudo*)	*B, L*
30F E2	*Enterococcus faecium*	1	not identified	Ripened cheese (*Toma*)	*B, L*
38F E1	*Enterococcus faecium*	1	*entA*	Ripened cheese (*Toma*)	*L*
4GL E2	*Enterococcus faecium*	1	*entA*	Fermented meat (*Salame crudo*)	*L*
4GL E3	*Enterococcus faecium*	1	*entA*	Fermented meat (*Salame crudo*)	*L*
4GL E4	*Enterococcus faecium*	1	*entA*	Fermented meat (*Salame crudo*)	*L*
28F E1	*Enterococcus faecium*	2	*entA, entP*	Ripened cheese (*Toma*)	*L*
28F E3	*Enterococcus faecium*	2	*ent A,ent P*	Ripened cheese (*Toma*)	*L*
4GL E1	*Enterococcus faecium*	–	*entA*	Fermented meat (*Salame crudo*)	*S, L*
1GL E5	*Enterococcus faecium*	–	*ent A*	Fresh meat (Salame da cuocere)	*B, L*
27SGL E7	*Enterococcus faecium*	4	*entA, entP*	Fermented meat (*Salame crudo*)	*L*
27SGL E2	*Enterococcus faecium*	4	*entA*	Fermented meat (*Salame crudo*)	*L*
27SGL E1	*Enterococcus faecium*	4	*entA*	Fermented meat (*Salame crudo*)	*L*
4GLL E2	*Enterococcus faecium*	–	*entA*	Fermented meat (*Salame crudo*)	*S, L*
4GL ET2	*Enterococcus* spp.	5	*entA, entP*	Fermented meat (*Salame crudo*)	*B*
4GL ET1	*Enterococcus* spp.	5	*entP*	Fermented meat (*Salame crudo*)	*B*
31F ET4	*Enterococcus* spp.	5	not identified	Ripened cheese (*Toma vaccina*)	*B, L*
10F E1	*Enterococcus faecalis*	3	not identified	Fresh cheese (*Robioletta di vacca*)	*B, L*
10F E3	*Enterococcus faecalis*	3	not identified	Fresh cheese (*Robioletta di vacca*)	*B, L*
10F E2	*Enterococcus faecalis*	3	not identified	Fresh cheese (*Robioletta di vacca*)	*B, L*
15F E4	*Enterococcus faecalis*	3	not identified	Ripened cheese (*Toma mista*)	*B*
30F E3	*Enterococcus faecalis*	3	not identified	Ripened cheese (*Toma vaccina*)	*B*
15F E5	*Enterococcus faecalis*	3	not identified	Ripened cheese (*Toma mista*)	*B*
30F E4	*Enterococcus faecalis*	3	not identified	Ripened cheese (*Toma vaccina*)	*B*
15F E6	*Enterococcus faecalis*	3	not identified	Ripened cheese (*Toma mista*)	*B*

[a] (*S*) *Staph. aureus* ATCC® 6538™; (*B*) *B. thermosphacta* ATCC® 11509™; (*L*) *L. monocytogenes* NCTC 10527; (*C*) *C. tyrobutyricum* DSM 2637.

Among 98 identified strains, for 15 Lactococcus and 19 Enterococcus we could not get any amplification by using the described primers for targeting the known bacteriocin genes. Further studies should be focused to understand the effective nature of these not identified substances.

4. Conclusions

In conclusion, the considerable presence in artisanal products of bacteriocin producing strains could be a good resource as bioprotective cultures to control the potential risks derived from foodborne pathogens. In this study a high incidence of bacteriocin producing Lactococcus and Enterococcus strains was observed. In the future, the possible use of these active strains could be a new way to preserve the quality of foods. Additional studies should be carried out in the future to understand which are the best bacteriocin

producing strains to employ, alone or combination, for application as starters or co-starters in food productions.

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References

M. Abdelbasset, K. Djamila Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk “Raïb” African Journal of Biotechnology, 7 (2008), pp. 2908–2914

T. Aymerich, H. Holo, L.S. Hâvarstein, M. Hugas, M. Garriga, I.F. Nes Biochemical and genetic characterization of enterocin A from Enterococcus faecium, a new antilisterial bacteriocin in the pediocin family of bacteriocins Applied and Environmental Microbiology, 62 (1996), pp. 1676–1682

M.J. van Belkum, J. Kok, G. Venema Cloning, sequencing, and expression in Escherichia coli of lcnB, a third bacteriocin determinant from the lactococcal bacteriocin plasmid p9B4-6 Applied and Environmental Microbiology, 58 (1992), pp. 572–577

M. Briggiler-Marcó, M.L. Capra, A. Quiberoni, G. Vinderola, J.A. Reinheimer, E. Hynes Nonstarter Lactobacillus Strains as adjunct cultures for cheese making: in vitro characterization and performance in two model cheeses Journal of Dairy Science, 90 (2007), pp. 4532–4542

J.R. Broadbent, C. Brotherson, M.E. Johnson, C.J. Oberg Cheese micro-ecology and the influence of adjunct/wash techniques Australian Journal of Dairy Technology, 57 (2002), pp. 137–142

P. Casaus, T. Nilsen, L.M. Cintas, I.F. Nes, P.E. Hernández, H. Holo Enterocin B, a new bacteriocin from Enterococcus faecium T136, which can act synergistically with enterocin Applied Microbiology, 143 (1997), pp. 2287–2294

H.J. Choi, C.I. Cheigh, S.B. Kim, Y.R. Pyun Production of a nisin-like bacteriocin by Lactococcus lactis subsp. lactis A164 isolated from kimchi Journal of Applied Microbiology, 88 (2000), pp. 563–571

J. Cleveland, T.J. Montville, I.F. Nes, M.L. Chikindas Bacteriocins: safe, natural antimicrobials for food preservation International Journal of Food Microbiology, 71 (2001), pp. 1–20

L. Cocolin, R. Foschino, G. Comi, M.G. Fortina Description of the bacteriocins produced by two strains of Enterococcus faecium isolated from Italian goat milk Food Microbiology, 24 (2007), pp. 752–758

L. Cocolin, K. Rantsiou, L. Iacumin, R. Urso, C. Cantoni, G. Comi Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods Applied and Environmental Microbiology, 70 (4) (2004), pp. 1883–1894

P.D. Cotter, C. Hill, R.P. Ross Bacteriocins: developing innate immunity for food Nature Reviews Microbiology, 3 (2005), pp. 777–788

V. Coueret, M. Gueguen, J.P. Vernoux In vitro screening of potential probiotic activities of selected lactobacilli isolated from unpasteurized milk products for incorporation into soft cheese Journal of Dairy Research, 71 (2004), pp. 451–460

L. De Vuyst, M.R. Foulquie Moreno, H. Revets Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins International Journal of Food Microbiology, 84 (2003), pp. 299–318

L.H. Deegan, P.D. Cotter, C. Hill, P. Ross Bacteriocins: biological tools for bio-preservation and shelf-life extension International Dairy Journal, 16 (2006), pp. 1058–1071

M.B. Diop, R. Dibois-Dauphin, E. Tine, A.N. Jacqueline, P. Thonart Bacteriocin producers from traditional food products Biotechnologie, Agronomie, Société et Environnement, 11 (2007), pp. 275–281

P. Dolci, V. Alessandria, K. Rantsiou, L. Rolle, G. Zeppa, L. Cocolin Microbial dynamics of Castelmagno PDO, a traditional Italian cheese, with a focus on lactic acid bacteria ecology International Journal of Food Microbiology, 122 (2008), pp. 302–311

P. Dolci, V. Alessandria, G. Zeppa, K. Rantsiou, L. Cocolin Microbiological characterization of artisanal Raschera PDO cheese: analysis of its indigenous lactic acid bacteria Food Microbiology, 25 (2008), pp. 392–399

V.G.H. Eijsink, M. Skeie, P.H. Middelhoven, M.B. Brurberg, I.F. Nes Comparative studies of class IIa bacteriocins of lactic acid bacteria Applied and Environmental Microbiology, 64 (1998), pp. 3275–3281

S. Ennahar, T. Sashihara, K. Sonomoto, A. Ishizaki Class IIa bacteriocins: biosynthesis, structure and activity FEMS Microbiology Reviews, 24 (2000), pp. 85–106

M.G. Fortina, G. Ricci, A. Acquati, G. Zeppa, A. Gandini, P.L. Manachini Genetic characterization of some lactic acid bacteria occurring in an artisanal protected denomination origin (PDO) Italian cheese, the Toma piemontese Food Microbiology, 20 (2003), pp. 397–404

M.R. Foulquié Moreno, P. Sarantinopoulos, E. Tsakalidou, L. De Vuyst The role and application of enterococci in food and health International Journal of Food Microbiology, 106 (2006), pp. 1–24

D. Gevers, G. Huys, J. Swings Applicability of rep-PCR fingerprinting for identification of Lactobacillus species FEMS Microbiology Letters, 205 (1) (2001), pp. 31–36

G. Giraffa Funcionality of enterococci in dairy products International Journal of Food Microbiology, 88 (2–3) (2003), pp. 215–222

L. González, H. Sandoval, N. Sacristán, J. Castro, J. Fresno, M. Tornadijo Identification of lactic acid bacteria isolated from Genestoso cheese throughout ripening and study of their antimicrobial activity Food Control, 18 (2007), pp. 716–722

M. Hugas, M. Garriga, M.T. Aymerich Functionality of enterococci in meat products International Journal of Food Microbiology, 88 (2003), pp. 223–233

G. Huys, M. Vancanneyt, K. D'Haene, V. Vankerckhoven, H. Goossens, J. Swings Accuracy of species identity of commercial bacterial cultures intended for probiotic or nutritional use Research in Microbiology, 157 (2006), pp. 803–810

T.D. Klingberg, L. Axelsson, K. Naterstadt, D. Elsser, B.B. Budde Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages International Journal of Food Microbiology, 105 (2005), pp. 419–431

M. Lasagno, V. Beoletto, F. Sesma, R. Raya, G. Font De Valdez, A. Eraso Selection of bacteriocin producer strains of lactic acid bacteria from a dairy environment Microbiologia, 25 (2002), pp. 37–44

B. Martinez, M. Fernandez, J.E. Suarez, A. Rodriguez Synthesis of lactococcin 972, a bacteriocin produced by Lactococcus lactis IPLA 972, depends on the expression of a plasmid-encoded bicistronic operon Microbiology, 145 (1999), pp. 3155–3161

B. Martinez, A. Rodriguez, M. Medina, P.E. Hernandez, J.M. Rodriguez Detection of specific bacteriocin-producing lactic acid bacteria by colony hybridization Journal of Applied Microbiology, 84 (1998), pp. 1099–1103

T. Mattila-Sandholm, J. Mättö, M. Saarela Lactic acid bacteria with health claims-interactions and interference with gastrointestinal flora International Dairy Journal, 9 (1999), pp. 25–35

G. Mauriello, A. Casaburi, G. Blaiotta, F. Villani Isolation and technological properties of coagulase negative staphylococci from fermented sausages of southern Italy Meat Science, 67 (2004), pp. 149–158

N. Murru, A. Kodjo, L. Villard, L. Fratino, G. Perrelli, M. Tozzi et al. Identification of coagulase negative Staphylococci isolated from dairy products using molecular methods Revue De Médicine Vétérinaire (Toulouse), 156 (8–9) (2005), pp. 455–459

E. Parente, S. Grieco, M.A. Crudele Phenotypic diversity of lactic acid bacteria isolated from fermented sausages produced in Basilicata (Southern Italy) Journal of Applied Microbiology, 90 (2001), pp. 943–952

J.C. Piard, O.P. Kuipers, H.S. Rollema, M.J. Desmazeaud, W.M. De Vos Structure, organization and expression of the lct gene for lacticin 481, a novel lantibiotic produced by Lactococcus lactis Journal of Biological Chemistry, 268 (1993), pp. 16361–16368

J. Prodělalová, A. Španová, B. Rittich Application of PCR, rep-PCR and RAPD techniques for typing of Lactococcus lactis strains Folia Microbiologica (Praha), 50 (2005), pp. 150–154

M. Quadghiri, M. Amar, M. Vancanneyt, J. Swings Biodiversity of lactic acid bacteria in Moroccan soft white cheese (Jben) FEMS Microbiology Letters, 251 (2005), pp. 267–271

C.L. Randazzo, E.E. Vaughan, C. Caggia Artisanal and experimental Pecorino Siciliano cheese: microbial dynamics during manufacture assessed by culturing and PCR-DGGE analyses International Journal of Food Microbiology, 109 (2005), pp. 1–8

K. Rantsiou, L. Cocolin New developments in the study of the microbiota of naturally fermented sausages as determined by molecular methods: a review International Journal of Food Microbiology, 108 (2006), pp. 255–267

F. Ravytz, S. Barbuti, M.A. Frustoli, G. Parolari, G. Saccani, L. De Vuyst et al. Competitiveness and antibacterial potential of bacteriocin-producing starter cultures in different types of fermented sausages Journal of Food Protection, 71 (9) (2008), pp. 1817–1827

J.M. Rodriguez, L.M. Cintas, P. Casaus, H.M. Dodd, P.E. Hernandez, M.J. Gasson Isolation of nisin-producing Lactococcus lactis strains from dry fermented sausages Journal of Applied Bacteriology, 78 (1995), pp. 109–115

M. Simonová, V. Strompfová, M. Marciňáková, A. Lauková, S. Vesterlund, M. Latorre Moratalla et al. Characterization of Staphylococcus xylosus and Staphylococcus carnosus isolated from Slovak meat products Meat Science, 73 (4) (2006), pp. 559–564

A. Sobrino-Lòpez, O. Martìn-Belloso Use of nisin and other bacteriocins for food preservation of dairy products International Dairy Journal, 18 (2008), pp. 329–343

M.E. Stiles Biopreservation by lactic acid bacteria Antonie Van Leeuwenhoek, 70 (1996), pp. 331–345

P. Svec, V. Drab, I. Sedlacek Ribotyping of Lactobacillus casei group strains isolated from dairy products Folia Microbiologica, 50 (3) (2005), pp. 223–228

R. Talon, S. Leroy, I. Lebert Microbial ecosystems of traditional fermented meat products: the importance of indigenous starters Meat Science, 77 (1) (2007), pp. 55–62

T. Toba, S.K. Samant, T. Itoh Assay system for detecting bacteriocin in microdilution wells Letters in Applied Microbiology, 13 (2) (1991), pp. 102–104

A. Vernile, G. Giammanco, G. Spano, T.P. Beresford, P.F. Fox, S. Massa Genotypic characterization of lactic acid bacteria isolated from traditional Pecorino Siciliano cheese Dairy Science Technology, 88 (2008), pp. 619–629

J. Versalovic, M. Schneider, F.J. de Bruijn, J.R. Lupski Genomic fingerprinting of bacteria using repetitive sequence based PCR (rep-PCR) Methods in Molecular and Cellular Biology, 5 (1994), pp. 25–40

F. Villani, M. Aponte, G. Blaiotta, G. Mauriello, O. Pepe, G. Moschetti Detection and characterization of a bacteriocin, garviecin L1-5, produced by Lactococcus garvieae isolated from raw cow's milk Journal of Applied Microbiology, 90 (2001), pp. 430–439

M. Zamfir, M. Vancanneyt, L. Makras, F. Vaningelgem, K. Lefebvre, B. Pot et al. Biodiversity of lactic acid bacteria in Romanian dairy products Systematic and Applied Microbiology, 29 (2006), pp. 487–495