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ORIGINAL ARTICLE

High biodiversity and potent anti-listerial action of complex red smear cheese microbial ripening consortia

Anne Bleicher · Tanja Obermajer · Bojana Bogovič Matijašić · Siegfried Scherer · Klaus Neuhaus

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Abstract When *Listeria monocytogenes* EGDe (serovar 1/2a) was cultivated in cell-free supernatants prepared from red smear cheese microbial ripening consortia grown for 8 h in liquid medium, 8 out of 49 supernatants exhibited a bactericidal activity, sometimes even reducing the inoculum of *L. monocytogenes* from 5×10^7 CFU/ml to zero after 24 h of incubation. Another five consortia displayed a bacteriostatic capacity. No inhibition in supernatants was observed when the complex consortia were incubated for a 10-min period only, indicating that the activity depends on actively growing consortia. Consortia displayed a very high biodiversity (Simpson's strain diversity index up to 0.97, species diversity up to 0.89). However, biodiversity did not correlate with anti-listerial activity. There was no obvious similarity between the anti-listerial consortia studied, and no general difference in comparison to non-inhibitory communities. The proportion of lactic acid bacteria (LAB) in the consortia ranged between 3 and 45%. Therefore, the presence of 23 different LAB bacteriocin genes was investigated using specific PCR primers, identifying one to five bacteriocin genes in several consortia. In situ transcription of lactococcin G mRNA on the cheese surface was demonstrated by RT-PCR in five samples, but this bacteriocin displayed no anti-listerial activity. Supernatants subjected to thermal and enzymatic treatment suggested the presence of heat-stable, non-proteinaceous molecules as well as heat-labile compounds which are sensitive to proteolytic digestion. Probably, substances other than LAB bacteriocins are responsible for the pronounced antilisterial action of some supernatants.

Keywords *Listeria monocytogenes* · Red smear cheese · Bactericidal activity · Biodiversity · Bacteriocin genes · Lactic acid bacteria

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A. Bleicher · S. Scherer · K. Neuhaus
Abteilung für Mikrobiologie, ZIEL,
Technische Universität München,
85354 Freising, Germany

T. Obermajer · B. B. Matijašić
Chair of Dairy Science, Biotechnical Faculty,
University of Ljubljana,
1230 Domžale,
Slovenia

S. Scherer (✉)
Lehrstuhl für Mikrobielle Ökologie,
Department für Grundlagen der Biowissenschaften, WZW,
Technische Universität München,
Weihenstephaner Berg 3,
85354 Freising, Germany
e-mail: siegfried.scherer@wzw.tum.de

Introduction

Bacterial surface-ripened cheeses have a long tradition in Germany, Austria, France, Italy and Switzerland. Brands like Munster, Limburger, Romadur and Appenzeller are artisanal high quality foods. Microbial consortia dwelling on the surface of the cheese ("smear") consist of coryneform bacteria, micrococci, staphylococci and yeasts, and contribute to cheese ripening by the production of proteolytic and lipolytic enzymes as well as aroma compounds (Bockelmann and Hoppe-Seyler 2001; Brennan et al. 2004). The smear is applied repeatedly by brushing, thereby beginning with the mature ("old") cheeses followed by the freshly produced ("young") cheeses. Old–young smearing provides an inexpensive

technique to propagate these complex surface consortia and ensures the plant-specific uniqueness of the product. However, the risk of spreading contaminating microorganisms by this procedure is a disadvantage of this approach (Little et al. 2008; Loncarevic et al. 1998).

Listeria monocytogenes, the causative agent of human listeriosis, is a Gram positive, non-sporeforming bacterium characterized by a remarkable resistance to environmental stresses (Gandhi and Chikindas 2007). Its psychrophilic, acid- and halotolerant, facultatively anaerobic character allows the pathogen to overcome many common approaches of food preservation. Contrary to most other pathogens targeting the gastrointestinal tract, infections with *L. monocytogenes* may not be self-limiting. The clinical symptoms of human listeriosis mainly manifest in infections of the central nervous system (meningitis and encephalitis), bacteremia and endocarditis (Lecuit 2005). The incidence of listeriosis in Europe is low but the mortality rate can reach 25–30% among persons developing the disease (de Valk et al. 2005).

The food-borne nature of *L. monocytogenes* was not recognized before the 1980s, when a number of listeriosis outbreaks were linked to the consumption of contaminated soft cheeses (Farber and Peterkin 1991). The production and ripening parameters of salty red smear cheeses provide excellent growth conditions for some undesirable microorganisms. As a consequence, smear cheeses are more frequently contaminated with *L. monocytogenes* than other soft cheeses, showing exceedingly high cell counts in some products (Jacquet et al. 1993; Loncarevic et al. 1995; Rudolf and Scherer 2001). Therefore, there is considerable interest in developing microbial ripening consortia which display anti-listerial activity (Carnio et al. 2000; Eppert et al. 1997; Loessner et al. 2003). Undefined cheese surface consortia with anti-listerial properties have been described in the past, sometimes containing bacteria-producing bacteriocins (Saubusse et al. 2007; Valdes-Stauber and Scherer 1994). Bacteriocins are small, ribosomally synthesized peptides usually acting on the cytoplasmic membrane of target bacteria, thereby causing cell lysis (for review, see Cotter et al. 2005; Jack et al. 1995). These findings raised hope that indigenous smear bacteria may serve as protective cultures to improve the microbial quality of these products (McAuliffe et al. 1999; O'Sullivan et al. 2006). Furthermore, unspecific and most likely synergistic inhibitory effects have been attributed to stable microbial consortia, preventing the establishment of undesired bacteria (Guillier et al. 2008; Saubusse et al. 2007).

The present study gives a survey over complex cheese microbial communities and their potential anti-listerial properties, reports on their biodiversity and explores the presence and transcription of 23 bacteriocin genes.

Materials and methods

Collection of microbial consortia

Smear cheeses produced in France, Italy and Germany were bought on the retail market or provided by Unité de Recherches Fromagères (INRA, Aurillac, France). The surface flora was recovered using a sterile knife and homogenized in saline solution containing 5% NaCl using a Stomacher Lab-Blender. Suspensions were centrifuged at 6,600 × *g* and washed twice in saline solution to remove excessive cheese fat. After addition of glycerol to the cells in a final concentration of 15%, aliquots were frozen at −80°C. All samples underwent routine analysis according to DIN EN ISO 11290-1 (1997) to detect natural contamination with *Listeria* sp. (see electronic supplementary material, ESM, Table S1).

Supernatant preparation

A sample of 500 µl of the thawed smear were used to inoculate 15 ml of Plate Count broth supplemented with 3% sodium chloride (PC3+ broth, initial cell density was approx. 3×10^8 CFU/ml) to mimic the conditions of cheese brines (Valdes-Stauber et al. 1991). Flasks were incubated for 10 min or 8 h at 30°C and 180 rpm. Cells were pelleted by centrifugation at 12,000 × *g*. Supernatants were filter-sterilized (Millex GP 0.2-µm filter units; Millipore, Schwalbach, Germany) and stored at −20°C until use. Preparation of cell-free supernatants (CFS) was carried out at least three times independently. The pH values of the CFS were not adjusted to avoid possible inactivation of unknown compounds (Hütt et al. 2006). pH was measured after thawing using universal indicator strips (Macherey-Nagel, Düren, Germany), the content of lactic acid and hydrogen peroxide was determined using the analytical systems of Merck (Darmstadt, Germany). The enzymes lactate dehydrogenase and peroxidase, respectively, are immobilized on strips allowing quantification via a colorimetric reaction. Bacteriophage involvement was excluded by monitoring plaque formation in poured plates using soft agar seeded with 10^8 CFU/ml *L. monocytogenes* mixed with CFS (data not shown).

Detection of anti-listerial activity in CFS

An overnight culture of *L. monocytogenes* EGDe WSLC 1993 from the Weihenstephan Listeria Collection WSLC (serovar 1/2 a) in Brain Heart Infusion broth (BHI; Merck) was diluted to an optical density of 0.1 at 600 nm (OD_{600}) with PC3+ broth and grown to a final OD_{600} of 0.3. Five ml of the CFS were inoculated with 0.5 ml of the cell suspension (the inoculation level was equivalent to 5×

10^7 CFU/ml) and incubated for 24 h at 30°C and 180 rpm. Samples of 100 µl were serially diluted and plated onto PC3+ agar in duplicate. Viable *Listeria* cells were counted after 2 days of incubation at 30°C (summarized in ESM Table S1). Bacteriostatic activity was assumed when *Listeria* was not able to proliferate in the CFS, but did not die; bactericidal activity was defined as a reduction of the initial inoculum of at least two log units.

In order to characterize possible inhibitory compounds, CFS were subjected to dilution 1:10 in PC3+ broth and to 100°C for 5 min, respectively. Sensitivity towards proteolytic digest was checked by adding proteinase K in a final concentration of 0.5 mg/ml (30 U/mg; Merck) and incubation for 2 h at 50°C. The enzyme was heat inactivated for 30 min at 75°C prior to an inoculation with *Listeria* (Table 1).

Determination of biodiversity

The composition of six surface consortia was studied in detail using Fourier Transform-Infra Red (FT-IR) spectroscopy (Wenning et al. 2008). Appropriate dilutions of the thawed smear were plated onto PC3+ agar plates. After 3–5 days of aerobic incubation at 30°C, 100–250 bacterial colonies were selected randomly for identification. The sample preparation to obtain reliable infrared spectra was carried out as described previously (Kümmerle et al. 1998; Oberreuter et al. 2002). Two independent measurements were conducted using a HTS-XT FT-IR spectrometer (Bruker, Karlsruhe, Germany). For data processing, OPUS software v. 6 (Bruker) was used. For identification purposes, the spectra obtained were compared to reference databases maintained at the TU Munich. For example, Gram positive non-sporeforming bacteria were compared to a database containing 2,200 infrared spectra of 360 species and 78 genera of 16S rRNA gene sequenced isolates (Oberreuter et al. 2002; Rieser et al., in preparation). A sublibrary, comprising 379 spectra of 92 species and 9 genera belonging to the lactic acid bacteria, was used for identification of this physiological group (Wenning et al. 2010). Ambiguous FT-IR identification results were clarified by 16S rRNA gene sequencing. The distances of two spectra, comprising the size of non-overlapping areas, were used to discriminate bacteria at the strain level. The term isolate is used if a colony has been identified to the species level. The term strain is used if different isolates belong to the same species but do not belong to the same clone. A subsequent cluster analysis was performed to visualize the abundance ratio of strains resulting in a dendrogram (average linkage algorithm).

The diversity, taking into account both richness and evenness of the microbiota, was estimated by calculating Simpson's Index of Diversity (SID). This index can be

Table 1 Characteristics and activity of CFS against *Listeria monocytogenes* EGDe

Number of consortium	Variety of cheese	Cell-free supernatant (CFS)		Number of viable *Listeria* after 24 h in CFS (CFU/ml)		
		pH	Lactic acid (mM)	Untreated[a]	5 min 100°C[b]	Proteinase K[c]
5a	Maroilles	6.0	5.5	0	0	0
6	Munster	6.0	4.6	4.2E+03	0	0
7	Munster	5.5	6.1	2.5E+03	3.3E+06	1.5E+07
9	Maroilles	6.0	3.4	1.9E+02	3.5E+06	8.7E+06
11a	Munster	5.5	8.4	0	0	0
15a	Maroilles	6.0	5.4	0	1.6E+07	0
20	Maroilles	6.0	3.9	0	9.6E+05	4.4E+06
21	Epoisses	5.5	3.2	3.0E+07	5.1E+07	3.0E+07
22	Munster	6.0	2.7	6.8E+06	5.1E+07	6.5E+06
23	Livarot	6.0	3.5	3.3E+06	2.5E+07	1.4E+07
24	Munster	6.0	4.4	0	9.8E+06	2.0E+06
11b	Munster	5.7	n.d.	2.3E+08	n.d.	n.d.
39b	St. Nectaire	6.0	n.d.	2.5E+09	n.d.	n.d.

The concentration of hydrogen peroxide was below 15 µM in all supernatants tested

n.d. Not determined

[a] Average number, calculated from three independent trials (see also ESM Table S1)

[b] Absolute number, performed only once

[c] Average number, calculated from two independent trials

calculated based on the number of different strains/ species in a sample, according to the following equation

$$SID = 1 - \frac{\Sigma_{i=1}^{S} ni(ni-1)}{N(N-1)} \quad (1)$$

where N is the total number of colonies identified (sample size), n_i is the number of colonies belonging to a certain strain/ species, and S is the total number of different strains/ species in a consortium (Simpson 1949).

Detection of bacteriocin genes

Genomic DNA of consortia 1–25 was extracted according to the Rotiphenol protocol (Roth, Karlsruhe, Germany), following the recommendations of the manufacturer. Then, 500 µl of thawed smear were mixed with 1 ml Rotiphenol. After a proteinase K treatment for digestion of disturbing proteins, the enzyme was heat inactivated. PCR reactions were performed using specific primer pairs for 23 different bacteriocin genes, listed in ESM Table S2 (oligonucleotide primers purchased from Invitrogen, Karlsruhe, Germany). PCR amplification was carried out in a 50-µl reaction mixture containing 1 pmol of specific primers, 200 µM dNTP mixture, 1.5 mM $MgCl_2$, 1 U Thermoprime Plus Polymerase (Thermo Scientific) and 100 ng of template. PCR conditions were as already described (see ESM Table S2). DNA extracts of bacteriocin producing strains, listed in ESM Table S3, served as positive controls. Amplified fragments were resolved by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. Resulting bands were excised and sequenced for verification. For a summary of all positive amplification reactions, see Table 2.

RNA extraction and reverse transcription

Total RNA of consortia tested positive in the above approach was extracted directly from the thawed smear using Trizol (Invitrogen,), following the instructions of the manufacturer. Reverse transcription was performed on extracts standardized to 1 µg RNA by spectroscopic measurements at 260 nm (Nanodrop ND-1000; Peqlab, Erlangen, Germany). The reaction volume of 20 µl contained DNase digested RNA (TURBO DNase; Ambion, Darmstadt, Germany), dNTP mixture (500 µM), DDT (5 mM), RNase OUT (40 U; Invitrogen) and first strand buffer. Then, 1 pmol of reverse primers was added for specific reverse transcription. Samples were completed by the addition of Superscript III (200 U; Invitrogen); in blank samples, no enzyme was added. The incubation conditions

Table 2 Presence of bacteriocin genes in 19 consortia as determined by PCR, using genomic DNA as a template

Consortium no.	Enterocin A	Enterocin B	Cytolysin	Lactococcin G	Helveticin J	Curvacin A	Acidocin B	Nisin
1	+	-	-	+/-	-	-	-	-
2	-	-	-	+	+	-	-	-
3	-	-	-	+/-	+	-	-	-
4	+	+	+	+	-	-	-	-
5a	+	-	-	+/-	-	-	-	-
6	-	-	-	+	+	+	-	-
7	-	-	+	+/-	-	+	-	-
8	+	-	-	+/-	-	-	-	-
11a	-	-	+	+/-	+	+	-	-
12	-	-	+	+/-	-	-	+	-
13	-	-	-	+/-	-	-	+	-
14	-	-	+	-	-	-	-	-
15a	-	-	-	+/-	+	-	-	-
17	-	-	-	+/-	+	-	+	-
18	-	-	-	+/-	-	+	-	+
19	-	-	-	-	+	-	-	-
22	-	-	-	+/-	+	-	-	-
23	+	+	+	+	-	-	+	-
24	-	-	+	+/-	+	-	+	-

Amplification products were sequenced for verification

+ Amplification positive, +/- weak bands, - no amplification product

for the RT reaction were as follows: 25°C for 5 min, 50°C for 90 min, and 70°C for 15 min. The amplification reactions using cDNA as template were carried out as described for the gDNA samples. No PCR amplification could be achieved when RNA samples did not undergo reverse transcription (blank samples) or when water was added instead of template (blind samples).

Results and discussion

Anti-listerial action depends on actively growing consortia

When 5×10^7 CFU/ml *L. monocytogenes* EGDe (serovar 1/2a) were inoculated in the cell-free supernatants, eight consortia (16%) displayed a bactericidal effect and another five (10%) acted bacteriostatically (Table S1). No inhibition was detected when CFS were prepared from microbial consortia cultured only for a 10-min period (data not shown). To the contrary, growth of *Listeria* was often stimulated in this case. It is concluded that, in the frozen smear, no antagonistic substance is present which can be washed off the cells. Actively growing consortia are necessary to produce inhibitory substances. Inhibition of the CFS was generally abolished upon 1:10 dilution in PC3+ (data not shown). The pH values and the concentrations of lactic acid and H_2O_2 determined in anti-listerial CFS (Table 1) were not considered to be listericidal (Gonzalez-Fandos and Dominguez 2006; Romanova et al. 2002; compare Table 1). Therefore, substances other than lactic acid or hydrogen peroxide must be active. In further experiments, the supernatants were heated and subjected to proteolytic digest, respectively (Table 1). The cell counts of viable *Listeria* in treated CFS suggest the presence of either heat-stable, non-proteinaceous molecules (CFS 5a, 6 and 11a) or heat-labile compounds sensitive to proteinase K.

Biodiversity of microbial consortia

Since only a fraction of the microbial consortia inhibited *Listeria*, the anti-listerial activity observed is not just a property of a complex microbial community due to general species competition, but depends critically on the species composition of a cheese microbial community. Therefore, in-depth taxonomical analyses were conducted using FT-IR spectroscopy. This technique has been applied successfully to analyze cheese surface floras even to the strain level (Larpin et al. 2006; Wenning et al. 2006). Cluster analysis allows grouping of strains for comparison of complex bacterial communities. Four anti-listerial and two non-inhibitory consortia were investigated, revealing each flora to have unique properties in terms of species composition and diversity (Table 3). The diversity index of the consortia was calculated according to Simpson, yielding values between 0.88 and 0.97 at the strain level, and between 0.67 and 0.89 at the species level (compare Table 3). As an example, the species composition of consortium 11a, having the lowest biodiversity and being anti-listerial, and consortium 39b, displaying the highest biodiversity but no anti-listerial activity, are shown in Fig. 1 for comparison. The non-inhibitory consortium 25 showed a low diversity

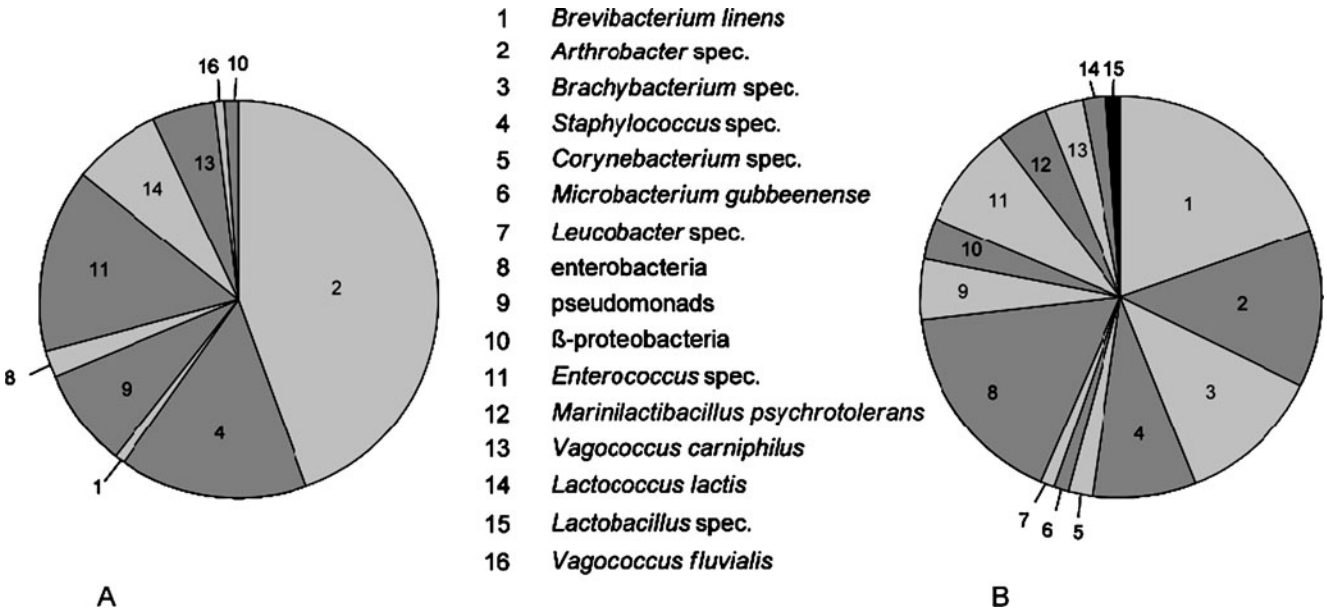

Fig. 1 Biodiversity at the species level of the anti-listerial consortium 11a (**a**) and the non-inhibitory consortium 39b (**b**), when 100 isolates were randomly analyzed

Table 3 Species composition and biodiversity of six microbial ripening consortia

Consortia		6[a]	11a[a]	23[a]	24[a]	25[b]	39b[b]
Number of colonies identified (N)		100	100	250	125	250	100
Coryneform bacteria	*Arthrobacter arilaitensis/ nicotianae/ protophormiae*	20 (9)	45 (9)	3 (4)	46 (19)	46 (9)	13 (10)
	Corynebacterium ammoniagenes/ casei/ variabilis	17 (7)	-	47.5 (7)	9 (4)	-	2 (2)
	Leucobacter komagatae	-	-	16 (2)	-	-	1 (1)
	Brachybacterium arcticum/ paraconglomeratum/ tyrofermentans	-	-	15 (7)	1 (1)	1 (1)	11 (3)
	Brevibacterium linens	1 (1)	1 (1)	2 (3)	-	5 (4)	20 (11)
	Microbacterium gubbeenense	2 (2)	-	5 (5)	-	-	1 (1)
	Staphylococcus equorum/ xylosus/ saprophyticus/ vitulinus	2 (1)	15 (1)	2 (2)	13 (6)	-	8 (6)
	Micrococcus luteus	1 (1)	-	-	-	-	-
	∑	43	61	90.5	69	52	56
Lactic acid bacteria	*Vagococcus carniphilus/ salmoninarum/ fluvialis*	22 (5)	6 (4)	1 (3)	6 (3)	-	3 (1)
	Marinilactibacillus psychrotolerans	16 (9)	-	2 (1)	16 (6)	16 (2)	4 (1)
	Enterococcus spec.	4 (3)	15 (7)	-	-	-	8 (4)
	Lactococcus lactis	3 (2)	7 (1)	-	1 (1)	-	2 (1)
	Lactobacillus spec.	-	1 (1)	-	-	-	1 (1)
	∑	45	29	3	23	16	18
Gram negative bacteria	pseudomonads	2 (1)	8 (2)	4	7 (5)	31 (5)	6 (4)
	enterobacteria	10 (7)	2 (1)	2	1 (1)	1 (3)	17 (5)
	others (ß-proteobacteria, γ-proteobacteria)	-	-	0.5 (1)	-	-	3 (2)
	∑	12	10	6.5	8	32	26
Total number of species/ groups		12	9	12	9	6	15
Diversity based on species		0.85	0.75	0.72	0.74	0.67	0.89
Total number of strains		48	27	41	46	24	53
Diversity based on strains		0.97	0.92	0.90	0.96	0.88	0.97

Species proportions are presented in percent of the total number of colonies identified; numbers in brackets indicate number of strains found for a given species/species group (n_i).

[a] Anti-listerial

[b] non-inhibitory

(the flora consisted of only 24 strains) with a high amount of Gram negative bacteria (32% of all isolates). Consortium 39b, on the other hand, was not anti-listerial although the number of strains reported (53) was the highest for all consortia. Gram negative bacteria accounted for 26% of all isolates in this flora. In the antilisterial consortia 6, 11a, 23 and 24, Gram negative bacteria were less frequently detected (between 6.5 and 12% of all isolates). There was no obvious correlation between biodiversity or species composition on the one hand and anti-listerial potential at the other hand.

Several ripening consortia, differing in their species mixture and also in terms of diversity, have been analyzed in the past. A low diversity and high temporal stability was reported by Goerges et al. (2008) for a Limburger consortium; a high species diversity was reported by Feurer et al. (2004) and Rea et al. (2007) for a French and an Irish smear cheese variety, respectively. There is no evidence that uncultivable organisms contribute significantly to such ripening consortia (Carnio et al. 1999), but the number of subdominant species detected and hence the diversity index may be larger when molecular techniques for species detection in situ are used (Feurer et al. 2004). In recent studies, flora analysis using strain typing methods revealed that species were often present as single clones (Brennan et al. 2004). This was not the case for the bacterial communities examined in this work. An unexpectedly high fraction of lactic acid bacteria (LAB) was detected in consortium 6 (45%, 19 different strains), followed by consortium 11 (29 %, 13 strains) and consortium 24 (23%, 10 strains). While this may partly be due to some carry over of LAB from the cheese curd during sampling of the microbial consortia, it should be noted that the majority of the lactic acid bacteria detected are not members of starter cultures typically used for cheese production (*Lactococcus lactis*, *Leuconostoc* sp.).

Presence of bacteriocin genes in RNA and DNA extracts

Three of the four anti-listerial consortia comprised a high proportion of LAB (Table 3). The ability of these organisms to produce bacteriocins in situ has been exploited to confer microbial safety to red smear cheeses (Izquierdo et al. 2009; Laukova et al. 2001). As shown by Loessner et al. (2003), the addition of *Lactobacillus plantarum* ALC01 culture supernatants containing pediocin resulted in a complete eradication of *L. monocytogenes* from the surface of soft cheeses. These findings encouraged us to investigate the presence of bacteriocin genes in the cheese surface communities. The presence of 23 bacteriocin genes typically produced by *Lactobacillus*, *Lactococcus* and *Enterococcus* species was explored in a PCR-based screening of 25 consortia. First, genomic DNA, extracted directly from the thawed smear, was used as a template. Nine consortia yielded positive results for one bacteriocin, five consortia for two bacteriocins and another five for three or more bacteriocin genes. The eight bacteriocins identified comprised classes I, IIa, IIb, IIc and III according to the classification of Drider et al. 2006 (Table 2). Only six consortia tested negative for all 23 primer pairs applied. Recently, it has been reported by Trmčić et al. (2008) that the microbial consortia of two Slovenian raw milk cheeses harbored three to nine bacteriocin genes, adding further emphasis to the antagonistic potential of complex microbial communities. However, the prevalence of these specific genetic determinants seems to be as equally diverse as the distribution of species in different consortia. Second, for samples tested positive in genomic DNA, the presence of bacteriocin mRNA in the smear was assayed. Amplification using cDNA was successful for only one of the eight genes investigated, i.e., lactococcin G. Products were obtained for five samples (consortia 2, 4, 7, 11a and 12) and could be verified by sequencing. To our knowledge, this is the first time that in situ transcription of bacteriocins on a cheese surface has been demonstrated. In other studies, bacteriocin activity in the food matrix was tested indirectly (Ryan et al., 1996 for lacticin 3147; Benech et al. 2002 for nisin; and Foulquié Moreno et al. 2003 for enterocin). Lactococcin G is produced by *Lactococcus lactis* LMG 2081 and displays a narrow inhibitory spectrum, acting only against other lactococci and *Clostridium* (Nissen-Meyer et al. 1992), but not against *Listeria*. Perhaps, mRNA species of bacteriocin genes detected in the DNA were degraded during storage of the consortia at −80°C.

No bacteriocin gene amplification was achieved when reverse transcribed RNA, which had been extracted after the 8-h incubation period for CFS production, was used as a template (data not shown). The regulation of bacteriocin expression is often mediated via a quorum-sensing mechanism involving peptide pheromones (Kleerebezem, 2004). Upon cultivation in broth, cells were diluted 1:30 and the external stimulus for bacteriocin production may thus have been eliminated and bacteriocin gene expression was down-regulated. Thus, we have no indication yet that the anti-listerial activity reported in this study is due to 1 or more of the 23 LAB bacteriocin genes investigated. It may also be due to unknown or non-investigated LAB bacteriocins or to inhibitory substances produced by coryneform bacteria (Maisnier-Patin and Richard, 1995; Valdes-Stauber and Scherer, 1994) or Gram negative bacteria.

Concluding remarks

In 8 out of 49 complex microbial consortia from the surface of red smear cheeses, the potential to produce highly potent anti-listerial substances was demonstrated. Challenge experiments revealed the participation of proteinaceous molecules but also heat-stable, most likely non-peptide substances, but their chemical nature remains to be determined. Intrinsic microbial food protection, often mediated by natural bacteriocinogenic starter cultures, becomes more and more important due to an increasing consumes awareness of chemical food preservatives. However, the development of resistances to single bacteriocins is a major concern in bacteriocin-based strategies for food preservation. Therefore, complex indigenous microbial ripening consortia, harboring several producers of inhibitory substances or exhibiting unspecific inhibitory activity, are a promising approach to develop an innate anti-listerial capacity while preventing the evolution of resistant pathogenic or spoilage bacteria. However, due to the enormous biodiversity of such consortia, there is still a long way to go in order to understand the chemical basis of anti-listerial activity as well as the temporal stability of the species composition and anti-listerial action.

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