Application of Single Strand Conformation Polymorphism — PCR method for distinguishing cheese bacterial communities that inhibit *Listeria monocytogenes*

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Abstract

The aim of this study was to compare the microbial communities of different cheeses where *Listeria monocytogenes* either grew or did not grow. For this purpose, (i) isolates from the most inhibitory cheese ecosystem were identified and their ability to produce anti-*Listeria* substances was determined, (ii) bacterial communities of cheeses with and without *L. monocytogenes* growth were compared using the Single Strand Conformation Polymorphism method.

The study showed SSCP to be an effective tool for differentiating between the bacterial communities of different cheeses manufactured with the same technology. All the cheeses with the lowest *L. monocytogenes* counts on day 8 were distinguished by the dominance in their SSCP profiles, after amplification of the V2 region of the 16S rRNA gene, of 3 peaks whose nucleotide sequences comigrated with *Enterococcus faecium* and *Enterococcus saccharominimus*, *Chryseobacterium* sp and *Corynebacterium flavescens*, *Lactococcus garvieae* and *Lactococcus lactis* respectively. However, no anti-*Listeria* compounds were produced under our experimental conditions.

These six bacterial species were inoculated, separately or together, into pasteurised milk and their anti-listerial activity in cheese was evaluated. The area of inhibition between the control and trial curves confirmed that *L. monocytogenes* is inhibited by *E. saccharominimus*, *C. flavescens*, *L. lactis*, *L. garvieae* and the mixture of all six bacterial strains.

Further studies should be performed to determine the metabolites involved in *L. monocytogenes* inhibition.

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Keywords: *Listeria monocytogenes*; Inhibition; Raw-milk cheeses; SSCP; Anti-*Listeria* compounds

1. Introduction

The safety of dairy products has been greatly improved throughout the food production chain by the implementation of Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) and by the development and increasing application of the semi-quantitative Hazard Analysis Critical Control Point (HACCP) system in “farm-to-fork” systems.

Safety can also be improved by eliminating milk pathogens by pasteurising or microfiltering raw milk. However, processing milk in these ways may have adverse effects on the typical organoleptic and sensory characteristics of traditional cheeses and does not prevent contamination by certain pathogens such as *Listeria monocytogenes*. Bio-preservation strategies based on the addition of inhibitory bacterial strains have been widely studied. Selecting bacterial strains that produce nisin (Maisnier-Patin et al., 1992; Olasupo et al., 1999) or other bacteriocins (Nunez et al., 1997; Ennahar and Deschamps, 2000; Buyong et al., 1998; Loessner et al., 2003) and/or organic acids (Ramsaran et al., 1998; Morgan et al., 2001; Millet et al., 2006) has been investigated as a method for inhibiting *L. monocytogenes* growth in cheeses. However, effective applications are scarce and the long-term effectiveness...
of inhibitory strains has been questioned, mainly because of the occurrence of pediocin- and nisin-resistant pathogenic strains (Gravesen et al., 2002). A promising strategy could result from recent studies based on the hypothesis that natural flora can compete with food pathogens in cheese. Indeed, it has been shown that some ripening consortia from smear-ripened cheese (Maoz et al., 2003), and microbial communities from raw milk (Millet et al., 2006; Gay and Amgar, 2005) can inhibit L. monocytogenes.

The mechanism of inhibition in smear-ripened cheeses has yet to be understood (Maoz et al., 2003). The inhibition observed by Millet et al. (2006) in some raw-milk cheeses may be partly due to lower pH and acid production, but the microbial populations involved have not been identified. In complex cheese ecosystems, one method for identifying the populations involved in inhibition is to isolate the different strains that make up the bacterial community with anti-Listeria properties (Maoz et al., 2003). Such an approach is time-consuming and the strains may behave quite differently in laboratory media and in food products. A new approach for identifying the microbial populations associated with inhibition can be proposed, based on comparing the microbial communities of raw-milk cheeses where L. monocytogenes grows while others do not. This can be achieved by applying molecular tools that help describe the dynamics of microbial communities as a whole without having to culture any micro-organisms. Among these techniques, Temporal Temperature and Denaturing Gradient Gel Electrophoresis (TTGE and DGGE) (Cocolin et al., 2004; Ercolini et al., 2004; Ogier et al., 2004) and Single Strand Conformation Polymorphism (SSCP) (Duthoit et al., 2003, 2005) have been applied to food microbial ecology with increasing success.

The aim of this study was to identify microbial populations which can be involved in the inhibition of L. monocytogenes in cheeses previously studied by Millet et al. (2006). Two approaches were combined for this purpose. In the first, the isolates from most of the inhibitory cheese ecosystem were identified and their ability to produce anti-Listeria substances was determined. In the second, the SSCP method was used to compare the bacterial communities of cheeses with and without L. monocytogenes growth.

2. Materials and methods

2.1. Cheese samples

The cheese samples were those studied by Millet et al. (2006). As described by those authors, they were manufactured according to a Saint-Nectaire type technology using raw milk from six different farms (F1, F2, F3, F4, F5 and F6). With each milk, cheese was made three times over a one-month period. This study specifically investigated cheeses ripened for 8 days, because the differences in L. monocytogenes counts have been shown to be greatest after this ripening period. Moreover, most of the microbial flora counted in different media have been shown to be at the highest level at day 8 (Millet et al., 2006). Two groups of cheeses were selected according to their respective L. monocytogenes counts. The first group, the F1, F2 and F3 cheeses, consisted of those with the highest L. monocytogenes counts, ranging between 1.26 and 3.58 log (CFU/ml). The second group, F3, F4 and F5, had the lowest L. monocytogenes counts (0.1 to 2.31 log CFU/ml).

2.2. Total genomic DNA extraction

Total genomic DNA was extracted from 1 g cheese samples on day 8 by a phenol–chloroform method including treatment with zirconium beads as described by Duthoit et al. (2003).

2.3. PCR amplification

Total DNA extracted from cheeses sampled on day 8 was amplified with two sets of primers (Table 1): w34–w49 and V2F–V2R.

Forward primers w49 and V2F were labelled with 5'-fluorescein phosphoramidite HEX and reverse primers w34 and V2R with 3'-fluorescein phosphoramidite NED. All the primers were synthesised by Applied Biosystems (Foster City, USA).

PfuUltra™ High-Fidelity DNA polymerase (Stratagene Europe, Amsterdam Zuidoost, Netherlands) was used for all PCR amplifications. PCR was performed in a total volume of 25 μl containing 10 μM of each primer, 0.2 mM of each deoxynucleoside triphosphate, 1 μl of cheese-extracted DNA, 1× buffer and 1.25 U of PfuUltra™ High-Fidelity polymerase.

All the reaction mixtures were subjected to 30 amplification cycles in a thermocycler model 9700 (Perkin Elmer, Applied Biosystems, Foster City, USA). PCR amplification conditions are reported in Table 1. PCR products were electrophoresed on a 0.8% agarose gel, in 0.5× Tris borate EDTA buffer (89 mM

<table>
<thead>
<tr>
<th>Primers Sequence</th>
<th>Amplification conditions</th>
<th>References</th>
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<td>w34 TTACCGCGGCGTGCTGCACTGCAC</td>
<td>3 min at 96 °C</td>
<td>Zunstein et al. (2000)</td>
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<td>w49 ACGTCCAGAATCTACGG</td>
<td>30 cycles of: 30 s at 96 °C; 30 s at 61 °C; 30 s at 72 °C; 10 min at 72 °C</td>
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<td>V2F CGCGAAGGGGGTGAGTA</td>
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<td>Duthoit et al. (2003)</td>
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<td>V2R ACTGCTGCCCCTCGTAG</td>
<td>30 cycles of: 30 s at 96 °C; 30 s at 55 °C; 45 s at 72 °C; 10 min at 72 °C</td>
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<td>w02 NTACCTTGTACGACTT</td>
<td>5 min at 96 °C</td>
<td>Godon et al. (1997)</td>
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<td>w18 GAGTTTGATCGTGGCTCAG</td>
<td>25 cycles of: 60 s at 96 °C; 30 s at 50 °C; 60 s at 72 °C</td>
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Tris base, 89 mM borate, 2 mM EDTA) containing 5% ethidium bromide. DNA bands were visualised under UV light.

2.4. SSCP capillary electrophoresis

PCR products were purified with a Strata Prep™PCR purification Kit (Stratagene Europe, Amsterdam Zuidoost, Netherlands) following the manufacturer’s recommendations. A mixture containing 18.75 μl deionised formamide (Applied Biosystems, Foster City, USA), 0.25 μM internal DNA molecular weight standard Genescan 400 HD Rox (Applied Biosystems, Foster City, USA) and 1 μl purified PCR product was denatured for 5 min at 95 °C and immediately cooled on ice. The mixture was then electrophoresed by SSCP capillary electrophoresis on an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, USA) as described by Duthoit et al. (2003).

2.5. Assignment of dominant peaks

Dominant peaks in the SSCP patterns were assigned by comparing their migration with the migration of cloned 16S rRNA gene sequences, or reference strains, or bacterial strains isolated from Saint-Nectaire cheese, or bacterial strains isolated from retentate raw-milk F5.

A clone library was prepared using genomic DNA extracted from the cheese sample showing the highest number of peaks on its SSCP profile. The 16S rRNA gene was amplified with universal primers w02 and w18. In a total volume of 50 μl, the PCR mixture contained 10 mM Tris HCl (pH 8.5), 25 mM KCl, 5 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1 μM of each primer, 0.2 mM of each deoxynucleoside triphosphate, 1 U of Pwo polymerase (Roche, Applied Science Division, Meylan Cedex, France) and 2 μl of extracted DNA. Amplification was performed as indicated in Table 1.

The amplified product was cloned into pCR-Blunt-II-TOPO vector and transformed in Escherichia coli cells following the manufacturer’s instructions (Invitrogen SARL, Cergy Pontoise Cedex, France). Plasmid inserts of 94 clones were amplified by PCR with M13 forward and M13 reverse.
primers. The 16S rRNA gene clones were sequenced with primer w34 on an ABI prism 310 genetic analyser (Applied Biosystems, Foster City, USA) using the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, USA). Each sequence (approximately 300 bp) was compared with sequences in the GenBank database, using the NCBI BLAST program.

2.6. Statistical analysis

The proportion of peaks present in the SSCP profiles obtained with each primer was calculated using the following formula: \( P_i = a_i \times 100/ \sum a_i \), where \( P_i \) was the ratio of peaks and \( a_i \) the area of one peak.

The relationship between the \( P_i \) values in the (V2F–V2R)–SSCP profiles of cheeses on day 8 was shown as a dendrogram constructed by the Unweighted Pair Group Method using Average (UPGMA) with statistical software (StatSoft 2003, Statistica version 6.1, Tulsa, USA).

Principal Component Analyses (PCA) were carried out with the \( P_i \) values in the (Gram1F–Gram2R)–SSCP profiles of cheeses on day 8 using statistical software (StatSoft 2003, Statistica version 6.1, Tulsa, USA).

2.7. Anti-Listeria activity assessment by the agar-well diffusion method

The anti-Listeria activity of the raw-milk microbial community expressing the strongest Listeria-inhibiting potential in cheese according to Millet et al. (2006) was assessed. The raw milk was then microfiltered on an MFS-1 microfiltration pilot plant with a capacity of 600 l/h (Alpha-Laval, Les Clayes-sous-Bois, France) and the composition of the microbial retentate (raw-milk microbial fraction trapped in a 1.2-μm pore membrane), which is representative of milk microbial community, was analysed. The various microbial populations composing the retentate were cultured in duplicate on 15 types of media (Table 2). All colonies grown on each nutrient agar medium were collected in a 5% solution of sterile glycerol and stored at −20 °C to be tested for their anti-Listeria activity as described below.

Ten to twelve colonies were also randomly picked from each duplicate culture agar medium, for identification. A total of 143 isolates were identified by sequencing approximately 300 bp of the 5′ end of the 16S rRNA gene (as described above) and comparing each sequence with sequences in GenBank using the NCBI BLAST program.

The whole colonies collected on different agar media and the isolates were each grown individually for 24 h in an appropriate broth medium and at a suitable incubation temperature. Culture broths were centrifuged at 5000 g for 15 min at 4 °C. The pH of supernatants obtained was adjusted to pH 6. Supernatants were filtered on a 0.45 μm membrane filter (Minisart Sartorius, Hannover, Germany). The anti-listerial properties of the supernatants were tested using the Tagg and McGiven (1971) agar-well diffusion method modified by the use of a double agar layer. Petri dishes were filled with 20 ml of neutral pH nutrient agar. The top layer consisted of 10 ml of Trypticase Soja–Yeast Extract (TS–YE) agar medium previously inoculated with 0.1 ml of an 18 h-old TS–YE broth culture of L. monocytogenes. Holes (Ø 6 mm) were pierced through the top agar, the wells were filled with 0.1 ml of culture filtrate and the pH was neutralised. The inoculated plates were incubated for 24 h at 37 °C. The diameter of the inhibition zone was measured.

2.8. Anti-Listeria activity assessment in pasteurised milk cheeses

The anti-listerial activities of some species were also evaluated by making pasteurised milk cheeses. The bacterial species tested were selected on the basis of the SSCP results.

All the milks pasteurised at 71 °C for 30 s were inoculated with the starter Streptococcus thermophilus at a concentration of 10^6 CFU/ml and L. monocytogenes strain S2 at 5 to 10 CFU/25 ml. Other species were then inoculated individually into the pasteurised milks as follows.

Chryseobacterium spp., Enterococcus faecium and Enterococcus saccharominimus were inoculated at 10^2 CFU/ml, Corynebacterium spp. at 10^5 CFU/ml, Lactococcus lactis and Lactococcus garvieae at 10^5 CFU/ml. The level of inoculation was chosen according to their bacterial counts in milk F5 (Millet et al., 2006 and unpublished data). One milk was only inoculated with S. thermophilus to act as a control.

Prior to inoculation, all the bacteria were prepared as described above (see above, Anti-Listeria activity assessment by the agar-well diffusion method).

For each set of experiment, seven batches of cheeses were made with milks inoculated in this way, according to Saint-Nectaire type technology.

After one day of ripening, the cheeses were coated with an orange wax containing 1000 ppm of natamycin (Coquard, Villefranche sur Saône, France).

All cheeses were ripened for 28 days in INRA’s ripening cellars under identical conditions, at 10 °C with 95% relative humidity.

The experiment was performed twice over a two-week period.

2.9. Sampling and pH determination of pasteurised milk cheeses

The cheeses were sampled for microbial and chemical analysis on days 1, 8, 18 and 28. For each cheese sample, pH was measured with a 926 VTV pH-meter with Ingold electrode 406 MX (Mettler-Toledo S.A., Viroflay, France). Cheese samples were prepared as described by Millet et al. (2006).

2.10. Enumeration of bacteria in pasteurised milk cheeses

L. monocytogenes concentrations were determined on PALCAM media. The genus and species of five presumed Listeria colonies were then confirmed according to norm ISO 11290-2.

Populations of S. thermophilus were enumerated on M17, Chryseobacterium spp. on PCA + M + I medium (Table 2),
3.1. Identification of isolates and the production of anti-
Listeria compounds by the raw-milk microbial community 
expressing the strongest Listeria-inhibiting potential in 
cheese

One hundred and forty-three isolates were assigned to a 
species, based on a 98% similarity between their partial 16S 
rRNA gene sequences and those in the databases (NCBI 
databases using the BLAST program) (Table 3). Ninety-five 
isolates were identified as Gram-positive bacteria (63 lactic and 
32 non-lactic bacteria), 48 as Gram-negative bacteria. The 143 
isolates were classified into 41 microbial species.

With the agar-well diffusion method we used, none of the 
tests, either with individual isolates or with the retentate or with 
microbial populations collected on 15 agar media, produced any 
L. monocytogenes-inhibiting compound.

3.2. SSCP patterns obtained with w34–w49 primers amplifying 
the V3 region of the 16S rRNA gene

The various cheeses, prepared with three sets of experiments 
with each type of 6 different milks (F1 to F6), were analysed with primer pair w34–w49 on day 8. The V3 region of the 16S rRNA gene of 16 cheeses was amplified. Comparison of the SSCP patterns obtained with these primers revealed a dominant peak, named A ($P_A > 30\%$), in all SSCP profiles regardless of the cheese-making batch studied. As peak A was dominant and present in all SSCP patterns, it hampered the distinction of other, subdominant peaks that might have been useful for distinguishing between the dif-
ferent cheeses’ bacterial communities (data not shown). The 
dominant peak corresponded to the S. thermophilus isolate 
sequence.

3.3. SSCP patterns obtained with V2F–V2R primers amplifying 
the V2 region of the 16S rRNA gene

The different cheeses prepared with three sets of experiment 
with each type of 6 different milks (F1 to F6) were also obtained 
on days 1 and 8 with the V2F–V2R primer pair (Fig. 1). As there 
were no marked differences in SSCP profiles between days 1 and 
8 and for the reasons explained in the Materials and methods 
section above, only SSCP profiles on day 8 were analysed. All 
the 8-day cheeses were classified in a dendrogram according 
to the SSCP pattern peak ratios obtained after amplifying the V2 
region of the 16S rRNA gene (Fig. 2). The cheeses with the 
highest L. monocytogenes counts were clustered in 5 groups 
deﬁned at an aggregative distance of 42: two cheeses prepared in 
two experiments from F2 and F3 were in group V and two 
cheeses from F1 were in group III. However, some F1, F2, F3 
cheeses manufactured in the different experiments were in groups 
II or IV, indicating some variation in bacterial community 
composition from one cheese-making day to another. In contrast, 
cheeses with low L. monocytogenes counts (<2.31 CFU/g) were 
clustered in group I (F4, F5 and F6 cheeses from all three 
experiments). This clustering shows that the balance between the 
different populations in the bacterial communities of these 
cheeses, as ampliﬁed by the V2F–V2R primer pair, was stable 
from one cheese-making day to another.

Peak F was the dominant peak in the SSCP patterns of 
cheeses clustering in groups II and IV, but was proportionately 
smaller in the SSCP patterns of group I, III and V cheeses. There 
was no peak clearly characteristic of the cheeses with the 
highest L. monocytogenes counts. The cheeses in group I were 
distinctive in that peaks C, G and D were dominant in their 
SSCP profiles, whereas these peaks were absent or subdominant 
in the SSCP profiles of cheeses in groups II to V.

Twenty-two clones were analysed to identify the peaks with the 
highest ratios in their SSCP profiles. Considering only 16S 
rDNA sequences with at least 98% similarity with database 
sequences, 52% of clones were identiﬁed as S. thermophilus, 
27% as L. lactis, 3% as Streptococcus parauberis and 14% 
as Streptococcus sp. As diversity was greater with isolate identi-
fication, the peaks were also assigned by comparing their 
migrations with those of the nucleotide sequences of the 
bacterial isolates and reference strains.

Peak F, whose ratios were among the highest in all 
SSCP patterns, comigrated with nucleotide sequences of S. 
thermophilus clones and isolates. Peak C comigrated with 
isolate sequences identiﬁed as E. faecium and E. saccharomi-
minus, and peak G with Chryseobacterium sp. and C. flavescens 
isolate sequences. Similarly, peak D coeluted with nucleotide 
sequences of L. garvieae isolate and also with nucleotide 
sequences of clones and isolates of L. lactis.

3.4. Evaluation of anti-listerial activity in pasteurised milk 
cheeses

The fact that peaks C, D and G have the highest ratios on 
SSCP patterns of cheeses with the lowest L. monocytogenes 
counts suggests that the bacterial species corresponding to
Fig. 1. Example of V2 SSCP patterns of PCR-amplified 16S rRNA gene fragments from bacterial communities of raw-milk cheeses on day 1 (A) and on day 8 (B) with highest counts in *L. monocytogenes* (I) and with lowest counts in *L. monocytogenes* (II). y axis, fluorescence; x axis, elution in scans (unit of Genescan software). F{i} (i from 1 to 6): origin farm of cheeses. f{j} (j from 1 to 3): experiment. Letters from A to G: name of peaks.
these sequences may be involved in \( L. \) monocytogenes inhibition. To verify this hypothesis, the ability of \( E. \) faecium, \( E. \) saccharominimus, Chryseobacterium sp., \( C. \) flavescens, \( L. \) garvieae and \( L. \) lactis to inhibit \( L. \) monocytogenes was evaluated in pasteurised milk cheeses. Cheeses made from milk inoculated with \( S. \) thermophilus, the species that was dominant in cheeses with high \( L. \) monocytogenes counts, acted as a control. \( L. \) monocytogenes, \( S. \) thermophilus and all other bacteria in the cheeses were enumerated (Table 4). All the bacterial species developed mainly during the first day. After day 1, their counts remained more or less stable. The highest counts were observed for \( L. \) garvieae and \( L. \) lactis and the lowest for \( E. \) faecalis. \( E. \) saccharominimus was not detected under our counting conditions. Between days 18 and 28, \( C. \) flavescens showed weaker growth when inoculated in the mixture of 6 bacterial strains than when inoculated alone.

The development of \( L. \) monocytogenes was evaluated by calculating Area of Inhibition (AI) values as described in the Materials and methods section. The AI data and pH values are shown in Table 5. The higher the AI values, the stronger the inhibition of \( L. \) monocytogenes.

At day 1, AI values were not significantly different. After day 1, \( L. \) monocytogenes developed differently depending on the bacterial inoculation. Throughout the ripening, \( L. \) monocytogenes AI values were the lowest in trial cheeses manufactured with Chryseobacterium sp. and \( E. \) faecium. After day 18, \( L. \) monocytogenes was not inhibited in presence of \( E. \) faecium (AI<0).

Between days 1 and 8, AI values were significantly the highest in cheeses manufactured with \( L. \) lactis. Between days 8 and 18, \( L. \) monocytogenes was most inhibited (AI values highest) in presence of \( L. \) garvieae and the mixture of the 6 bacterial strains.

On days 1, 8 and 28, the pH values of the cheeses did not differ significantly. On day 18, the pH value was significantly lowest in the control cheese, whose \( L. \) monocytogenes count was among the highest.

4. Discussion

The SSCP technique was successfully used for monitoring microbial community dynamics in cheeses (Duthoit et al., 2003). This study revealed that SSCP after DNA amplification with the proper primer set can also be an effective tool for distinguishing between cheese bacterial communities. The dominance of one peak on the SSCP profiles resulting from amplification of the V3 region of the 16S rRNA gene shows one of the limitations of the direct molecular approach when analysing bacterial communities in which one species is dominant. In the Saint-Nectaire cheeses studied, \( S. \) thermophilus was dominant since it is routinely used as a starter culture for Saint-Nectaire cheese. Amplification of the V2 region of the 16S rRNA gene proved more relevant for distinguishing the bacterial communities of the different cheeses and for forming a hypothesis on the bacterial flora involved in the inhibition of \( L. \) monocytogenes. The only feature common to the cheeses with the highest \( L. \) monocytogenes counts was the peak assigned to \( S. \) thermophilus. Their SSCP profiles had no other common characteristics. By contrast, all the cheeses with the lowest \( L. \) monocytogenes counts on day 8 were characterised by the dominance of 3 peaks whose nucleotide sequences comigrated with \( E. \) faecium and \( E. \) saccharominimus, Chryseobacterium spp. and \( C. \) flavescens and \( L. \) garvieae and \( L. \) lactis sequences respectively. By inoculating these species in pasteurised milk with \( L. \) monocytogenes, it was shown that not all of them were able to inhibit it in cheeses prepared with these milks. Chryseobacterium spp. and \( E. \) faecium may not be involved in the inhibition under our experimental conditions. Inhibition occurred when \( L. \) lactis and \( L. \) garvieae were inoculated and, to a lesser extent, when \( E. \) saccharominimus and \( C. \) flavescens were inoculated. According to the literature, \( L. \) lactis can inhibit \( L. \) monocytogenes (1) by producing bacteriocins such as nisin and pediocin (Olasupo et al., 1999; Rodriguez et al., 2005), (2) by producing organic acids (Wenzel and Marth, 1990; Hicks and Lund, 1991) and (3) by lowering pH values as observed by Pitt et al. (2000) in pasteurised milk. However, our results reveal that \( L. \) monocytogenes inhibition was not due to a decrease of pH alone, nor to the ability of strains to produce anti-listerial compounds. Lowering pH values below 5.2 was not a sufficient condition to inhibit \( L. \) monocytogenes, although this value has been considered important for controlling \( L. \) monocytogenes in raw-milk Saint-Nectaire cheeses (Millet et al., 2006). A number of studies have found it difficult to identify the compounds involved in the inhibition of \( L. \) monocytogenes in cheeses. Maoz et al. (2003) failed to understand why \( L. \) monocytogenes was inhibited on cheese surfaces as the different microbial isolates of the inhibitory consortium exhibited no anti-listerial activity. Gay and Amgar (2005) suggest that the microbial community had some role in explaining why \( L. \) monocytogenes developed less in raw-milk cheeses than in pasteurised milk cheeses but they did not study the nature of the inhibition (pH, organic acids, bacteriocins, etc.).
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All cheeses were manufactured with pasteurised milk inoculated with S. thermophilus and L. monocytogenes.
Values are the mean of 2 experiments, D: day; ±S: Standard deviation, ND: not detected.
Control: no inoculation of other bacteria, Trial: inoculation with single bacteria, Mixture: inoculation with 6 bacterial species.
L. Listeria, S. Streptococcus, L. Lactococcus, E. Enterococcus, C. Corynebacterium.
Table 5
Al and pH values in pasteurised milk cheeses

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### Acknowledgements

The authors are indebted to ARILAIT RECHERCHES and CNIEL (Centre National Interprofessionnel de l’Economie Laitière) for intellectual and financial support. They also sincerely thank Saint-Nectaire trade cheese. This work was financially supported by the AQ5 program of the French Ministry of Agriculture. English proofreading by Philip Rousseau-Cunningham and Harriet Colman.

### References


