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Design and application of a *Staphylococcus*-specific single strand conformation polymorphism-PCR analysis to monitor *Staphylococcus* populations diversity and dynamics during production of raw milk cheese

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ABSTRACT

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Aim: Development of a nested-PCR single strand conformation polymorphism (SSCP) assay targeting the 16S rRNA genes of the *Staphylococcus* genus, to monitor staphylococci in cheese.

Methods and Results: New primer sets to specifically amplify 16S rDNA of staphylococci were designed to be used in a nested-PCR SSCP assay. The method was efficient in discriminating the staphylococcal species most frequently found in cheese. It was validated by monitoring *Staphylococcus* populations in three productions of raw milk cheese. Analysis of milk samples revealed dominant SSCP peaks corresponding to *Staphylococcus aureus*, *Staphylococcus equorum* and *Staphylococcus saprophyticus*. After 12 h, the *S. aureus* peak became dominant.

Conclusions: The combination of specific *Staphylococcus* nested-PCR and SSCP allows rapid and direct monitoring of staphylococci diversity and dynamics in milk and cheese. In the core of the cheeses studied, *S. aureus* may have ecological advantages against other *Staphylococcus* populations.

Significance and Impact of the Study: This approach is a promising tool to study the ecology of staphylococci in cheeses and in other food samples.

Keywords: 16S rRNA gene, cheese, nested-PCR, single strand conformation polymorphism, *Staphylococcus*.

INTRODUCTION

Staphylococci are of the utmost interest for microbiological safety and quality of traditional cheeses. Coagulase-positive staphylococci such as *Staphylococcus aureus* can produce enterotoxins in foods and may cause toxic infections (Meyrand *et al.* 1998). Many other staphylococcal species such as *Staphylococcus equorum*, *Staphylococcus xylosus*, *Staphylococcus vitulinus*, *Staphylococcus lentus*, *S. fleurettii* and *Staphylococcus saprophyticus* are also found in dairy products (Devriese and De Keyser 1980; Vernozy-Rozand *et al.* 1996) and can be important in the cheese ripening process (Irlinger *et al.* 1997). Food derived *S. aureus* strains have been intensively studied. However, only limited

information is available concerning the ecology of staphylococci populations in cheeses and especially in the core of cheeses.

The PCR-based methods targeting 16S–23S rRNA spacer (Couto *et al.* 2001) or 16S rRNA gene (Mason *et al.* 2001; Vandecasteele *et al.* 2001) were used to identify isolates of staphylococcal human or animal infections. However, methods based on the isolation of staphylococci remain too time-consuming to follow populations dynamics. A few culture-independent studies have been carried out on the microbial diversity of dairy products by using molecular techniques targeting 16S rDNA, such as denaturing gradient gel electrophoresis (DGGE) (Coppola *et al.* 2001; Randazzo *et al.* 2002; Ercolini *et al.* 2003), temporal temperature gradient gel electrophoresis (Ogier *et al.* 2002), and recently single strand conformation polymorphism (SSCP) analysis was applied to describe the bacterial

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community dynamics during the production of RDO Salers cheese (Duthoit *et al.* 2003). Duthoit *et al.* (2003) highlighted the need for a strategy to point out subdominant populations. Staphylococci can remain undetected on bacterial DGGE or SSCP patterns, although they have been recovered from culture plates (Duthoit *et al.* 2003; Ercolini *et al.* 2003).

To our knowledge, no direct method for ecology studies of staphylococci in food products has yet been proposed. The objective of the present study is the development of a *Staphylococcus* genus-specific SSCP-PCR assay based on 16S rRNA gene analysis. The method was validated by investigating the diversity and dynamics of staphylococci during the production and ripening of cheese made from raw milk.

MATERIALS AND METHODS

Milk and cheese samples

Cheese productions chosen for this study are artisanal farmhouse productions of a noncooked semi-hard cheese variety made from cow raw milk. A commercial starter culture was added to the milk along with calf rennet. Coagulation proceeded for about 45 min then the curd was cut, filled into moulds and prepressed to remove the whey. Then, 20 g of salt were added to each cheese and pressing was carried out for about 10 h. Ripening took place at about 10°C and 96% relative humidity for 28 days. Milk and cheese samples were collected in three farms named SA1, SA2 and SA3. Samples were taken aseptically from raw milk, coagulated milk, 12-h cheese, and then from cheese at 1, 5 and 28 days. In each farm, three cheeses from the same production batch were sampled at each stage and the rind was discarded. All samples were stored at -20°C for further bacteriological and molecular analyses.

Enumeration of coagulase-positive *Staphylococcus* in milk and cheese

Cheese samples were emulsified in sterile phosphate buffer, diluted, plated on rabbit plasma fibrinogen (RPF) agar (EN ISO 6888-2, De Buyser *et al.* 2003) and incubated for 24 h at 37°C.

Extraction of total bacterial DNA from milk and cheese

Total bacterial DNA was extracted by a phenol-based method as previously described (Duthoit *et al.* 2003), starting from 1 ml of milk or 1 g of cheese. Cells lysis was performed by blending sample with zirconium beads in a reciprocating shaker and heating at 80°C.

Specific amplification of *Staphylococcus* 16S rRNA gene and SSCP-PCR

The primer Staph 69B (5'-GAACRGAYRAGRAGCTTG-3'), specific for the *Staphylococcus* genus, was selected from a conserved region at the 5' end of the 16S rRNA gene on the basis of sequences of 32 most frequent *Staphylococcus* species available in databases (Genbank and RDP) (Maidak *et al.* 1999). The specific primer Staph 1416 (5'GAAGCCGGTGGAGTAACCA-3') was adapted from Vandecasteele *et al.* (2001).

The primer set Staph 69B degenerated/Staph 1416 was used to amplify the 16S rRNA gene of *Staphylococcus*. The amplification was carried out in a volume of 25 µl containing: 1 µl of genomic DNA solution (2–10 ng of DNA extracted from milk or cheese), 1X PCR reaction buffer, 200 µmol l⁻¹ of each dNTP, 2 mmol l⁻¹ MgCl₂, 0.3 µmol l⁻¹ of Staph 1416, 1 µmol l⁻¹ of Staph 69B degenerated and 0.2 U *Taq* DNA polymerase (Qbiogene, Montréal, Canada). The PCR was performed under the following conditions: 3 min initial denaturation at 94°C; 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 60°C) and extension (1 min 30 s at 72°C); a final extension at 72°C for 10 min. After amplification, the PCR product was purified with the StrataPrep PCR purification kit as specified by the manufacturer (Stratagene, La Jolla, CA, USA).

For SSCP-PCR, owing to the sensitivity of SSCP to single-base changes, a nondegenerated version of primer Staph 69B (Staph 69B NED; 5'-GAACA GATGAGGAGCTTG-3') was used along with the primer V2R HEX (5'-ACTGCTGCCTCCCGTAG-3') in a semi-nested PCR to amplify the V2 region of the 16S rRNA gene of staphylococci. The primers were labelled with 5'fluorescein phosphoramidite: NED for Staph 69B and hexachloro derivative of fluorescein for V2R. The amplification reaction contained 1 µl of purified PCR product, 1X PCR reaction buffer, 200 µmol l⁻¹ of each dNTP, 2 mmol l⁻¹ MgCl₂, 0.5 µmol l⁻¹ of each primer and 1.25 U *Pfu* Ultra DNA polymerase (Stratagene). The PCR was performed under the following conditions: 3 min at 96°C; 25 cycles of 30 s at 96°C, 30 s at 60°C and 45 s at 72°C; a final extension at 72°C for 10 min. Quality of the amplification products was checked on 1.5% (w/v) agarose gel before SSCP electrophoresis.

Staphylococcus strains used as references for SSCP analysis

Fourteen strains belonging to different species of the *Staphylococcus* genus which can be found in milk and cheese ecosystems were selected: *S. xylosus* (CIP 8166 T), *S. vitulinus* (ATCC 51145T), *S. equorum* (CIP 103502 T),

S. lentus (CIP 8163 T), *S. fleurettii* (CIP 106114), *S. saprophyticus* (CIP 76125 T), *Staphylococcus cohnii* (DSM 20260), *Staphylococcus simulans* (DSM 20322), *Staphylococcus capitis* (DSM 20326), *Staphylococcus sciuri* (DSM 20345), *Staphylococcus schleiferi* (DSM 6628), *Staphylococcus hyicus* (DSM 20459), *S. aureus* subsp. *anaerobius* (DSM 20714) and *S. aureus* subsp. *aureus* (CNRZ 873) strains were obtained from the Collection Institut Pasteur, Paris, France (CIP), the American Type Culture Collection, Rockville, MD, USA (ATCC), the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DSMZ) and the Centre National de Recherches Zootechniques, INRA, Jouy-en-Josas, France (CNRZ). Genomic DNA from these strains was isolated from 5 ml of fresh brain-heart infusion cultures by a phenol-based method as described above. In order to prepare a mixture of equal amounts of genomic DNA from each strain, DNA was quantified with PicoGreen (Molecular Probes, Eugene, OR, USA) on a fluorimeter as specified by the manufacturer.

SSCP electrophoresis

The SSCP-PCR products were analysed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Applied, Foster City, CA, USA) as previously described (Duthoit *et al.* 2003).

Partial sequencing of nested-PCR clones and 16S rRNA gene of *Staphylococcus* isolates

The nested SSCP-PCR reaction was performed with unlabelled primers as described above and the PCR product was ligated in pCR4Blunt-TOPO vector and transformed into *Escherichia coli* TOP10 OneShot cells as specified by the manufacturer (Invitrogen, Carlsbad, CA, USA). The 450 bp of the 5' ends, comprising the V2 region, of the 16S rRNA gene of clone inserts and isolates were sequenced by using the dye-terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase FS (Applied Biosystems) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Each sequence obtained was compared with sequences available in databases (Genbank and RDP) (Maidak *et al.* 1999).

RESULTS

Semi-nested PCR and SSCP analysis

New primer sets to specifically amplify 16S rDNA of staphylococci in complex microbial ecosystems and especially in cheese were designed. The first primer set, Staph69B-Staph 1416 allowed amplification of almost the whole 16S rRNA gene. The optimal annealing temperature

was empirically determined by raising the temperature in steps of 1°C from 56 to 66°C and was found to be 60°C. At this temperature, a 1400-bp amplicon was obtained with DNA from the 14 *Staphylococcus* strains tested. A range of nontarget bacteria found in cheese (*Streptococcus thermophilus*, *Lactococcus lactis*, *E. coli* and *Macrocococcus caseolyticus*) was tested to evaluate the specificity of the primers and gave negative results after 35 PCR cycles. This primer set was used in a 35-cycle PCR assay on DNA extracted from milk with low levels of staphylococci (<100 CFU ml⁻¹). No amplicon could be detected on an agarose gel. Hence, staphylococci were under the detection limit of this first PCR but no other species from cheese was amplified after 35 cycles. To improve the sensitivity of the assay for low levels of staphylococci and to generate an amplicon convenient for analysis by SSCP, a semi-nested PCR was performed. The first amplicon was purified in order to remove all remaining oligonucleotides and then served as a template during the semi-nested PCR step using the nondegenerated genus-specific primer Staph 69B NED and a bacterial primer V2R HEX to amplify the V2 region of staphylococcal 16S rDNA.

Figure 1 shows the fingerprints obtained after specific amplification of 16S rDNA of staphylococci via the two PCR steps and SSCP analysis. A 200-bp amplicon was obtained with all of the 14 *Staphylococcus* species used as references and most of them gave rise to distinguishable patterns with the forward strand of the amplicon labelled with Staph 69B-NED, except *S. cohnii* and *S. hyicus* that

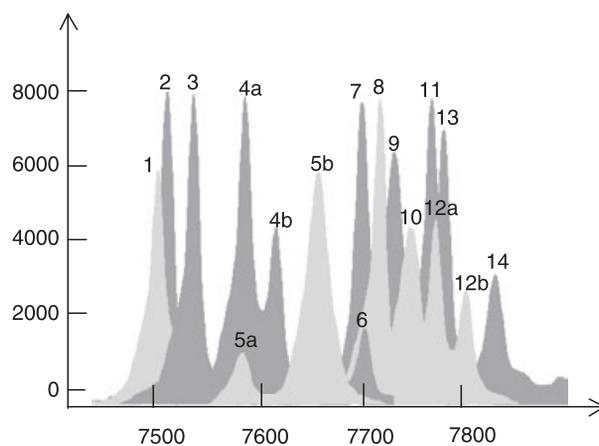


Fig. 1 SSCP analysis of PCR-amplified 16S rRNA gene fragments from reference strains of staphylococci. Comparison of patterns of individual species (forward strand of amplicon). Peak number 1: *Staphylococcus vitulinus*; 2: *S. sciuri*; 3: *S. fleuretti*; 4a,b: *S. lentus*; 5a,b: *S. aureus*; 6: *S. hyicus*; 7: *S. cohnii*; 8: *S. aureus* subsp. *anaerobius*; 9: *S. schleiferi*; 10: *S. capitis*; 11: *S. xylosum*; 12a,b: *S. saprophyticus*; 13: *S. equorum*; 14: *S. simulans*. y axis: fluorescence intensity; x axis: elution in scans (unit of GeneScan software)

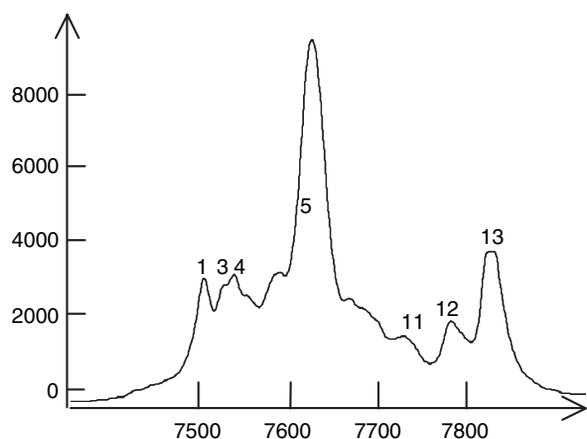


Fig. 2 SSCP pattern obtained after amplification of a mixture of equal amounts of DNA from seven species (forward strand of amplicon). Correspondence between peak numbers and species: see legend of Fig. 1

co-eluted (Fig. 1). Some strains, such as *S. aureus* subsp. *aureus* and *S. lentus*, showed a two-peak pattern. Patterns obtained with the reverse strand (labelled with V2R-HEX) allowed discrimination between *S. cohnii* and *S. hyicus* but showed comigration of the peaks of *S. aureus* and *S. equorum* (data not shown). Consequently, for milk and cheese analysis, only patterns obtained with the forward strand (Staph 69B labelled with NED) are shown. The discriminatory potential of the semi-nested SSCP-PCR was further validated on an artificial mixture of equal amounts of total DNA (5 ng each) from seven strains frequently found in cheese (Fig. 2). All seven strains were successfully detected; however, a higher signal corresponding to the *S. aureus* pattern was noticed.

Diversity and dynamics of *Staphylococcus* populations during manufacture and ripening of cheeses of three farms

The growth kinetics of coagulase-positive staphylococci during cheese making and ripening was determined by enumeration on RPF agar. Coagulase-positive staphylococci reached their maximum in 12-h-old cheese, with an increase of $1.3 \log_{10}$, $1.7 \log_{10}$ and $2 \log_{10}$ in SA1, SA2 and SA3 cheeses, respectively, from the count in raw milk to the count at 12 h. Afterwards, populations either remained stable (cheeses SA1) or decreased slightly (cheeses SA3 and SA2) during ripening.

Staphylococcus-specific SSCP patterns of DNA extracted from coagulated milk were different for each cheese production SA1, SA2 and SA3 (Fig. 3A–C) and comprised three to four main peaks (peaks a–d) with various relative intensities. Patterns from milks SA1 and SA3 were similar, comprising peaks a, c and d in different ratios. The pattern

from milk SA2 distinguished itself by the presence of one additional peak (peak b). Dramatic changes in peak ratio were observed on patterns from 12-h cheeses: peak 'a' became dominant in the three cheese productions (Fig. 3D). No more shift in peak ratio was observed during ripening (28-day cheese, Fig. 3E).

Most of the peaks were identified by comparison with the patterns of the reference species. Peak a/a' was found to correspond to the *S. aureus* pattern. Peak c comigrated with the *S. saprophyticus* pattern and peak d with the *S. equorum* pattern. Peak b did not correspond to any species included in our reference.

In order to confirm this identification through a direct approach, the nested SSCP-PCR products of the coagulated milk and the 28-day cheese from farm SA1 were cloned. Twenty-five clones were analysed by the nested SSCP-PCR assay. Twenty isolates of coagulase-positive staphylococci were also randomly picked from count plates of cheeses from the three farms and subjected to SSCP-PCR analysis. All SSCP patterns from clones and isolates were found to comigrate with the *S. aureus* pattern and with peak 'a' of the cheese patterns, and 16S rDNA sequences all corresponded to *S. aureus* 16S rDNA (ATCC 700699, Genbank accession number AP003363).

DISCUSSION

This study describes the development of a *Staphylococcus* genus-specific SSCP-PCR assay used to investigate the diversity and dynamics of *Staphylococcus* populations during cheese making. To our knowledge, this is the first report of specific amplification of the *Staphylococcus* genus directly from food samples and not from isolates.

Through analysis by the nested SSCP-PCR approach, *S. aureus* gave a two-peak SSCP pattern with a dominant peak. This may be because of intraspecies 16S rRNA operon sequence heterogeneity or to different conformations of the same sequence (Coenye and Vandamme 2003). When applying the PCR assay on an artificial mixture of equal amounts of total DNA from seven species, a higher signal corresponding to *S. aureus* was noticed. This discrepancy may result from preferential amplification of *S. aureus* 16S rRNA gene, differences in genome size of the different species or interspecies 16S rRNA operon copy number heterogeneity (Coenye and Vandamme 2003). According to currently available data about 16S rRNA operon copy number in the *Staphylococcus* genus, four copies were detected in *Staphylococcus carnosus* strain TM300, five copies in *Staphylococcus epidermidis* strain PCI 1200, five in *S. aureus* strains Mu50 and N315, and six in *S. aureus* strains MW2 and BB255 (Klappenbach *et al.* 2001). These observations point out the limits of the SSCP approach and have to be carefully considered while interpreting the results.

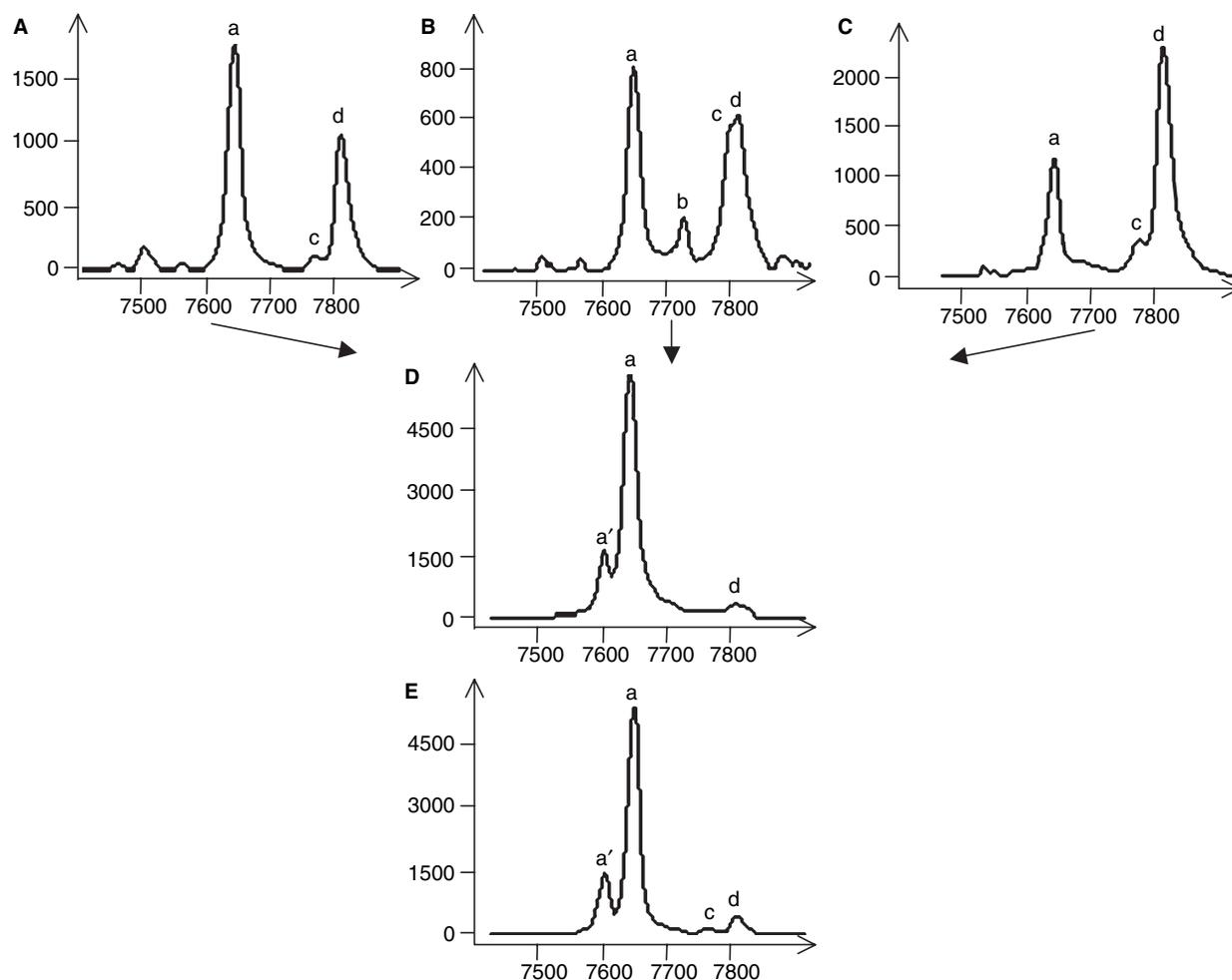


Fig. 3 SSCP patterns obtained after specific amplification of *Staphylococcus* genus 16S rRNA genes from milk and cheeses at different stages of ripening (forward strand of amplicon). (A–C) Coagulated milk from farms SA1, SA2 and SA3 respectively; (D) representative pattern of cheeses SA1, SA2 and SA3 at 12 h; (E) representative pattern of cheeses SA1, SA2 and SA3 at 28 days. Peaks a, a': *S. aureus*; peak b: unidentified; peak c: *S. saprophyticus*; peak d: *S. equorum*

Analysis of the V2 region of the 16S rRNA gene discriminated the staphylococcal species most frequently found in cheese. Other regions of the 16S rRNA gene were not discriminative enough (Takahashi *et al.* 1999; Ercolini *et al.* 2003). The nested PCR–SSCP approach was efficient in distinguishing species such as *S. xylosum*, *S. equorum* and *S. saprophyticus*, for which phenotypic identification is problematic (Irlinger *et al.* 1997; Morot-Bizot *et al.* 2003).

Apart from *S. aureus*, *S. equorum* was the second species of *Staphylococcus* detected by SSCP–PCR in milk from all three farms. *Staphylococcus saprophyticus* was also found at a lower level. We did not find any of the other species used as reference at detectable levels. This diversity of species closely matches the results of conventional or molecular studies in other cheeses, that found *S. equorum* as the prevailing coagulase-negative *Staphylococcus* in cheese, followed by

S. xylosum, *S. saprophyticus* and *S. vitulinus* (Irlinger *et al.* 1997; García *et al.* 2002; Ercolini *et al.* 2003). Our approach has several advantages. It is independent of strain isolation, gives a direct pattern of *Staphylococcus* populations and was efficient in showing the diversity of staphylococcal species present in each milk sample. As a consequence of the possible bias in favour of *S. aureus* as discussed above, peak ratios between the different species from an isolated pattern should be considered cautiously. However, this bias does not prevent the drawing up of conclusions from changes in these ratios in respect to population dynamics. Successive SSCP patterns revealed marked population dynamics with a major increase in *S. aureus* population ratio from milk to cheese at 12 h. This observation was corroborated by the growth kinetics of coagulase-positive staphylococci, as determined on plated medium.

In conclusion, our results suggest that in the core of the cheeses studied, *S. aureus* may have ecological advantages against other *Staphylococcus* populations. The strategy described in the present study allows direct monitoring of staphylococci diversity and dynamics in ecosystems in which these populations can be subdominant, such as milk and cheese. Combined with real-time PCR for a more accurate and culture-independent quantification of *Staphylococcus* populations, this approach may be a helpful tool to study the ecology of staphylococci on the cheeses surface, where coagulase-negative staphylococci grow more particularly (Irlinger *et al.* 1997) and in other food samples.

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REFERENCES

- Coenye, T. and Vandamme, P. (2003) Intragenomic heterogeneity between multiple 16S ribosomal RNA operons in sequenced bacterial genomes. *FEMS Microbiol Lett* **228**, 45–49.
- Coppola, S., Blaiotta, G., Ercolini, D. and Moschetti, G. (2001) Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese. *J Appl Microbiol* **90**, 414–420.
- Couto, I., Pereira, S., Miragaia, M., Sanches, I.S. and de Lencastre, H. (2001) Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. *J Clin Microbiol* **39**, 3099–3103.
- De Buyser, M.L., Lombard, B., Schulten, S.M., In't Veld, P.H., Scotter, S.L., Rollier, P. and Lahellec, C. (2003) Validation of EN ISO standard methods 6888. Part 1 and Part 2: 1999 – Enumeration of coagulase-positive staphylococci in foods. *Int J Food Microbiol* **83**, 185–194.
- Devriese, L.A. and De Keyser, H. (1980) Prevalence of different species of staphylococci on teats and in milk samples from dairy cows. *J Dairy Res* **47**, 155–158.
- Duthoit, F., Godon, J.J. and Montel, M.C. (2003) Bacterial community dynamics during production of registered designation of origin salers cheese as evaluated by 16S rRNA gene single strand conformation polymorphism analysis. *Appl Environ Microbiol* **69**, 3840–3848.
- Ercolini, D., Hill, P.J. and Dodd, C.E.R. (2003) Bacterial community structure and location in stilton cheese. *Appl Environ Microbiol* **69**, 3540–3548.
- García, M.C., Rodríguez, M.J., Bernardo, A., Tornadijo, M.E. and Carballo, J. (2002) Study of enterococci and micrococci isolated throughout manufacture and ripening of San Simón cheese. *Food Microbiol* **19**, 23–33.
- Irlinger, F., Morvan, A., El Sohl, N. and Bergere, J.L. (1997) Taxonomic characterization of coagulase-negative staphylococci in ripening flora from traditional French cheeses. *Syst Appl Microbiol* **20**, 319–328.
- Klappenbach, J.A., Saxman, P.R., Cole, J.R. and Schmidt, T.M. (2001) rrndb: The ribosomal RNA operon copy number database. *Nucleic Acids Res* **1**, 181–184.
- Maidak, B.L., Cole, J.R., Parker, C.T., Garrity, G.M., Larsen, N., Li, B., Lilburn, T.G., McCaughey, M.J. *et al.* (1999) A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res* **27**, 171–173.
- Mason, W.J., Blevins, J.S., Beenken, K., Wibowo, N., Ojha, N. and Smeltzer, M.S. (2001) Multiplex PCR protocol for the diagnosis of staphylococcal infection. *J Clin Microbiol* **39**, 3332–3338.
- Meyrand, A., Boutrand-Loei, S., Ray-Gueniot, S., Mazuy, C., Gaspard, C.E., Jaubert, G., Perrin, G., Lapeyre, C. *et al.* (1998) Growth and enterotoxin production of *Staphylococcus aureus* during the manufacture and ripening of Camembert-type cheeses from raw goats' milk. *J Appl Microbiol* **85**, 537–544.
- Morot-Bizot, S., Talon, R. and Leroy-Setrin, S. (2003) Development of specific PCR primers for a rapid and accurate identification of *Staphylococcus xylosum*, a species used in food fermentation. *J Microbiol Methods* **55**, 279–286.
- Ogier, J.C., Son, O., Gruss, A., Tailliez, P. and Delacroix-Buchet, A. (2002) Identification of the bacterial microflora in dairy products by temporal temperature gradient gel electrophoresis. *Appl Environ Microbiol* **68**, 3691–3701.
- Randazzo, C.L., Torriani, S., Akkermans, A.D.L., de Vos, W.M. and Vaughan, E.E. (2002) Diversity, dynamics, and activity of bacterial communities during production of an artisanal sicilian cheese as evaluated by 16S rRNA analysis. *Appl Environ Microbiol* **68**, 1882–1892.
- Takahashi, T., Satoh, I. and Kikuchi, N. (1999) Phylogenetic relationships of 38 taxa of the genus *Staphylococcus* based on 16S rRNA gene sequence analysis. *Int J Syst Bacteriol* **49**, 725–728.
- Vandecasteele, S.J., Peetermans, W.E., Merckx, R. and Van Eldere, J. (2001) Quantification of expression of *Staphylococcus epidermidis* housekeeping genes with Taqman quantitative PCR during in vitro growth and under different conditions. *J Bacteriol* **183**, 7094–7101.
- Vernozy-Rozand, C., Mazuy, C., Perrin, G., Haond, F., Bes, M., Brun, Y. and Fleurette, J. (1996) Identification of Micrococaceae isolated from goat's milk and cheese in the Poitou-Charente region. *Int J Food Microbiol* **30**, 373–378.