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Staphylococcal community of a small unit manufacturing traditional dry fermented sausages

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Abstract

The level and the diversity of the staphylococcal community occurring in the environment and meat products of a small unit manufacturing traditional dry fermented sausages were investigated at two seasons: winter and spring. Gram-positive cocci were enumerated and a collection of 412 *Staphylococcus* isolates was made. Multiplex PCR, pulse-field gel electrophoresis (PFGE) and sequencing of the *sodA* gene were used to identify and characterize the isolates. High counts of *Staphylococcus* were found in final traditional sausages, reaching about 6 log CFU/g in winter and about 8 log CFU/g in spring. In the environment, the counts varied from 2 log to 7 log/100 cm^2, the higher colonisation being observed on the surface of the drying and cold rooms, cutting tables and the butcher's block. The combination of the three methods allowed the identification of seven species of *Staphylococcus* in spring and five in winter. *S. equorum* and *S. succinus* dominated both in environment and in meat products, 49% and 33% of the isolates, respectively. The other identified species were in decreasing order *S. saprophyticus* (6%), *S. xylosus* (5%), *S. carnosus* (5%), *S. simulans* (1%) and *S. warneri* (1%). The two species *S. xylosus* and *S. carnosus* were sporadically isolated during the spring. PFGE allowed the assignment of *S. equorum* to eight pulsotypes showing a wide diversity among this species. But the entire environment and the meat products were dominated by one pulsotype. For *S. succinus*, three pulsotypes were found with one dominant mainly isolated during the spring sampling. This study highlighted the diversity of staphylococci isolated in the environment and the meat products of a small processing unit manufacturing traditional dry fermented sausages. The *S. equorum* and *S. succinus* species rarely described in meat products and never in the environment had great capacity to colonise the entire small processing unit and the meat products.

Keywords: *Staphylococcus*; Diversity; Sausages; Environment

1. Introduction

Traditional dry sausages are essentially made with pork lean and fat that are mixed with salt, spices and are processed without the addition of starter cultures. The fermentation of these products only relies on the indigenous microbial flora whose composition is variable and the growth promoted by the environmental conditions (Cocolin et al., 2001a; Rebecchi et al., 1998). This indigenous flora is composed by a wide variety of micro-organisms derived from raw materials contaminated during slaughter. This flora could colonize the batter, the products or the environment of the small processing units (Larrouture et al., 2000; Talon et al., 2003). The micro-organisms of technological interest isolated from the indigenous flora of naturally fermented sausages generally belong to the Lactic Acid Bacteria (LAB) group and to the Gram-positive Catalase-positive Cocci (GCC^+) group mainly represented by the *Staphylococcus* and *Kocuria* genera. The LAB ensure the stability of the products mainly by producing lactic acid, which prevents the growth of pathogens. The GCC^+ are involved in color development thanks to the nitrate reductase activity and enhance the sensory properties of fermented sausages mainly through the amino and fatty acid degradations (Talon et al., 1999, 2002).

Among the GCC^+, many staphylococcal species have been isolated from dry fermented sausages. The *Staphylococcus xylosus* and *S. saprophyticus* species were found dominant in Greek fermented sausages (Samelis et al., 1998; Papamanoli et al., 2002; Drosinos et al., 2005). In Spanish sausages, *S. xylosus* was the dominant species (Garcia-Varona et al., 2000) whereas

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in low-acid chorizos the staphylococcal flora was more diversified, dominated by *S. xylosus*, *S. carnosus* and *S. epidermidis* (Aymerich et al., 2003). In Italian sausages, the indigenous flora was composed of *S. xylosus*, *S. saprophyticus* and *S. equorum* but many other species have been identified such as *S. succinus*, *S. warneri*, *S. vitulinus*, *S. pasteuri*, *S. epidermidis*, *S. lentus* and *S. haemolyticus* (Coppola et al., 2000; Cocolin et al., 2001a; Rossi et al., 2001). In traditional French dry fermented sausages, the main species identified were *S. xylosus*, *S. carnosus*, *S. warneri* and *S. saprophyticus* (Montel et al., 1992, 1996).

The identification of staphylococci to the species level by phenotypical methods has limitations and may have resulted in misidentifications (Rhoden and Miller, 1995; Blaiotta et al., 2003). In order to provide increasingly reliable identifications, several molecular methods have been developed, including PCR-based methods such as randomly amplified polymorphic DNA-PCR analysis (Rossi et al., 2001), species-specific PCR (Aymerich et al., 2003; Morot-Bizot et al., 2003), multiplex PCR (Corbière Morot-Bizot et al., 2004), partial sequencing of the highly conserved *tuf*, *sodA* or *hsp60* genes for the identification of species of the genus *Staphylococcus* (Martineau et al., 2001; Poyart et al., 2001; Kwok and Chow, 2003), and oligonucleotide array targeting *sodA* gene (Giammarinaro et al., 2005). Many typing methods have also been used to characterize staphylococci, such as pulse-field gel electrophoresis (PFGE) (Snopkova et al., 1994), ribotyping (Villard et al., 2000), denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments (Cocolin et al., 2001b) and amplification of the 16S-23S intergenic spacer region (ISR) (Rossi et al., 2001; Blaiotta et al., 2003).

Most of the studies on the staphylococcal species isolated from dry fermented sausages focused on the flora of the meat products and its evolution along the process. The *Staphylococcus* house-flora occurring in the environment of processing unit manufacturing naturally fermented sausages has been poorly studied although the environmental flora could contribute to the contamination of the meat products. In this study, we aimed to characterize by molecular methods the staphylococcal flora of a traditional small processing unit through the study of the raw materials, the sausages and the environment. The GCC^+ isolates were subjected to a multiplex PCR identifying the ones belonging to the *Staphylococcus* genus and to the *S. xylosus*, *S. saprophyticus*, *S. epidermidis* and *S. aureus* species. Staphylococci, which did not belong to the former species, were typed and grouped by the PFGE technique. Then, each PFGE group was identified to species level thanks to the partial sequencing of the *sodA* gene. Two species *S. equorum* and *S. succinus* were found to mainly colonize the meat products and environment of this small processing unit.

2. Materials and methods

2.1. Staphylococcal reference species

Strains belonging to the *Staphylococcus* species described in dry naturally fermented sausages flora were used in this study as reference (Table 1). They were grown at 30 or 37 °C on Brain Heart Infusion (BHI) broth or agar (Difco, Detroit, USA).

2.2. Sampling procedures and isolation of strains

A collection of GCC^+ strains was obtained by sampling a small unit manufacturing naturally fermented sausages during two seasons, winter and spring. This unit is located in a medium mountain area in France. Dry sausages were manufactured with pork lean (2/3), pork fat (1/3), NaCl (2.5%), spices (mainly pepper) and without use of starter cultures and nitrate or nitrite. The batter was stuffed into natural casings and ripening was performed as follows: one week of fermentation with a relative humidity (RH) of 76% to 88% at 12 °C and eight weeks of drying with 76% to 86% RH in a natural drying room. The average ambient temperature in the drying room varied to 9.5 °C in winter to 13.5 °C in spring. In the processing room, the average ambient temperature varied to 12.5 °C in winter to 16.5 °C in spring. After processing, the unit was cleaned and decontaminated with a solution of quaternary ammonium at 1% (v/v), pH 9 (Bactégil, Sodevi, Saint Etienne, France). The decontaminating solution was applied 5 min and rinsed.

Samples were collected at 14 control points during winter and spring samplings. Ten surface samples (1 from carcass, 3 from machines, 2 from tools, 4 from environment) were collected by swabbing a surface of 100 cm^2 (Tables 2 and 3). Surfaces and equipments were sampled prior to processing and after cleaning and disinfection. Four samples were collected from the meat products (raw material, batter, sausage after one week of fermentation and sausage after nine weeks). GCC^+ were enumerated by spread plating in Chapman agar (Merck, Darmstadt, Germany) at 30 °C for two days. For each sampling point, 3 to 40 colonies on plates of Chapman agar were randomly selected, plated on BHI agar. The strains were screened by Gram staining and catalase test and stored at −80 °C in BHI broth containing 20% glycerol before being subjected to molecular analysis.

Table 1
Staphylococcal species encountered in dry fermented sausages used as reference

Species or subspecies	Strain
S. aureus	CIP 65.8 T[a]
S. carnosus	DSM 20501 T[b]
S. cohnii	DSM 20260 T
S. epidermidis	DSM 20044 T
S. equorum subsp. *equorum*	DSM 20674 T
S. equorum subsp. *linens*	CIP 107656 T
S. haemolyticus	CIP 81.56 T
S. intermedius	CIP 81.60 T
S. kloosii	DSM 20676 T
S. lentus	CIP 81.63 T
S. pasteuri	CIP 103540 T
S. saprophyticus	CIP 76.125 T
S. simulans	DSM 20322 T
S. succinus	CIP 107307 T
S. vitulinus	CIP 104850 T
S. warneri	DSM 20316 T
S. xylosus	DSM 20266 T

[a] CIP, Collection Institut Pasteur.
[b] DSM, Deutsche Sammlung von Mikroorganismen.

Table 2
Distribution of pulsotypes and identification of the staphylococcal species among the small processing unit during winter sampling

PFGE group	Surfaces and equipment									Meat samples				Total	$sodA_{int}$ identification (% identity)
	Cold room	Wall	Tables	Block	Knives	Mincing machine	Stuffing machine	Drying room	Carcass	Fat/ lean	Batter	Sausage, 1 week	Sausage, 9 weeks		
A			2	3	1			1		1		1	2	11	*S. equorum* (100%)
B	2	1	2	1				1			2	3	2	14	*S. equorum* (100%)
C	2				1	1	7	9	1	3		3	3	30	*S. equorum* (98%)
D		1	4		1			2	3			1		12	*S. equorum* (98%)
E	2		1	2	1			5		1		2	2	16	*S. equorum* (99%)
F	2		1											3	*S. equorum* (98%)
G								3		1		1		5	*S. equorum* (98%)
H			1	1	4			2		2				10	*S. equorum* (99%)
J			1											1	*S. simulans* (97%)
L	1		2	1										4	*S. succinus* (99%)
M							2							2	*S. succinus* (100%)
N	1		1				2	2		1		1	3	11	*S. succinus* (100%)
P													1	1	*S. warneri* (100%)
															PCR identification
			4			2	4							10	*S. saprophyticus*
Total	10	2	19	8	8	3	15	25	4	9	2	12	13	130	

A total of 436 GCC^+ were isolated, 149 and 287 strains from the winter and spring samplings, respectively.

2.3. PCR multiplex protocol

The 436 GCC^+ isolates grown on BHI agar medium for 24 h at 30 °C were submitted to multiplex PCR. Amplifications were performed from one colony of each isolate with the primers TstaG422, Tstag765, Se705-1, Se705-2, Sa442-1, Sa442-2, XYL F, XYL R, Sap1 and Sap2 allowing the identification of the *Staphylococcus* genus and of the *S. epidermidis*, *S. aureus*, *S. xylosus* and *S. saprophyticus* species, respectively, as previously described (Corbière Morot-Bizot et al., 2004).

2.4. Typing of the isolates by PFGE

The 366 *Staphylococcus* spp. isolates unidentified by the multiplex PCR and the reference strains were submitted to PFGE analysis. Agarose plugs containing chromosomal DNA were prepared, digested with the endonuclease *Sma*I (Promega, Lyon Charbonnières, France) according to the manufacturer's instructions and separated by PFGE in a contour-clamped homogeneous electric field apparatus CHEF-DRIII (Bio-Rad, Ivry, France) as previously reported (Morot-Bizot et al., 2003). The pulse-field program was defined as follows: (i) pulse times of 10 to 30 s, 6 V/cm, with a running time of 23 h, and (ii) pulse times of 50 to 100 s, 6 V/cm, with a running time of 1 h. Lambda ladder (Promega) was used as molecular weight marker. Gels were stained with ethidium bromide and digitalized with the Gel Doc 2000 apparatus (Bio-Rad). Analysis of *Sma*I macrorestriction profiles and phylogenetic analyses were performed with Diversity Database software (Bio-Rad). Isolates with a DNA band pattern differing by more than three band shifts were interpreted as being genetically unrelated and of different pulsotype (Tenover et al., 1995). The pulsotypes were assigned a distinct capital letter. Cluster analyses using the Dice correlation for band matching with a 2% position tolerance and a hierarchic unweighted pair group method (UPGMA) were used to generate a dendrogram.

2.5. DNA sequence analysis of the $sodA_{int}$ gene

The internal fragment of *sodA* gene ($sodA_{int}$) from a representative strain of each pulsotype was amplified by the d1 and d2 primers as reported by Poyart et al. (2001). The PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and both strands were directly sequenced with the d1 and d2 oligonucleotides by using the 'BigDye terminator cycle sequencing ready reaction' kit with an automatic DNA sequencer 'ABI PRISM 310' (Perkin-Elmer). The sequences were aligned with those in GenBank with the BLAST program to determine the closest known relatives of the $sodA_{int}$ sequence obtained (Altschul et al., 1990).

2.6. Accession numbers

The GenBank accession numbers of the partial *sodA* sequences of *Staphylococcus* isolates belonging to pulsotypes A to Q are AY818174, AY821519, AY818175, AY818176, AY818177, AY818178, AY818179, AY818180, AY795886, AY795887, AY795888, AY795892, AY795893, AY795894, AY795889, AY795890 and AY795891, respectively.

3. Results

3.1. GCC^+ counts

The counts and distribution of GCC^+ are shown in Fig. 1. All the environmental samples were colonized by the GCC^+ flora with counts ranging between 2×10^2 and 1×10^7 CFU/100 cm^2. The GCC^+ counts did not differ (<1 log CFU/100 cm^2) between the winter and spring samplings but the tables, which were more

contaminated in spring. The most colonized environmental samples were the surfaces of drying room and the butcher's block. The counts of GCC^+ in the raw materials (fat and lean) were similar in winter and spring samplings (Fig. 1). However, the level of GCC^+ was higher in the batter in spring compared to the winter samples (3×10^5 versus 1×10^2 CFU/g). Then, in both sampling periods, during the first week of fermentation the GCC^+ flora quite rapidly increased to 7×10^7 and 2×10^5 CFU/g in spring and winter, respectively. During the eight following weeks of process the GCC^+ continued to grow slowly. At the end of ripening the counts of GCC^+ were 2 log CFU/g higher in spring than in winter (2×10^8 versus 2×10^6 CFU/g).

3.2. Identification of Staphylococcus isolates by PCR

The 149 and 287 GCC^+ strains collected during the winter and spring samplings, respectively, and isolated from the environment and meat samples of the small processing unit were analyzed by multiplex PCR. Among the 436 isolates, 412 gave a PCR product of 370 bp, confirming that they belonged to the *Staphylococcus* genus and 24 did not give any fragment, showing that they belonged to another genus of Gram-positive cocci (data not shown). These 24 isolates were not further categorized. Among the 412 *Staphylococcus* isolates, 24 were identified as *S. saprophyticus* by producing a specific PCR product of 221 bp (data not shown). Ten *S. saprophyticus* isolates were recovered from the winter sampling (Table 2). They were only isolated from environment samples. From spring sampling, 14 other *S. saprophyticus* isolates were recovered from the environment and meat samples (Table 3). Twenty two isolates were identified as *S. xylosus* by giving a specific PCR product of 539 bp (data not shown). All of the *S. xylosus* isolates were recovered from the spring sampling (Table 3). Fourteen isolates were recovered from the environment. From the meat samples, 8 isolates were found but only in the batter. *S. aureus* and *S. epidermidis* were never isolated from any location.

3.3. Staphylococcal pulsotypes and cluster analysis

The 366 *Staphylococcus* isolates unidentified by multiplex PCR (120 and 246 from winter and spring samplings, respectively) were characterized by PFGE after *Sma*I restriction of DNA. They were divided into 17 distinct PFGE patterns named pulsotypes and designated A to Q (Fig. 2). The dominant pulsotypes were C and N, recovering 21% (77 of 366) and 32% (117 of 366) of the staphylococcal isolates, respectively. Each of the remaining pulsotypes represented less than 10% of the isolates. In order to evaluate the relatedness between each of the pulsotypes determined, a cluster analysis was performed and a phylogenetic tree was constructed (Fig. 3). The pulsotypes of the reference strains belonging to the most frequently staphylococcal species encountered in fermented sausages (Table 1, Fig. 2) were included in the analysis. The analysis of pulsotypes revealed that the different groups of isolates and the reference strains were poorly clustered with less than 40% relatedness among the pulsotypes. The low similarity observed between the different pulsotypes is in accordance with the results obtained by Snopkova et al. (1994) showing the wide diversity of PFGE patterns among the staphylococcal species. Only two pulsotypes among the 17 were closely related to reference strains, such as the pulsotype N, which was related to the *S. succinus* reference strain showing a similarity of 63% and the pulsotype I, which was 100% identical to pulsotype of the *S. carnosus* reference strain.

Table 3
Distribution of pulsotypes and identification of the staphylococcal species among the small processing unit during spring sampling

PFGE group	Surfaces and equipment											Meat samples				Total	$sodA_{int}$ identification (% identity)
	Cold room	Wall	Tables	Block	Knives	Bowls	Mincing machine	Mixing machine	Stuffing machine	Drying room	Carcass	Fat/ lean	Batter	Sausage, 1 week	Sausage, 9 weeks		
A				1			3	2			1		1	3	2	13	*S. equorum* (100%)
B			1	1			2	4		2				2	3	15	*S. equorum* (100%)
C	4	1	10	2	3	5	1	1		5		1		2	12	47	*S. equorum* (98%)
D					1	1				4		1		1		8	*S. equorum* (98%)
E	2		4				1	1		2		1		2		13	*S. equorum* (99%)
F			1													1	*S. equorum* (98%)
G	1		1													2	*S. equorum* (98%)
H															1	1	*S. equorum* (99%)
I						1				18						19	*S. carnosus* (99%)
J				1							1		1			3	*S. simulans* (97%)
K													1			1	*S. simulans* (98%)
L	11								1							12	*S. succinus* (99%)
M												1				1	*S. succinus* (100%)
N	6	1	13	4	4	7	9	11	11	5	3	13	9	9	1	106	*S. succinus* (100%)
O				1									1	1		3	*S. warneri* (99%)
Q														1		1	*S. warneri* (97%)
																	PCR identification
			1		2			1		4		2	3		1	14	*S. saprophyticus*
	3		1			1		3		6			8			22	*S. xylosus*
Total	27	2	32	10	10	15	16	23	12	46	5	19	24	21	20	282	

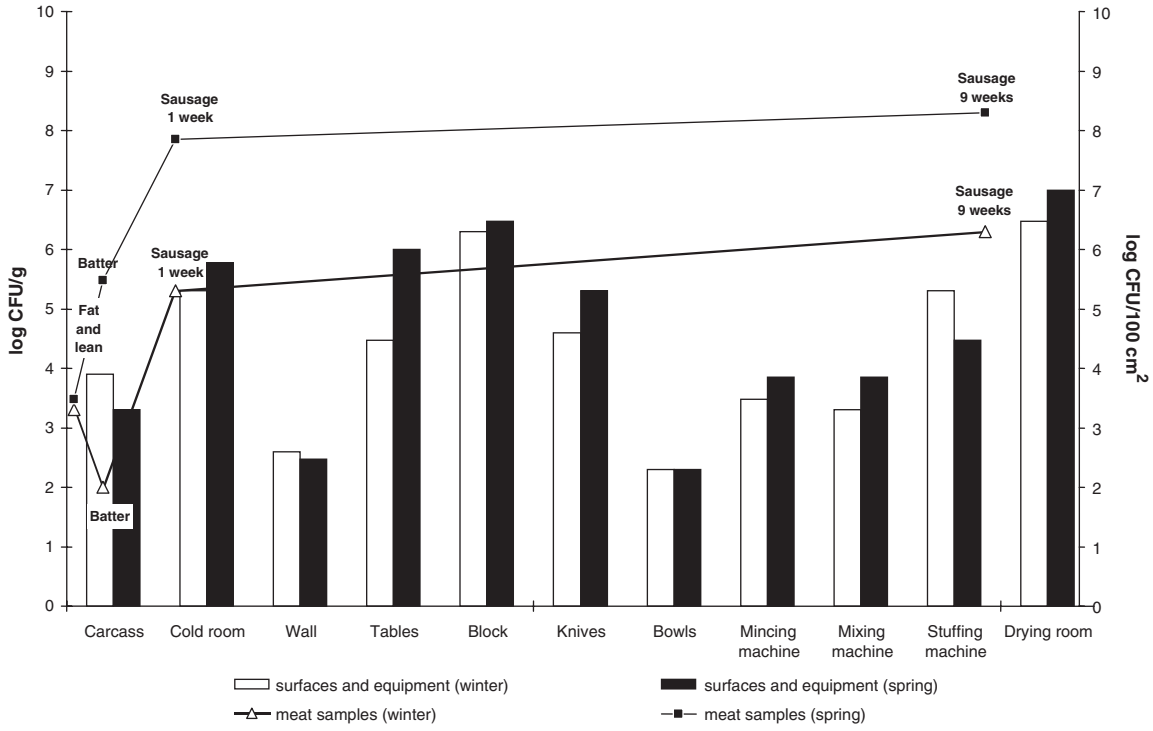

Fig. 1. GCC+ counts from samples taken throughout the environment and the meat products of the small processing unit.

3.4. Identification of Staphylococcus isolates by $sodA_{int}$ sequencing

In order to identify at the species level the strains belonging to the different pulsotypes, the $sodA_{int}$ gene sequences of one representative strain per pulsotype were determined. The comparisons of sequences were done using BLAST (Altschul et al., 1990). The strains belonging to the pulsotypes A to H were identified as *S. equorum*, the $sodA_{int}$ sequences of representative strains showing identity values of 100% to 98% (Tables 2 and 3).

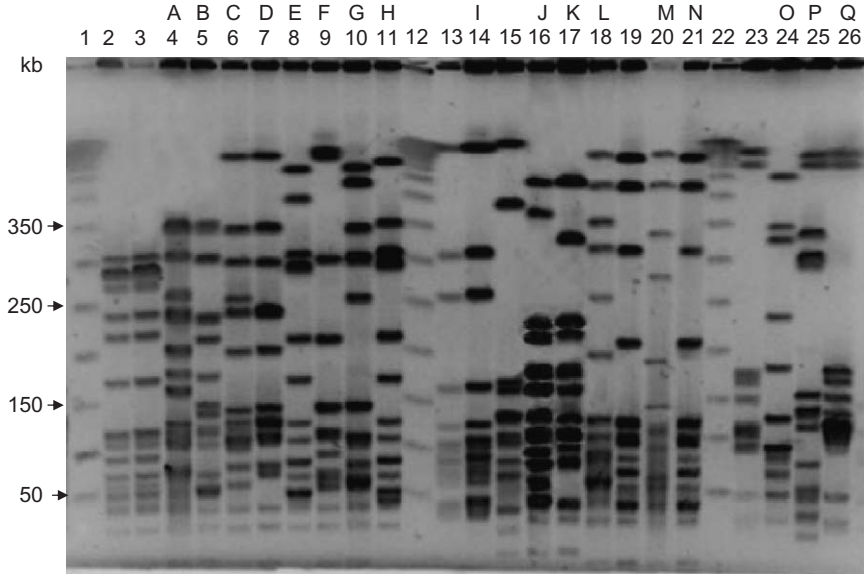

Fig. 2. PFGE profiles of *Sma*I restricted genomic DNA of the *Staphylococcus* isolates and reference strains. Lanes 1, 12 and 22: Lambda ladder (Promega); lane 2: *S. equorum* subsp. *linens* CIP 107656 T; lane 3: *S. equorum* subsp. *equorum* DSM 20674 T; lane 4: CIT S00-004 (pulsotype A); lane 5: CIT S00-034 (B); lane 6: CIT S00-175 (C); lane 7: CIT S00-422 (D); lane 8: CIT S00-024 (E); lane 9: CIT S00-130 (F); lane 10: CIT S00-092 (G); lane 11: CIT S00-023 (H); lane 13: *S. carnosus* DSM 20501 T; lane 14: CIT S00-298 (I); lane 15: *S. simulans* DSM 20322 T; lane 16: CIT S00-420 (J); lane 17: CIT S00-437 (K); lane 18: CIT S00-032 (L); lane 19: *S. succinus* CIP 107307 T; lane 20: CIT S00-161 (M); lane 21: CIT S00-182 (N); lane 23: *S. warneri* DSM 20316 T; lane 24: CIT S00-147 (O); lane 25: CIT S00-237 (P); lane 26: CIT S00-438 (Q).

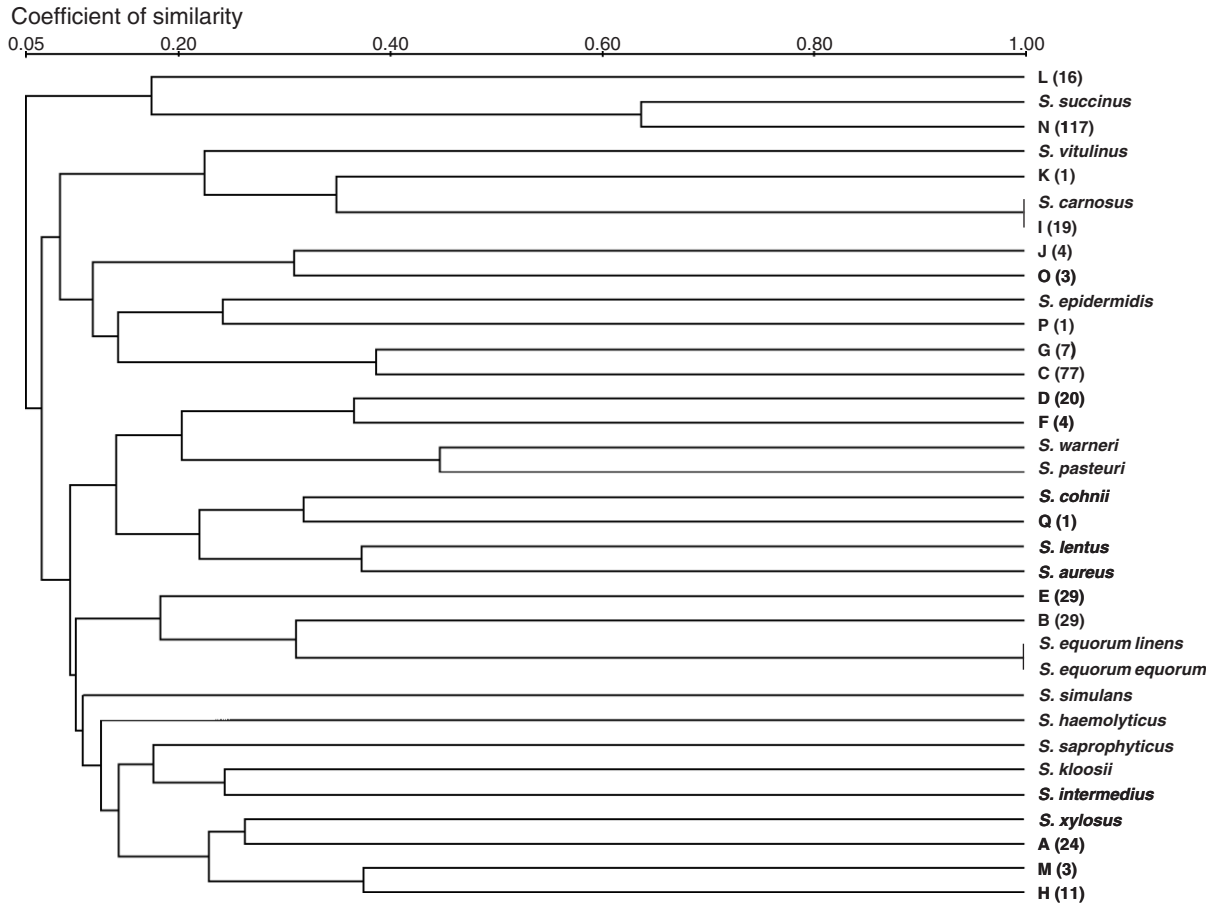

Fig. 3. Relatedness of the staphylococcal pulsotypes analyzed with the Dice similarity coefficient and the UPGMA method. The dendrogram was constructed with 2% band tolerance. The letters A to Q indicate the pulsotypes and the figures in brackets represent the number of isolates found in each pulsotype.

The representative strain of the pulsotype I which was identical to the pulsotype of the *S. carnosus* reference strain was confirmed to belong to the *S. carnosus* species, the $sodA_{int}$ sequence showing identity value of 99%. The strains of pulsotypes J and K were identified as *S. simulans*, the $sodA_{int}$ sequences of the two representative strains showing identity values of 98% to 97%. The strains belonging to the pulsotypes L to N were identified to *S. succinus*, the $sodA_{int}$ sequences of representative strains showing identity values of 100% to 99%. The strains of pulsotypes O and Q were identified to *S. warneri*, the $sodA_{int}$ sequences of the representative strains showing identity values of 100% to 97%.

3.5. Distribution of the staphylococcal pulsotypes in the environment and the meat products

The distribution of the pulsotypes of the staphylococcal isolates recovered from samples is shown in the Tables 2 and 3. Twelve of the pulsotypes (A to H, J, L, M, N) were found independently of the period of sampling, whereas the pulsotype P was sporadic in winter and the pulsotypes I, K, O and Q were sporadic in spring. From the winter sampling, the isolates displayed 13 different pulsotypes. All of the winter sampling points were colonized by staphylococci except the bowls and the mixing machine. The pulsotype C was predominant in winter representing 25% of the isolates (30 of 120). This pulsotype was relatively ubiquitous and colonized surfaces, equipment and meat products. The B and E pulsotypes represented 12% and 13% of the isolates, respectively, and were found in most samples. From the spring sampling, the isolates displayed 16 different pulsotypes. All of the sampling points were colonized by staphylococci. The pulsotype N was dominant with 43% of the spring isolates (106 of 246). The pulsotype C was the second one with 19% of the isolates showing this pattern (47 of 246). These two pulsotypes were relatively ubiquitous because they were found from almost all sampling points. The pulsotypes F, G, I and L only colonized the environment whereas the H, M and Q pulsotypes were only found in the meat samples. No correlation could be established between the pulsotypes and the origin of sample.

4. Discussion

In this study, the diversity of the staphylococcal community of a small unit manufacturing traditional dry fermented sausages was evaluated by the identification and characterization of 412 *Staphylococcus* strains isolated from the meat products and the environment. Counts of GCC^+ colonies dominated by staphylococci (94%) ranged from 1×10^2 to 3×10^5 CFU/g in the batter to 2×10^6 to 2×10^8 CFU/g in the sausages at the end of ripening.

The level of GCG^+ in the final product was comparable with the level observed in the artisanal low-acid sausages (fuets and chorizos), which ranged from 2×10^5 to 1×10^8 CFU/g (Aymerich et al., 2003). In the environment counts of GCC^+ colonies ranged from 2×10^2 to 1×10^7 CFU/100 cm^2. The density of colonization was relatively high in the entire small processing unit which offered conditions suitable for the survival of the staphylococci. The most colonized samples of environment were the surfaces of drying and cold rooms, the butcher's block and the cutting tables. To our knowledge, the evaluation of the GCG^+ colonization of the environment of food plants manufacturing dry sausages had not been studied previously.

The *Staphylococcus* isolates were identified by using multiplex PCR, PFGE patterns and determination of the $sodA_{int}$ gene sequences. The combination of these methods allowed the identification of seven *Staphylococcus* species and showed that the staphylococcal flora of the environment and meat products of this small processing unit was dominated by the *S. equorum* and *S. succinus* species, 49% and 33% of the isolates, respectively. These two species dominated over the other staphylococci species, namely *S. saprophyticus*, *S. xylosus*, *S. carnosus*, *S. simulans* and *S. warneri*. During the winter sampling, only five species among the seven were found: *S. equorum*, *S. succinus*, *S. saprophyticus*, *S. simulans* and *S. warneri*. The season had an effect on the ambient temperature of the processing unit. The higher temperature in the unit in spring (>4 °C) could explain the different repartition of the staphylococci species during the two seasons.

The dominant *S. equorum* and *S. succinus* species were ubiquitous and isolated in the environment and in the meat products. These species have been already described in the flora of fermented sausages. Blaiotta et al. (2004) have studied the diversity of staphylococci from naturally fermented sausages and found that about 10% of the strains belonged to *S. equorum* and less than 2% to *S. succinus* species (Blaiotta et al., 2004). In other Italian traditional fermented sausages, Mauriello et al. (2004) found that *S. equorum* was one of the most common species and could represent up to 40% of the staphylococcal flora. The *S. equorum* species was not yet often described in fermented sausages but was frequently described in other foods such as cured hams and cheeses (Blaiotta et al., 2004). Moreover, some authors agreed with the fact that this species can be misidentified because of the difficulty of separating this species from *S. xylosus*, mostly when the identification is based on phenotypic methods (Blaiotta et al., 2004; Mauriello et al., 2004). The 201 strains of *S. equorum* isolated in this study belonged to eight distinct unrelated pulsotypes (A to H) showing a wide diversity among this species. However the dominant pulsotype C with 77 clones was found in the environment of the small processing unit and in the meat products. The presence of a dominant pulsotype indicates that some strains may be capable for adaptation to food plant environment and processing and for survival in the small processing unit. The presence of eight other pulsotypes suggests a continuous source for colonization of this small processing unit. One hundred and thirty six strains of *S. succinus* were also isolated in this study. The *S. succinus* species has been rarely described in food. It was found at low levels in the smear cheese flora (Place et al., 2002) in Italian fermented sausages (Blaiotta et al., 2003, 2004) and in fresh sausages (Rantsiou et al., 2005). The strains isolated belonged to three pulsotypes but one was mainly dominant with 86% of strains. These strains were largely isolated during the spring sampling suggesting a better colonization or survival when the outside temperature increased.

The 75 remaining isolates were identified as *S. saprophyticus* (24 strains), *S. xylosus* (22), *S. carnosus* (19), *S. simulans* (5) and *S. warneri* (5). The *S. saprophyticus* species has been already described in other studies, essentially in Greek fermented sausages in which this species was found dominant (Samelis et al., 1998; Papamanoli et al., 2002; Drosinos et al., 2005). The *S. warneri* and *S. simulans* species have also sporadically been isolated from Italian traditional fermented sausages (Blaiotta et al., 2004; Mauriello et al., 2004). The two technologically important species, *S. xylosus* and *S. carnosus*, occurred at a relatively low percentage (<10%) of the isolates and were sporadically isolated during the spring sampling. In many studies, *S. xylosus* was the predominant species of staphylococci isolated from naturally fermented sausages (Blaiotta et al., 2004; Mauriello et al., 2004; Drosinos et al., 2005). Surprisingly, in this study, *S. xylosus* was not frequently isolated. But more surprisingly, the *S. xylosus* and *S. carnosus* strains were never isolated in the final products even though some strains of *S. xylosus* were isolated in the batter before the fermentation.

In conclusion, the study of a small unit manufacturing naturally fermented sausages showed that there is a variety of staphylococci found in meat and food plant environment with seven species identified. The analysis of the staphylococcal flora at two seasons pointed out differences in the diversity of species through the seasons, because more species were found in spring than in winter.It revealed the occurrence of two species, *S. equorum* and *S. succinus*, which are rarely described in the meat products and never in the environment. These species showed a high capacity to colonize the surfaces, the equipment and the meat products. A further study should be done in other small processing units manufacturing traditional dry fermented sausages to determine the prevalence of these two species in such environments.

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