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Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units

Sabine Leroy, Philippe Giammarinaro, Jean-Paul Chacornac, Isabelle Lebert, Régine Talon*

INRA, UR 454 Microbiologie, F-63122 Saint-Genès Champanelle, France

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ABSTRACT

The staphylococcal community of the environments of nine French small-scale processing units and their naturally fermented meat products was identified by analyzing 676 isolates. Fifteen species were accurately identified using validated molecular methods. The three prevalent species were *Staphylococcus equorum* (58.4%), *Staphylococcus saprophyticus* (15.7%) and *Staphylococcus xylosus* (9.3%). *S. equorum* was isolated in all the processing units in similar proportion in meat and environmental samples. *S. saprophyticus* was also isolated in all the processing units with a higher percentage in environmental samples. *S. xylosus* was present sporadically in the processing units and its prevalence was higher in meat samples. The genetic diversity of the strains within the three species isolated from one processing unit was studied by PFGE and revealed a high diversity for *S. equorum* and *S. saprophyticus* both in the environment and the meat isolates. The genetic diversity remained high through the manufacturing steps. A small percentage of the strains of the two species share the two ecological niches. These results highlight that some strains, probably introduced by the meat, will persist in the manufacturing environment, while other strains are more adapted to the meat products.

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1. Introduction

The production of fermented products is one of the oldest food processes known to humans. Even if fermentation has today become an industrial process controlled by the use of starter cultures, some traditional fermentation processes remain in use. These traditional dry fermented sausages are still produced without the addition of selected starter cultures. The fermentation of these meat products only relies on indigenous microflora whose composition is variable. The contamination of raw materials occurs during slaughtering and the level of microbial flora increases during manufacturing promoted by the environmental conditions (Rebecchi et al., 1998; Cocolin et al., 2001a). The microflora of the traditional dry fermented sausages is composed of a wide variety of micro-organisms. Those of fundamental technological interest belong to two groups, the lactic acid bacteria (LAB) and the gram-positive catalase-positive cocci (GCC^{+}), specifically coagulase-negative staphylococci (CNS). LAB, mainly through lactic acid production, are responsible for the decrease in pH, which contributes to the firmness of the dry fermented sausages and ensures their stability by preventing the growth of pathogens (Leroy et al., 2006). CNS contribute to the development and stability of the color and avoid the rancidity of sausages due to their anti-oxidant activities (Barrière et al., 2001, 2002). They enhance the flavor of fermented sausages mainly through amino and fatty acid degradations (Talon and Leroy, 2006). CNS are ubiquitous in nature. They are natural hosts of skin and mucous membranes of warm-blooded animals, but are also isolated from a wide range of foods such as meat, milk and cheese, and from environmental sources (Talon et al., 2007; Irlinger, 2008). They are part of the microflora of traditional cheeses (Irlinger et al., 1997; Ercolini et al., 2003; Irlinger, 2008) and dry fermented sausages (Blaiotta et al., 2003; Rantsiou et al., 2005). The staphylococcal ecosystem of traditional dry fermented sausages is diverse. In Italian sausages, it is mainly composed of *Staphylococcus xylosus*, *Staphylococcus saprophyticus* and *Staphylococcus equorum*, but other species have been identified such as *Staphylococcus succinus*, *Staphylococcus warneri*, *Staphylococcus vitulinus*, *Staphylococcus pasteuri*, *S. epidermidis*, *Staphylococcus lentus* and *Staphylococcus haemolyticus* (Coppola et al., 2000; Cocolin et al., 2001a; Rossi et al., 2001). *S. xylosus* and *S. saprophyticus* are dominant in Greek fermented sausages (Samelis et al., 1998; Papamanoli et al., 2002; Drosinos et al., 2005). In Spanish sausages, *S. xylosus* can be the dominant species (Garcia-Varona et al., 2000), whereas in low-acid chorizos the staphylococcal flora is more diversified, dominated by three species, *S. xylosus*, *Staphylococcus carnosus* and *S. epidermidis* (Aymerich et al., 2003). In traditional French dry

* Corresponding author. Tel.: +33 4 73 62 41 70; fax: +33 4 73 62 45 81.
E-mail address: regine.talon@clermont.inra.fr (R. Talon).

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fermented sausages, the main identified species were *S. xylosus*, *S. carnosus*, *S. warneri* and *S. saprophyticus* (Montel et al., 1996), and in another study, *S. equorum* and *S. succinus* were dominant (Corbière Morot-Bizot et al., 2006). While the staphylococcal species isolated from natural dry fermented sausages have been relatively well identified, the *Staphylococcus* house-microflora surviving in the environment and the equipment of processing units (PUs) manufacturing naturally fermented sausages has been poorly studied, although this microflora could contribute to the contamination of meat products (Corbière Morot-Bizot et al., 2006).

In this study, we have accurately identified the staphylococcal species of nine small-scale units manufacturing traditional dry fermented sausages through the meat products at different steps of manufacturing, the equipment and the environment. First, the GCC$^+$ isolates were subjected to a genus-specific PCR to identify the ones belonging to the *Staphylococcus* genus. Second, the *Staphylococcus* species were accurately identified by a *sodA* based species-specific oligonucleotide array (Giammarinaro et al., 2005). Then, by focusing on a small-scale PU in which environment and meat product were colonized by the three species, *S. equorum*, *S. saprophyticus* and *S. xylosus*, we genotyped all the strains.

2. Materials and methods

2.1. Sampling procedures and isolation of strains

A collection of GCC$^+$ strains was obtained by sampling nine small-scale PUs manufacturing naturally dry fermented sausages located in a medium-size mountain area. These PUs were chosen from 108 located in the center of France and were representative of the sensorial diversity of the naturally dry fermented sausages manufactured (Rason et al., 2007). No commercial starters were used in these PUs; for details concerning the process see Lebert et al. (2007a). Four environmental samples were collected in each PU by swabbing a surface of 500 cm^2 with a sterile wet cloth: two from equipment (machines, knives) and two from surfaces (cutting table, cold room for storage). They were sampled prior to processing and after cleaning and disinfecting procedures. The clothes were transferred into 25 ml of buffered peptone water (AES, Combourg, France). One casing and three meat samples per PU were collected: batter, sausages after fermentation and sausages at the end of ripening. Twenty-five grams of each sample were transferred into 225 ml of buffered peptone water. The resulting suspensions were homogenized with a stomacher for 4 min. GCC$^+$ were enumerated by surface plating on Mannitol salt agar (MSA, Merck). Plates were incubated at 30 °C for 48 h. For each samples, 5 to 20 colonies showing typical morphology on countable plates were randomly picked and plated on Brain Heart Infusion (BHI, Difco) agar. The isolates were stored at −80 °C in BHI broth containing 20% glycerol.

2.2. Staphylococcus-specific PCR

The 766 GCC$^+$ isolates grown on BHI agar medium for 24 h at 30 °C were submitted to PCR. Amplifications were performed from one colony of each isolate with the primers TstaG422 and Tstag765, allowing the identification of the *Staphylococcus* genus as described by Martineau et al. (2001).

2.3. Species identification of staphylococcal isolates

Species identification of the 676 isolates of *Staphylococcus* was achieved by a validated species-specific oligonucleotide array targeting the manganese-dependent superoxide dismutase (*sodA*) gene as described by Giammarinaro et al. (2005). Briefly, the primers D1 (CCITAYICITAYGAYGCIYTIGARCC) and D2 (ARRTARTAIGCRTGYTCCCAIACRTC) were used to amplify by PCR the internal part of the *sodA* gene (Poyart et al., 2001), but D2 was 5′-terminal digoxigenin-labeled. The labeled PCR products were heat denatured and hybridized with species-specific oligonucleotide probes fixed to a nylon membrane. The hybridized targets were detected with the Dig color detection kit (Roche, Meylan, France) (Giammarinaro et al., 2005).

2.4. Pulsed-field gel electrophoresis (PFGE) analysis

One hundred and thirty three selected *Staphylococcus* strains were submitted to PFGE analysis. High-molecular-weight DNA was isolated from log-phase cultures grown on BHI broth and prepared in low-melting-point agarose plugs as described by Morot-Bizot et al. (2003), and digested with the endonuclease *Sma*I (Promega, Lyon Charbonnières, France) according to the manufacturer's instructions. DNA fragments were resolved on a 1% gel with a contour-clamped homogeneous electric field apparatus CHEF-DRIII (Bio-Rad, Ivry, France). PFGE was performed at 6 V/cm and an including angle of 120° with switch times of 40–100 s for 2 h and 5–35 s for 22 h, or switch times of 100 s for 12 h, 70 s for 16 h and 1–30 s for 12 h depending on the sizes of the DNA fragments to be resolved with 0.5× Tris base-borate-EDTA running buffer maintained at 14 °C. Lambda DNA concatemers (Promega) and *Staphylococcus cerevisae* chromosomal DNA (Bio-Rad) were used as molecular weight markers. Gels were stained with ethidium bromide and digitalized with the Gel Doc 2000 apparatus (Bio-Rad). The analysis of *Sma*I macrorestriction profiles and phylogenetic analyses were performed with Diversity Database software (Bio-Rad). Dendrograms were generated using the Dice correlation coefficient and by clustering by the unweighted pair group method analysis (UPGMA) using average linkages with a maximum position tolerance of 2%. Isolates were defined putatively as the same strain if they showed an indistinguishable PFGE pattern. They were defined as clusters or being closely related if they showed PFGE patterns with at least 85% similarity, corresponding to a two- to three-band difference (Tenover et al., 1995).

3. Results

3.1. GCC$^+$ population in environments and sausages

The GCC$^+$ population was enumerated in the nine PUs both in the environments and the meat products. All the PU environments were colonized by GCC$^+$ to various degrees. PU F04, for example, had three samples contaminated at a level ≥ 5.0 log CFU/100 cm^2, while PU F06 and PU F09 had no samples contaminated to this level (Table 1). All the equipment and working surfaces were contaminated to various degrees, the tables often the most: five tables with a level ≥ 5.0 log CFU/100 cm^2, followed by the knives with three with a level ≥ 5.0 log CFU/100 cm^2 (Table 1). Conversely, 44% of samples from cold room surfaces were not contaminated (level of detection below 2 log CFU/100 cm^2).

GCG$^+$ counts of the casings averaged 3.9 log CFU/g, and ranged from 2.0 to 5.7 log CFU/g for the PUs (Table 1). The contamination of the nine batters was more homogenous and averaged 4.2 log CFU/g. This population increased during the process, except in one PU (F05), and averaged 6.4 log CFU/g in the ripened sausages.

3.2. Diversity of the staphylococcal microflora

A total of 766 GCC$^+$ were isolated from the nine PUs, including 676 isolates of the *Staphylococcus* genus, using specific PCR. These 676 isolates could be divided in 288 originating from all the

Table 1
Level of GCC⁺ population in the environments and the meat products of the nine processing units.

PU	F01	F02	F03	F04	F05	F06	F07	F08	F09
Environment (log CFU/100 cm²)									
Cold room	<2.0	5.4	4.8	4.8	2.5	<2.0	<2.0	3.9	<2.0
Table	<2.0	2.8	6.9	6.3	5.6	3.1	5.9	6.2	2.0
Knife	5.1	2.8	5.3	5.1	2.9	3.7	<2.0	4.1	3.0
Machine	4.4	3.5	4.8	5.0	3.0	3.6	3.5	2.7	2.3
Meat Product (log CFU/g)									
Casing	2.8	3.7	5.0	3.6	4.8	3.1	4.4	5.7	2.0
Batter	3.5	4.4	5.2	4.4	4.5	4.6	4.2	4.3	3.1
Fermented	4.9	6.1	6.6	6.0	4.1	5.3	4.2	5.4	5.7
Ripened	6.6	7.0	6.6	7.1	4.0	6.6	6.3	6.8	7.0

GCC⁺: Gram-positive catalase-positive cocci; PU: processing unit; <2.0: level of detection 2 log CFU/100 cm², Machine: mincing, mixing or stuffing machines.

environments and 388 from all the meat products (Table 2). Staphylococci were the dominant isolates in all the PUs except F03 (Table 2). No effort was made to identify the 90 remaining GCG + isolates.

All the *Staphylococcus* spp. isolated from meat products, casings, equipment or environments were successfully identified by the *sodA* based species-specific oligonucleotide array. Results revealed a wide variety of *Staphylococcus* with a total of 15 species identified and between 5 and 8 species identified in each PU; all species were coagulase-negative (Table 2). Among those, *S. equorum* was the dominant species (58.4%), followed by *S. saprophyticus* (15.7%), *S. xylosus* (9.3%), *S. succinus* (5.3%), *S. warneri* (2.8%) and *S. carnosus* (2.5%) (Table 2). *S. equorum* was isolated in all the samples of all the PUs and its distribution between meat and environmental samples was similar. *S. saprophyticus* was also isolated in all the PUs, but its percentage was higher in environmental than in meat samples. The opposite was observed with *S. xylosus* and *S. succinus*: their prevalence was higher in meat samples. The remaining nine species identified represented 5.6% of the total of *Staphylococcus* (Table 2). The species, *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus fleurettii* and *Staphylococcus hominis* were only sporadically isolated from the environments of the PUs. Two species, *Staphylococcus arlettae* and *Staphylococcus sciuri*, were sporadically present in the meat products.

3.3. Staphylococcal microflora in the environment

The machines (mixing, mincing, stuffing) were contaminated by a wide diversity of species as 10 species could be identified, followed by the knives (7 species) and the tables (5 species), and cold rooms (3 species) (Table 3). *S. equorum* was the prevalent species isolated in all the environmental samples in most of the PUs (Table 3). This species seemed adapted to low temperature as it was found in 44% of the samples from the cold rooms. *S. saprophyticus* was the second microflora present in all the environmental samples. *S. xylosus* was identified only on machine surfaces and knives in three of the nine PUs studied. The other species were episodically isolated on machine surfaces and knives.

3.4. Staphylococcal succession during the process

The widest species diversity was noted in the raw materials, with 7 species identified in the casings and 10 in the batters of the 9 PU (Table 3). Six species were common in these raw materials, with *S. equorum* the dominant one. The number of species decreased during the process from 10 in the batters to 6 after the fermentation steps and 5 in the ripened sausages of the 9 PU (Table 3). Considering each PU, the batter could be contaminated by up to 5 species and 78% of the batters were contaminated by at least 3 species, while only 44% of the fermented sausages and 33% of the final sausages were contaminated by 3 species (data not shown). The species *S. equorum* was isolated in all the final products, *S. saprophyticus* was the second species detected in 67%, while *S. succinus*, *S. vitulinus*, *S. xylosus* were present episodically in the final dry fermented sausages (Table 3). Furthermore, these five species were found at each step of sausage manufacturing, and seemed well adapted to the meat ecosystem and process.

3.5. Diversity of the strains of the three prevalent species

The three prevalent species encountered in the environments and the meat products, *S. equorum*, *S. saprophyticus* and *S. xylosus*, were co-dominant in the environment and meat products of the PU F02 (Table 2). The isolates of F02 belonging to these three species were characterized by PFGE analysis to evaluate the diversity within each species and to determine if the same strains were

Table 2
Prevalence of staphylococcal species in the environments and the meat products of the nine processing units.

Processing unit	F01		F02		F03		F04		F05		F06		F07		F08		F09		Total		Total env		Total meat	
GCC⁺	env[a]	meat	env	meat	env	meat	env	meat	env	meat	env	meat	env	meat	env	meat	env	meat						
	21	31	44	99	54	36	42	30	63	25	41	35	17	32	66	97	9	24	766	%[b]	357	%[b]	409	%[b]
Staphylococcus	16	31	44	99	13	18	42	30	62	25	38	35	17	32	47	94	9	24	676		288		388	
S. arlettae										1									1	0.1	0	0.0	1	0.3
S. capitis					1														1	0.1	1	0.3	0	0.0
S. carnosus		6		2			3	4		2									17	2.5	3	1.0	14	3.6
S. cohnii													2						2	0.3	2	0.7	0	0.0
S. epidermidis		6							2		2					1		1	12	1.8	4	1.4	8	2.1
S. equorum	4	15	27	44	9	14	22	19	40	11	15	15	4	11	43	75	5	22	395	58.4	169	58.7	226	58.2
S. fleurettii	1														1				2	0.3	2	0.7	0	0.0
S. hominis									1										1	0.1	1	0.3	0	0.0
S. pasteuri				5				3	1							1			10	1.5	1	0.3	9	2.3
S. saprophyticus	11	2	10	21		3	16	4	8	11	6	1	8	3		1	1		106	15.7	60	20.8	46	11.9
S. sciuri		1																	1	0.1	0	0.0	1	0.3
S. succinus											1		2	15	3	15			36	5.3	6	2.1	30	7.7
S. vitulinus		1		3		1	1						1	2		1			9	1.3	2	0.7	8	2.1
S. warneri									10		8							1	19	2.8	18	6.3	1	0.3
S. xylosus			7	24	3						6	19		1			3		63	9.3	19	6.6	44	11.3

[a] env: environment.
[b] % of each species on a total of 676 *Staphylococcus* isolates, 288 *Staphylococcus* isolates isolated from environments or 388 from meat products.

Table 3
Prevalence of the CNS species in the environments and the meat products of the nine processing units.

Species	Environment				Meat product			
	Cold room	Table	Knife	Machine	Casing	Batter	Fermented	Ripened
S. arlettae	0[a]	0	0	0	0	1	0	0
S. capitis	0	0	0	1	0	0	0	0
S. carnosus	0	0	1	1	2	0	2	0
S. cohnii	0	1	0	0	0	0	0	0
S. epidermidis	0	0	1	1	1	3	0	0
S. equorum	4	5	5	8	4	8	9	9
S. fleurettii	0	0	0	2	0	0	0	0
S. hominis	0	0	0	1	0	0	0	0
S. pasteuri	0	0	1	0	1	3	0	0
S. saprophyticus	1	2	3	6	0	4	5	6
S. sciuri	0	0	0	0	0	1	0	0
S. succinus	0	1	1	2	2	2	2	2
S. vitulinus	0	0	0	2	1	1	4	1
S. warneri	1	1	0	0	0	1	0	0
S. xylosus	0	0	3	3	1	3	2	1
Total species	3	5	7	10	7	10	6	5

[a] Prevalence of the species: absence in the 9 processing units.

present in the environment and in the meat products. A diversity of PFGE patterns was found among the isolates of the three species (Fig. 1). The isolates that showed indistinguishable macrorestriction patterns were presumed to belong to the same clone (*i.e.*, putatively the same strain).

The analysis of the 71 *S. equorum* isolates showed 28 distinct PFGE patterns, 12 of which were constituted by only one isolate and were considered as singletons (Fig. 1a, Table 4). Employing a cut-off similarity value of 85%, no PFGE patterns were clustered (Fig. 1a). A large diversity of PFGE patterns was thus found between isolates. This diversity was observed both in the environment (18 patterns) and in the meat products (17 patterns). The diversity of the strains did not decrease during the process as 9, 7 and 9 PFGE profiles were noted for the batter, the fermented and the ripened sausages, respectively, and only two profiles were common at these different steps of manufacturing (Table 4). Only 7 PFGE profiles including the three dominant ones (SE2, SE12 and SE17) were common between meat and environment (Table 4). These 7 strains were distributed along the processing line up to the ripened sausage.

The analysis of the 31 *S. saprophyticus* isolates showed 13 distinct PFGE patterns, 6 of which were singletons (Fig. 1b, Table 5). Only two patterns (SS2a and SS2b) were clustered with similarity of 85% and formed the PFGE type SS2 including 5 *S. saprophyticus* isolates (Fig. 1b). The diversity within these *S. saprophyticus* isolates was thus rather high both in the environment (7 profiles) and in the meat products (9 profiles) (Table 5). Three PFGE patterns (SS1, SS2a, SS7) were found in environmental and meat product samples. As mentioned for *S. equorum*, the process did not simplify this diversity as 5, 4 and 4 PFGE profiles were noted for the batter, the fermented and the ripened sausages, respectively (Table 5).

The analysis of the 31 *S. xylosus* isolates showed only 6 distinct PFGE patterns (Fig. 1c, Table 5). Three patterns were clustered with similarity higher than 85% and formed the prevalent PFGE type SX1 including 27 *S. xylosus* isolates. Among them, 20 isolates presented indistinguishable patterns (subtype SX1a), while the remaining seven isolates showed closely related patterns (subtypes SX1b containing six isolates and SX1c). The isolates belonging to the predominant PFGE SX1 pattern were present on the surface of the equipment and in the meat products, but only at the beginning of the process: batter and fermentation step (Table 5). The four isolates of the three other PFGE patterns were present only on the equipment (SX3) or in the meat products (SX2, SX4) (Table 5).

4. Discussion

The GCC$^+$ ecosystem of the products and the environments of the nine small-scale French PUs was dominated by coagulase-negative *Staphylococcus* (88%). All the staphylococcal isolates could be identified at species level by the oligonucleotide array described by Giammarinaro et al. (2005), which is to date the only tool that identifies 36 species accurately in one shot. The 676 CNS isolates from the sausage and manufacturing environment ecosystems were identified as belonging to 15 species. In other studies, the number of CNS strains unidentified by phenotypic methods is often high (Iacumin et al., 2006). By PCR-denaturing gradient gel electrophoresis (PCR-DGGE), some species remained also unidentified or two species could not be distinguished (Rantsiou et al., 2005; Rantsiou and Cocolin, 2006; Villani et al., 2007).

In our study, the most common species were in decreasing order *S. equorum, S. saprophyticus, S. xylosus, S. succinus, S. warneri* and *S. carnosus* with the remaining nine species identified representing a minor fraction of the staphylococcal population. The prevalence of these species varied within the Mediterranean traditional sausages. These sausages are low-acid fermented meat products (final pH 5.3 to 6.2) with great diversity between countries and regions (Lebert et al., 2007b). *S. equorum* was the prevalent species along the process from environmental manufacturing samples to dry fermented sausages in the nine French traditional producers we studied. The dominance of *S. equorum* has been already observed in a French small-scale facility manufacturing traditional sausages (Corbière Morot-Bizot et al., 2006). This species was not mentioned in traditional Spanish products: fuet, salchichon and chorizo (Garcia-Varona et al., 2000; Martín et al., 2006) and traditional Greek sausages (Papamanoli et al., 2002; Drosinos et al., 2005). It has been identified in artisanal Italian sausages; in soppressata and sausages from Basilicata and Argentina, it was detected at low percentage (Blaiotta et al., 2004a, 2004b; Mauriello et al., 2004; Fontana et al., 2005; Iacumin et al., 2006; Villani et al., 2007), whereas in the Ciauscolo salami, it was found to contribute to the fermentation process (Aquilanti et al., 2007). By contrast, in other Italian salami, it was not detected (Rebecchi et al., 1998; Mauriello et al., 2004). *S. saprophyticus* was dominant or co-dominant with *S. xylosus* in Greek products and in some Italian products (Papamanoli et al., 2002; Mauriello et al., 2004; Drosinos et al., 2005), while *S. xylosus* dominated in Spanish and certain Italian sausages (Rebecchi et al., 1998; Garcia-Varona et al., 2000; Cocolin et al., 2001b; Blaiotta et al., 2004b; Iacumin et al., 2006; Martín et al., 2006; Silvestri et al., 2007). For the other species episodically isolated, it is very difficult to draw a picture as their percentages are totally different in each product.

CNS constitute also a non-negligible part of the microflora of soft and smear cheeses and semi-hard and hard cheeses (Irlinger et al., 1997; Irlinger, 2008). Many CNS species are common in the ecosystems of cheeses and traditional fermented sausages, raising the question of the origin of the contamination. These fermented foods have in common that the raw materials used (meat or milk) come from domestic animals. It is well known that staphylococci are found living naturally on the skin and mucous membranes of warm-blooded animals. Thus the species inventoried in sausages or in cheeses have been identified in various farm animals, sheep, cattle, pig, goat, horse, bird and pet (Gillespie et al., 2009; Zadoks and Watts, 2009), and also on human skin, in particular for *Staphylococcus epidermidis*, *S. capitis* and *S. hominis* which are the most prevalent and persistent (Nagase et al., 2002; Piette and Verschraegen, 2009). Furthermore, the prevalence of the staphylococcal microflora in these fermented foods is certainly favored by their halo-tolerance and their few nutritional requirements (Irlinger, 2008). Up to now very few species of CNS have been developed as commercial starter cultures. *S. xylosus* and *S. carnosus* are commonly used for sausage manufacturing

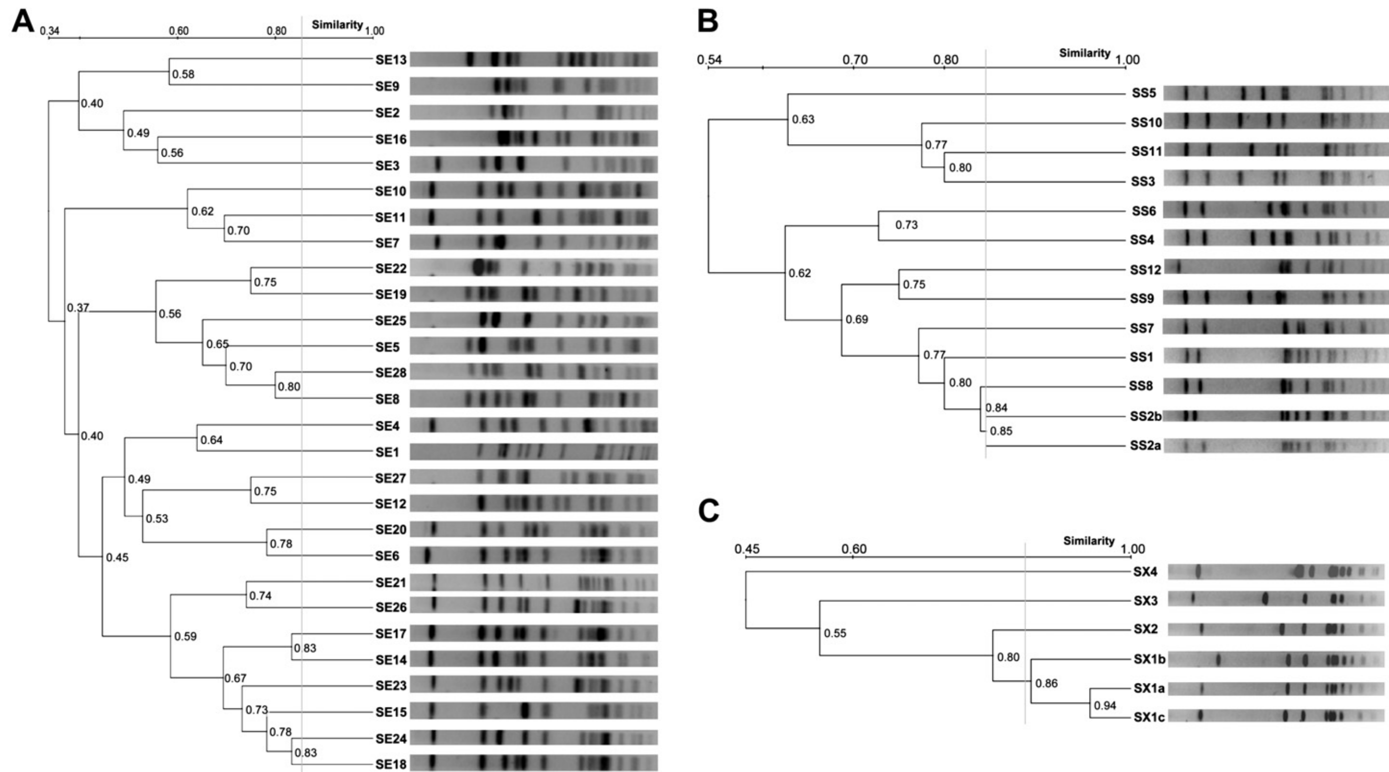

Fig. 1. Dendrograms generated by the unweighted pair group method of analysis with Dice coefficient of *Sma*I PFGE profiles of *S. equorum* (A), *S. saprophyticus* (B) and *S. xylosus* (C) isolates.

Table 4
Diversity of the 71 *S. equorum* isolates inside the small-scale processing unit F02.

PFGE profile	Environment				Meat Product			
	Cold room	Table	Knife	Machine	Casing	Batter	Fermented	Ripened
SE1 (3)		1				1	1	
SE2 (7)				4			3	
SE3 (3)			3					
SE4 (1)			1					
SE5 (2)			1				1	
SE 6 (1)			1					
SE 7 (1)			1					
SE 8 (1)			1					
SE 9 (2)			1					1
SE 10 (1)			1					
SE 11 (2)				2				
SE 12 (8)	2	1				2	2	1
SE 13 (2)	2							
SE 14 (1)	1							
SE 15 (1)		1						
SE 16 (1)		1						
SE 17 (10)		1				1	1	7
SE 18 (5)		1					2	2
SE 19 (1)					1			
SE 20 (5)						3		2
SE 21 (2)								2
SE 22 (2)						1		1
SE 23 (1)						1		
SE 24 (1)						1		
SE 25 (1)						1		
SE 26 (2)								2
SE 27 (2)						1		1
SE 28 (2)							2	

(): Number of isolates.

(Corbière Morot-Bizot et al., 2007). *S. equorum* and *S. succinus* have been proposed as starters for smear ripened cheeses and typical Swiss semi-hard cheeses (Place et al., 2002, 2003). *S. equorum* with *S. succinus* have been tested as autochthonous starter cultures for meat fermentation (Talon et al., 2008).

Even though the ubiquity of CNS is well known, very few studies have concerned their persistence in the manufacturing environment of food processing. In our study, CNS have been isolated in the environments of nine PUs. Their presence and persistence in such environments could depend on their capacity to colonize surfaces (Moretro et al., 2003). In the food industry, biofilm formation may be undesirable for hygienic and safety reasons. Nevertheless, colonization of surface by bacteria used or frequently isolated in food may be desired to inhibit the colonization by spoilage or pathogenic bacteria. Among the CNS species, *S. sciuri*, *S. xylosus* and *S. equorum* were described as biofilm-positive on abiotic surfaces (Leriche and Carpentier, 2000; Planchon et al., 2006; Leroy et al., 2009). Furthermore, *S. sciuri* biofilm was able to prevent adhesion and growth of *Listeria monocytogenes* to stainless steel surfaces (Leriche and Carpentier, 2000). Moreover, some strains of *S. sciuri* and *S. xylosus* produced inhibitory substances in laboratory media that prevent development of *L. monocytogenes* (Villani et al., 1997; Leriche and Carpentier, 2000; Norwood and Gilmour, 2000). Thus, their role as protective barrier microflora in the environment of the food industry is questioned.

Table 5
Diversity of the 31 *S. saprophyticus* and the 31 *S. xylosus* isolates inside the small-scale processing unit F02.

PFGE profile	Environment			Meat Product		
	Table	Knife	Machine	Batter	Fermented	Ripened
Staphylococcus saprophyticus						
SS1(3)			2	1		
SS2a (2)	1				1	
SS2b (3)			3			
SS3 (1)	1					
SS4 (1)		1				
SS5 (1)		1				
SS6 (1)						1
SS7 (8)			1		3	4
SS8 (2)				1	1	
SS9 (1)				1		
SS10 (3)				1	2	
SS11 (4)				1		3
SS12 (1)						1
Staphylococcus xylosus						
SX1a (20)		3	1	10	6	
SX1b (6)		1		3	2	
SX1c (1)	1					
SX2 (2)				1	1	
SX3 (1)		1				
SX4 (1)					1	

(): Number of isolates.

The diversity of species is often accompanied by a diversity of strains within a species. In our study, the genetic diversity within the *S. equorum* and the *S. saprophyticus* species isolated from the processing unit F02 was high and comparable. The genetic diversity was observed both in the environment and the meat isolates. A small percentage of the strains, 24% for *S. equorum* and 23% for *S. saprophyticus*, colonized these two ecological niches. Such a high diversity has been reported for strains of *S. equorum* isolated from the processing unit F08 manufacturing sausages (Leroy et al., 2009). These authors have already reported that few strains were persistent along the processing line. The bacterial exchange between the meat and the surface of the equipment is difficult to evaluate. However, if we assume that the meat is the principal source of contamination, these results highlight that some strains more adapted to the manufacturing environments will persist and could re-contaminate the meat products, while for other strains the meat products are more suitable. The genetic diversity within the *S. equorum* and *S. saprophyticus* species remained high throughout the manufacturing steps: from the batter (9 and 5 strains, respectively) to the ripened sausages (9 and 4 strains, respectively); moreover few strains were common in these two steps. Even if the process, curing, drying and acidifying, often reduces the diversity of the species as observed in our study and in the literature (Rebecchi et al., 1998; Cocolin et al., 2001a; Fontana et al., 2005) it did not decrease the diversity of the strains within a species. For *S. xylosus*, we only observed the presence of 6 strains in the processing unit F02, with one dominant (87% of the isolates). Other studies have emphasized wide heterogeneity among the *S. xylosus* strains from different sausages (Rossi et al., 2001; Di Maria et al., 2002; Dordet-Frisoni et al., 2007).

In conclusion, there is great diversity in the coagulase-negative staphylococci found in the traditional dry fermented sausages and the environments of small-scale PUs. The identification of this microflora at the species and also the strain levels increases our knowledge and could lead to the development of new starters composed of different species and above all including different strains to keep the typicality of the traditional sausages. This development will require the evaluation of CNS diversity as we performed it, but also should include food safety criteria as recommended by the French Agency for Sanitary and Food Safety and the European Food Safety Agency in the Qualified Safety Presumption approach.

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