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Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters

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Abstract

This paper reviews the diversity of microbiota, both in the environment and in traditional fermented European sausages. The environments of processing units were colonised at variable levels by resident spoilage and technological microbiota, with sporadic contamination by pathogenic microbiota. Several critical points were identified such as the machines, the tables and the knives – knowledge crucial for the improvement of cleaning and disinfecting practices. Traditionally fermented sausages generally did not present a sanitary risk. The great diversity of lactic acid bacteria and staphylococci was linked to manufacturing practices. Development of indigenous starters is very promising because it enables sausages to be produced with both high sanitary and sensory qualities. Our increasing knowledge of the genomes of technological bacteria will allow a better understanding of their physiology in sausages.

Keywords: Microbial ecosystem; Traditional fermented sausage; Indigenous starter; Lactic acid bacteria; Coagulase-negative cocci

1. Introduction

In Europe, naturally fermented sausages have a long tradition originating from Mediterranean countries during Roman times (Comi et al., 2005). Production then spread to Germany, Hungary and others countries including the United States, Argentina and Australia (Demeyer, 2004). Europe is still currently the major producer and consumer of dry fermented sausages (Talon, Leroy-Sétrin, & Fadda, 2004).

There is a wide variety of dry fermented products on the European market as a consequence of variations in the raw materials, formulations and manufacturing processes, which come from the habits and customs of the different countries and regions. However, Northern products have a pH below 5, while Mediterranean products have a pH of 5.3–6.2 and are highly desiccated. In both categories, industrial development has led to the use of starter cultures to standardise and control sausage manufacturing. However, artisanal slightly fermented sausages form a group of traditional Mediterranean products with a great regional diversity, both between and within countries. These traditional sausages can be defined as a meat product made of a mixture of meat (often pork), pork fat in variable ratio and salt including eventually sugar, nitrate and/or nitrite (Fontana, Cocconcelli, & Vignolo, 2005; Lebert et al., 2007). The fermentation and ripening/drying do not always constitute two separated steps and they can be carried out in a natural dryer depending on local climatic conditions (Corbière Morot-Bizot, Leroy, & Talon, 2006; Lebert et al., 2007). Traditional dry sausages rely on natural contamination by environmental microflora. This contamination occurs during slaughtering and increases during manufacturing.

The objective of this paper is to review the diversity of microbiota both in the environment and in traditional fermented sausages. The diversity of the technological microbiota (lactic acid bacteria or LAB, and coagulase-negative

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cocci or CNC) is also highlighted. Finally, the importance of developing indigenous starters and of increasing knowledge on starters is underlined.

2. Microbiota in the environment of small-scale processing units

Many authors support the belief that the microorganisms present in traditional sausages are derived from the raw materials or from the environment of manufacturing (Mauriello, Casaburi, Blaiotta, & Villani, 2004; Rantsiou, Urso et al., 2005). This microbiota is usually referred to as "house flora" (Garcia-Varona, Santos, Jaime, & Rovira, 2000). If the microbiota isolated from traditional sausages is well described (see paragraph below), the resident microbiota in the environment of the processing units is still poorly known.

We have shown by studying a French small-scale processing unit manufacturing sausages that all the processing surfaces and the equipments were colonised by CNC and yeasts/moulds (Chevallier et al., 2006; Corbière Morot-Bizot et al., 2006). Their level and particularly for CNC could reach up to 4.7 log cfu/cm^2 (cfu = colony forming unit). Sporadic cases of contamination was recorded for *Staphylococcus aureus* and *Listeria monocytogenes*. By extending our research to 10 traditional French processing units (PUs) during the European project Tradisausage (QLK1-CT-2002-02240, http://www2.clermont.inra.fr/tradisausage), we have shown that regardless of the microorganisms, cold rooms and mixing machines had the lowest levels of contamination with median levels of 2.2 log cfu/100 cm^2, while knives and tables had the highest ones (from 2.1 to 4.4 log cfu/100 cm^2) (Table 1) (Lebert et al., 2007). CNC and yeasts/moulds but also *Pseudomonas* colonised all the surfaces and equipments of these processing units (Table 1). Considering the pathogenic bacteria, *Salmonella* and *S. aureus* were not detected in the environment while *L. monocytogenes* was enumerated in the table and the knife of one processing unit (Lebert et al., 2007). In the European project Tradisausage, the residual microbiota was also assessed in the environment of 43 traditional processing units of Spain, Portugal, Italy, Greece and Slovakia (Talon et al., 2007). All the PUs' environments were colonised at variable levels by spoilage and technological microbiota with some PUs having too high contamination. *Salmonella* and *L. monocytogenes* were detected in 4.8% and 7.6% of the samples and *S. aureus* was enumerated in 6.1%. Several critical points were identified such as the machines for *S. aureus* and the tables and the knives for *L. monocytogenes*; their knowledge is crucial for the improvement of the control systems. The variability of the residual contamination highlighted the different cleaning, disinfecting and manufacturing practices of the small-scale processing units (Talon et al., 2007). In fact, unclean, insufficiently or inadequately cleaned pieces of equipment have often been identified as the source of pathogens (Reij & Den Aantrekker, 2004). Many studies investigated the pathogen flora of food processing environments and food processing lines such as *L. monocytogenes* in pork and poultry processing plants and products (Chasseignaux et al., 2002) and *Salmonella* species in pork slaughter and cutting plants (Giovannacci et al., 2001).

3. Microbiota of products

Fermentation of traditional dry sausages relies on the indigenous microbiota. We have shown that this microbiota was enumerated in the batters manufactured in French traditional processing units at average level of 4.0 log cfu/g (Fig. 1) (Lebert et al., 2007). It was composed of useful microorganisms (CNC, LAB, yeasts/moulds) for the fermentation and flavour of sausages, but also spoilage microbiota (*Pseudomonas*, enterobacteria) and enterococci. This indigenous microbiota could arise from the raw materials or from the environment of manufacturing (microbiota described in Table 1) as mentioned by several authors. However, in this study, we found no evidence of cross-contamination between environment and meat. We assumed that the contamination of the batter was mainly due to the microbiota of the raw materials and sometimes of the casings. We have shown that lean meat and fat used to prepare the batter were sometimes contaminated with high levels of CNC, yeasts/moulds, coliforms and *Pseudomonas* (Chevallier et al., 2006). Comi et al. (2005) also showed that raw meats had total aerobic counts of between 4.3 and 5.8 log cfu/g and casings of between 3.8 and 6.1 log cfu/g.

Table 1
Microbiota of the environments of 10 French processing units manufacturing traditional fermented sausages

	Tables			Knives			Cold room			Mincing machines			Mixing machines			Stuffing machines		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
Y/M	4.0	<0.7	6.8	3.7	2.7	6.7	1.1	<0.7	6.7	2.6	1.5	4.7	1.8	<0.7	5.4	2.9	<0.7	6.7
LAB	4.0	<1.7	7.1	3.3	1.7	5.6	<1.7	<1.7	5.0	2.6	<1.7	5.5	<1.7	<1.7	5.8	2.2	<1.7	5.4
CNC	4.4	<1.7	6.9	4.0	<1.7	5.3	0.9	<1.7	4.8	3.4	1.7	5.5	2.3	<1.7	5.3	2.7	<1.7	5.7
ENC	2.2	<0.7	3.1	2.6	<0.7	5.0	0.2	<0.7	4.7	2.2	<0.7	3.4	<0.7	<0.7	4.0	1.7	<0.7	3.9
ENB	3.2	<0.7	6.4	2.7	<0.7	6.0	< 0.7	<0.7	1.7	<0.7	<0.7	3.1	<0.7	<0.7	2.1	1.7	<0.7	6.1
PSE	4.3	<0.7	7.1	4.2	2.7	7.3	1.5	<0.7	3.9	2.2	<0.7	5.3	1.5	<0.7	3.5	2.7	<0.7	7.1

Data expressed in log cfu/100 cm^2. Y/M, yeasts and moulds; LAB, lactic acid bacteria; CNC, coagulase-negative cocci; ENC, enterococci; ENB, enterobacteria; PSE, *Pseudomonas*.

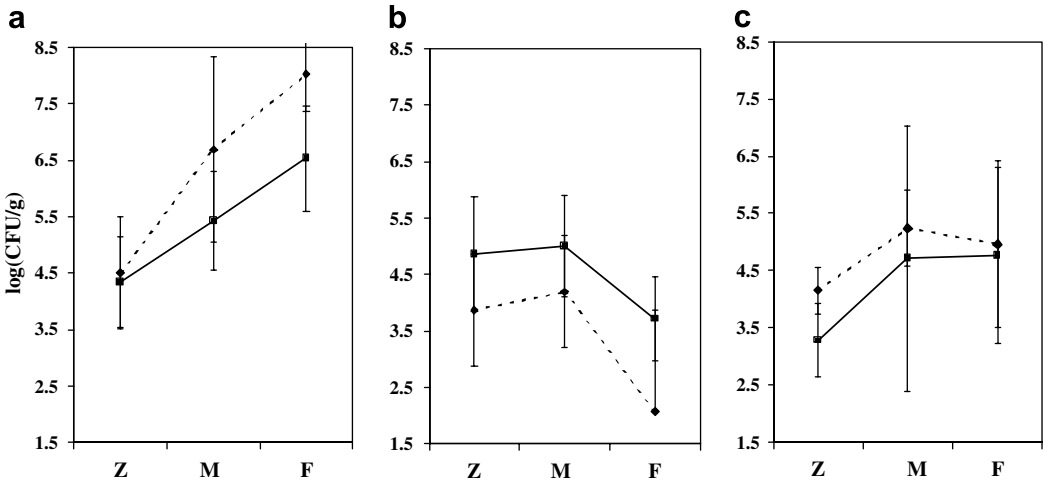

Fig. 1. Evolution of the microbiota during the processing of 10 traditional French fermented sausages: batter (Z), after fermentation (M) and final product (F). (a) (—■—) Coagulase-negative cocci; (--◆--) lactic acid bacteria. (b) (—■—) *Pseudomonas*; (--◆--) *Enterobacteriaceae*. (c) (—■—) *Enterococcus*; (--◆--), yeasts and moulds. Data were calculated from the average of the 10 processing units (log cfu/g). Vertical lines: standard deviation.

The type of microbiota that develops in traditional sausages is related to the diversity in formulation, and to the fermentation and ripening practices. These practises could be very different in terms of temperature, duration and relative humidity (Lebert, Leroy, & Talon, 2007). In Europe, fermentation can be carried out at high temperature (18–24 °C) between 1 and 2 days (Cocolin, Manzano, Cantoni, & Comi, 2001; Comi et al., 2005; Mauriello et al., 2004) or at low temperature (10–12 °C) during 1 week (Lebert et al., 2007; Mauriello et al., 2004). Similarly, temperatures of drying/ripening ranged mainly between 10 and 14 °C for French (Lebert et al., 2007), Italian (Cocolin et al., 2001; Cocolin, Manzano, Aggio, Cantoni, & Comi, 2001; Comi et al., 2005; Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000; Rantsiou, Drosinos et al., 2005) and Greek sausages (Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003). The duration of this step varied from 4 to 12 weeks. The diversity in the relative humidity leads to variable water content of traditional sausages at the end of drying ranging from 0.83 to 0.93 in French, Spanish, Portuguese and Italian sausages (Lebert, Leroy et al., 2007).

Despite the different practises within the countries, or the regions, the microbial populations showed similar evolutions as those we have shown for French sausages (Fig. 1). In traditional sausages, LAB constituted the major microbiota at the end of the ripening stage (Fig. 1a). Even if their initial levels varied their final levels were close to the one of industrial products manufactured with starter cultures. LAB usually increased the very first days of fermentation and they remained constant during ripening at 7–9 log cfu/g (Cocolin et al., 2001; Comi et al., 2005) or they can increased during all the process as shown for French sausages in Fig. 1a and reached similar final value. The initial population can be low, as observed in Salame Milano in Italy (Rebecchi, Crivori, Sarra, & Cocconcelli, 1998) and in a French sausage manufactured at low temperature (Chevallier et al., 2006) but it was generally comprised between 3.2 and 5.3 log cfu/g. LAB growth was often correlated with the decrease in pH in the first stage of maturation (Cocolin et al., 2001; Cocolin et al., 2001).

CNC constituted the second microbiota at the end of ripening with a population of 6–8 log cfu/g, population generally inferior to that of the LAB and closed to the one reached in industrial sausages (Fig. 1a). Their initial level varied from 3.1 to 4.4 log cfu/g (Lebert et al., 2007). CNC sometimes grew during the fermentation period to 10^6–10^8 cfu/g or they can grow during ripening (Comi et al., 2005) or during all the process (Fig. 1a).

Spoilage bacteria such as *Pseudomonas* and enterobacteria had far different initial levels according to the type of sausages. They ranged from 1.7 to 4.4 log cfu/g for enterobacteria and from 1.5 to 5.2 log cfu/g for *Pseudomonas* (Lebert et al., 2007). In French sausages, they remained constant during the fermentation and decreased during the ripening (Fig. 1b). In Greek sausages, they were progressively eliminated regardless of their initial population (Drosinos et al., 2005; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). Other authors found that enterobacteria and *Pseudomonas* increased during the fermentation (Comi et al., 2005). Then enterobacteria remained constant until the end while *Pseudomonas* remained constant or disappeared (Chevallier et al., 2006; Comi et al., 2005).

Yeasts and moulds were usually detected in all sausages in the batter at levels varying from 2.0 to 4.5 log cfu/g (Lebert et al., 2007). Some authors observed growth during the fermentation period with levels not increasing above 5 log cfu/g (Fig. 1c), then a stability or decrease in the population (Comi et al., 2005; Drosinos et al., 2005). Yeasts and moulds were not detected in Italian dry sausages Salame Milano at the end of ripening (Rebecchi et al., 1998).

Enteroccocci had an initial level between 2 and 4 log cfu/g (Fig. 1c). Enterococci usually grew during early fermentation and remained constant at a level of 4–6 log until the end of the whole process (Fig. 1c) (Comi et al., 2005; Drosinos et al., 2005; Rebecchi et al., 1998). In few

cases their counts declined. Enterococci are poor acidifiers and in traditional sausages of high pH they find good conditions for survival and growth (Hugas, Garriga, & Aymerich, 2003). There is still controversy over considering them as Generally Recognised as Safe (GRAS) microorganisms (Giraffa, 2002). However, studies point out that meat enterococci, especially *Enterococcus faecium* have a much lower pathogenicity potential than clinical strains and some strains of *E. faecium* are already used as starter culture or probiotic (Hugas et al., 2003; Martın, Garriga, Hugas, & Aymerich, 2005).

Considering the pathogenic bacteria, we have shown in the European project Tradisausage, that *Salmonella* was detected in 5.6% of 54 ripened sausages, *S. aureus* was enumerated in 7.4% of the sausages at a level in excess of the limit of 2.7 log cfu/g (Commission Regulation (EC) No. 2073/2005, 2005) and *L. monocytogenes* was numerated at a level in excess of the limit of 2.0 log cfu/g (Commission Regulation (EC) No. 2073/2005, 2005) in only one sample. In Salame Milano, *S. aureus* was observed at the beginning of the production process, decreased during ripening until it was undetectable at the end of the process (Rebecchi et al., 1998). While, it was still enumerated in sausages produced in Italian artisanal plants from level ranging from 2 to 4 log cfu/g (Blaiotta et al., 2004). *L. monocytogenes* was sometimes present in initial samples, but diminished or was not detected by the end of fermentation in Greek sausages (Drosinos et al., 2005; Samelis et al., 1998).

4. Diversity of technological microbiota

Lactic acid bacteria and *Staphylococcus* or *Kocuria* belonging to the CNC group are considered as technological microbiota because they are involved in the development of hygienic and sensory qualities of the final product. Lactic acid bacteria are involved mainly through their acidification. *Staphylococcus* and *Kocuria* contribute to the development of colour and flavour in fermented meat products mainly by degrading free amino acids and inhibiting the oxidation of unsaturated free fatty acids (Talon & Leroy, 2006; Talon et al., 2004).

4.1. Lactic acid bacteria (LAB)

Phenotypical methods have been widely used to identify LAB, however, methods relying on these methods are ambiguous and time consuming. Molecular methods such as species-specific PCR (Aymerich et al., 2006), PCR-denaturing gel electrophoresis (Rantsiou, Drosinos et al., 2005) and real time PCR (Furet, Quenee, & Tailliez, 2004) have been developed.

By using these molecular methods, it has been shown that the LAB species the most commonly identified in traditional fermented sausages were *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* (Lebert, Leroy et al., 2007). *L. sakei* is often the dominant one and can represent more than 42% of the isolates (Comi et al., 2005; Coppola et al., 2000; Greco, Mazette, De Santis, Corona, & Cosseddu, 2005; Papamanoli et al., 2003; Urso, Comi, & Cocolin, 2006). Aymerich et al. (2006) showed that *L. sakei* was identified in all of the Spanish sausages and represented 89% in chorizo and 76% in a traditional Spanish sausage "fuet". In a French sausage, *L. sakei* represented 100% of the isolates on the final products although it was minor in the raw materials (Ammor et al., 2005). *L. curvatus* is the second species identified; it is dominant in some Greek or Italian sausages (Comi et al., 2005; Rantsiou, Drosinos et al., 2005). *L. plantarum* is the third one; it dominates the LAB flora in a Greek sausage (Drosinos et al., 2005). Many other LAB are identified but represent a minor population (*L. alimentarius*, *L. casei*, *L. delbrueckii*, *L. farciminis*, *L. paraplantarum*, *L. pentosus* and *L. sharpeae*).

The diversity at strain level inside the dominant species is important. The combination of results obtained with plasmid profiling and randomly amplified polymorphic DNA (RAPD)-PCR, allowed to distinguish 112 different strains out of 185 isolates of *L. sakei* and 23 profiles for 53 isolates of *L. curvatus* (Aymerich et al., 2006). The analysis by RAPD-PCR of 100 strains of *L. curvatus* isolated from Greek, Hungarian and Italian naturally fermented sausages revealed that nine profiles were obtained, while 168 strains of *L. sakei* from the same samples gave 19 major clusters (Rantsiou, Drosinos et al., 2005). Urso et al. (2006) have also used RAPD to determine the diversity and the distribution of 353 strains of *L. sakei* and 67 strains of *L. curvatus* during a natural fermentation of three Italian sausages. Clusters containing strains isolated from different plants but also clusters formed by strains isolated from a specific fermentation were observed.

4.2. Coagulase-negative cocci (CNC)

The identification of staphylococci to the species level by phenotypical methods has limitations and may have resulted in misidentifications (Giammarinaro, Leroy, Chacornac, Delmas, & Talon, 2005). To provide increasingly reliable identifications, several molecular methods have been developed, including PCR-based methods such as PCR-DGGE (Cocolin et al., 2001), species-specific PCR (Aymerich, Martın, Garriga, & Hugas, 2003; Morot-Bizot, Talon, & Leroy-Sétrin, 2003), multiplex PCR (Corbière Morot-Bizot et al., 2006) and oligonucleotide array targeting *sodA* gene (Giammarinaro et al., 2005). This last method developed in our laboratory identifies the 36 validated species and constitutes presently a powerful tool for the identification of staphylococci (Giammarinaro et al., 2005).

Staphylococcus xylosus is the most common species in Greek, Italian and Spanish traditional products at the end of ripening (Blaiotta et al., 2004; Cocolin et al., 2001; Garcia-Varona et al., 2000; Iacumin, Comi, Cantoni, & Cocolin, 2006a; Martın et al., 2006; Mauriello et al.,

2004; Papamanoli et al., 2003; Rebecchi et al., 1998). It represents from 17% to 100% of the isolates according to the type of sausages. Even if it was not the dominant species in the batter, *S. xylosus* became rapidly dominant in Salame Milano (Rebecchi et al., 1998). *S. saprophyticus* is the second dominant species identified. It is dominant in some Greek and Italian sausages (Drosinos et al., 2005; Mauriello et al., 2004; Papamanoli et al., 2003). In the Italian products, *Staphylococcus equorum* and *Staphylococcus succinus* were also isolated (Mauriello et al., 2004). In three French small producers, we have shown that the staphylococcal microflora of the product and the environment was dominated by *S. equorum* (49%, 56% and 71% of the isolates), the second species was *S. succinus* for two producers (33% and 12%) while it was *S. xylosus* (19%) and *S. saprophyticus* (19%) for the third producer (Corbière Morot-Bizot et al., 2006; Leroy, Chevallier, Lebert, Chacornac, & Talon, 2006). Many other minor species were identified in the different traditional sausages (*S. aureus*, *S. auricularis*, *S. carnosus*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. intermedius*, *S. lentus*, *S. pasteuri*, *S. vitulus* and *S. warneri*).

The diversity of *S. xylosus* strains have been characterised by different typing methods. The combination of plasmid profiling and RAPD-PCR allowed the discrimination of 169 different profiles out of 194 *S. xylosus* isolates (Martín et al., 2006). By genotypic clustering based on comparison of RAPD-PCR profiles (Rossi, Tofalo, Torriani, & Suzzi, 2001) distinguished 22 clusters at 70% of homology. A total of 249 strains of *S. xylosus* isolated from three plants manufacturing sausages were genetically characterised using RAPD, Rep-PCR and Sau-PCR techniques (Iacumin, Comi, Cantoni, & Cocolin, 2006b). The results obtained allowed the discrimination of the strains coming from different plants.

5. Importance of the indigenous starter

The manufacture of traditional sausages is more an art depending on the skill and experience of the meat manufacturer rather than a process fully based on scientific and technological means. This is because meat fermentation is a complex biological phenomenon accelerated by the desirable action of certain microbes in the presence of a great variety of competing or synergistically acting species. These traditional practises lead to a great variability in the quality of the products. Few sporadic studies conducted on traditional products have shown that hygienic shortcomings can lead up to 25% of product loss with high economic consequences and may undermine consumer confidence for traditional products.

The development of starters from the natural fermentative communities of traditional fermented products may avoid or limit this variability in the production. Moreover, the development of indigenous starters may diversify the market that will be able to produce many typical regional fermented sausages with specific flavours.

The development of indigenous starters improving hygienic quality and keeping the sensorial one is a real challenge. This challenge was considered in the European project Tradisausage. We have developed a starter composed of a mixture of the indigenous bacteria isolated from a traditional sausages manufactured by a French producer, it consisted of *L. sakei*, *S. equorum* and *S. succinus* (Lebert, Leroy, Lebecque, Chacornac, & Talon, 2006; Talon, 2006). This starter was added to the batter and positive results were observed. Its addition resulted in sausages with higher sanitary qualities compared to the control: reduction of the level of *L. monocytogenes* (1.1 log cfu/g versus 2.7 for the control) and enterococci (4.2 log cfu/g versus 6.2 for the control), reduction of 1.7-fold the level of biogenic amines (233.8 mg/kg DM versus 404.4 mg/kg DM), reduction of lipid oxidation (0.61 mg MDA/kg versus 0.71 mg MDA/kg) and total cholesterol oxides (0.95 mg/kg versus 4.75 mg/kg). Finally this starter did not modify the flavour of the sausages as evaluated by a panel of jury but improved slightly the texture. Similar experiences have been carried out in different countries in the EU project Tradisausage and have lead to close conclusion.

6. Towards genomics and post-genomics to characterise starters

Up to recently, classical methods based on biochemical and physiological traits have been used to select the most performing strains for technological use. They were mainly based on acidification and antimicrobial properties for lactic acid bacteria and colour and flavour developments for staphylococci (Talon & Leroy, 2006). During the last decade, genetic studies have provided basic knowledge on targeted metabolic activities. Now global approaches with the sequencing of whole genome of bacteria are developed and will allow a better understanding of their physiology in meat ecosystem (Champomier-Vergès et al., 2007; Talon & Leroy, 2006).

Most of the genetic information concerned *L. sakei* 23K, initially isolated from a fermented dry sausage (Champomier-Vergès, Chaillou, Cornet, & Zagorec, 2002). Its chromosome map was obtained by the use of 47 genetic loci (Dudez et al., 2002). In parallel, a proteomic approach was developed to study the genes involved in adaptation to its environment (Marceau, Mera, Zagorec, & Champomier-Vergès, 2001). In 2005, the complete genome sequence of *L. sakei* 23K was published (Chaillou et al., 2005). From the known involvement of *L. sakei* as sausage starter, the genome analysis confirmed that the main role of *L. sakei* is to ferment sugars into lactic acid, and that it lacks main aroma production pathways. *L. sakei* 23K also lacks genes responsible for biogenic amine production. A battery of functions was identified which might explain adaptation or resistance of this species to stressing environmental conditions used during sausage manufacturing (presence of curing agents, spices, smoke, low temperature) (Chaillou et al., 2005).

The physical and genetic map of *S. carnosus* TM300 was established and the size of the chromosome was estimated to be 2590 kb (Wagner, Doskar, & Götz, 1998). Eighteen genetic markers were located within them genes related to sugar metabolism and to nitrate and nitrite reduction which are important traits for meat fermentation and colour development. Other genes of *S. carnosus* involved in technological properties such as the branched-chain amino acid aminotransferase producing flavour compounds (Madsen et al., 2002), the superoxide dismutase and the catalase contributing to the control of lipid oxidation (Barrière, Brückner, & Talon, 2001) have been also identified, sequenced and characterised. The annotation of the completed sequence of the chromosome of *S. carnosus* TM300 is under way (Rosenstein, Nerz, Resch, & Götz, 2005). The first comparison of the gene products revealed that about 20% were specific of this species. Their identification will reveal the originality of this species.

We have established the physical and genetic map of *S. xylosus* C2a by locating 47 restriction fragments and 33 genetic markers (Dordet-Frisoni, Talon, & Leroy, 2007). Twenty-three previously identified loci mainly concerned carbohydrate utilization and carbon catabolite repression (Bassias & Brückner, 1998; Brückner, Wagner, & Götz, 1993; Egeter & Brückner, 1996; Fiegler, Bassias, Jankovic, & Brückner, 1999) and antioxidant capacities (Barrière et al., 2001; Barrière, Leroy-Sétrin, & Talon, 2001) and 10 were newly identified (Dordet-Frisoni et al., 2007). The *S. xylosus* C2a chromosome contains six *rrn* operons, this number varies among strains from 5 to 6 as already observed in other staphylococcal species. The sequencing of the complete genome of *S. xylosus* in collaboration with the genoscope (http://www.genoscope.cns.fr) is just achieved. In parallel we have developed a proteomic approach to study the physiology of *S. xylosus* C2a strain in biofilm to allow a better understanding of its survival in food processing plants (Planchon, 2006).

7. Conclusion

This review outlined the diversity in the microbiota of naturally fermented sausages but also the strong impact of the process on its growth. Traditional fermented sausages generally did not present a sanitary risk. The great diversity in the LAB and staphylococci species and also within the species was certainly linked to the formulation, fermentation and ripening practices. The most promising strains for starter cultures are those which are isolated from the indigenous microbiota of traditional products. These strains are well adapted to the meat environment and to the specific manufacturing process and thus are capable of dominating the microbiota of products. Above all, development of indigenous starters leads to sausages with high sanitary quality and typical sensorial quality. The exploitation of the data of the genomes of species of technological interest will offer new research opportunities by revealing some properties that could explain their adaptation to the meat environment. Global approaches based on proteomics and transcriptomics are in progress and will allow a better understanding of their interactions with the ecosystem and the meat substrate.

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References

Ammor, S., Rachman, C., Chaillou, S., Prévost, H., Dousset, X., Zagorec, M., et al. (2005). Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. *Food Microbiology, 22*, 373–382.

Aymerich, T., Martín, B., Garriga, M., & Hugas, M. (2003). Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages. *Applied and Environmental Microbiology, 69*(8), 4583–4594.

Aymerich, T., Martín, B., Garriga, M., Vidal-Carou, M. C., Bover-Cid, S., & Hugas, M. (2006). Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *Journal of Applied Microbiology, 100*, 40–49.

Barrière, C., Brückner, R., & Talon, R. (2001). Characterization of the single superoxide dismutase of *Staphylococcus xylosus*. *Applied and Environmental Microbiology, 67*(9), 4096–4104.

Barrière, C., Leroy-Sétrin, S., & Talon, R. (2001). Characterization of catalase and superoxide dismutase in *Staphylococcus carnosus* 833 strain. *Journal of Applied Microbiology, 91*(3), 514–519.

Bassias, J., & Brückner, R. (1998). Regulation of lactose utilization genes in *Staphylococcus xylosus*. *Journal of Bacteriology, 180*(9), 2273–2279.

Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R., & Parente, E. (2004). Diversity and dynamics of communities of coagulase-negative staphylococci in traditional fermented sausages. *Journal of Applied Microbiology, 97*, 271–284.

Brückner, R., Wagner, E., & Götz, F. (1993). Characterization of a sucrase gene from *Staphylococcus xylosus*. *Journal of Bacteriology, 175*(3), 851–857.

Chaillou, S., Champomier-Vergès, M. C., Cornet, M., Crutz-Le Coq, A. M., Dudez, A. M., Martín, V., et al. (2005). The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology, 23*(12), 1527–1533.

Champomier-Vergès, M. C., Chaillou, S., Cornet, M., & Zagorec, M. (2002). *Lactobacillus sakei*: Recent developments and future prospects. *Research in Microbiology, 153*(2), 115–123.

Champomier-Vergès, M. C., Crutz-Le Coq, A. M., Zagorec, M., Leroy, S., Dordet-Frisoni, E., Planchon, S., et al. (2007). Genetics of microbial starters – Chapter 17. In F. Toldra, Y. H. Hui, I. Astiasaran, W. K. Nip, J. G. Sebranek, E. T. F. Silveira, L. H. Stahnke, & R. Talon (Eds.), *Handbook of fermented meat and poultry* (pp. 177–186). Blackwell publishing.

Chasseignaux, E., Gerault, P., Toquin, M. T., Salvat, G., Colin, P., & Ermel, G. (2002). Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. *FEMS Microbiology Letters, 210*(2), 271–275.

Chevallier, I., Ammor, S., Laguet, A., Labayle, S., Castanet, V., Dufour, E., et al. (2006). Microbial ecology of a small-scale facility producing traditional dry sausage. *Food Control, 17*(6), 446–453.

Cocolin, L., Manzano, M., Cantoni, C., & Comi, G. (2001). Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. *Applied and Environmental Microbiology, 67*(11), 5113–5121.

Cocolin, L., Manzano, M., Aggio, D., Cantoni, C., & Comi, G. (2001). A novel polymerase chain reaction (PCR) -denaturing gradient gel

electrophoresis (DGGE) for the identification of *Micrococcaceae* strains involved in meat fermentations. Its application to naturally fermented Italian sausages. *Meat Science, 58*, 59–64.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., et al. (2005). Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science, 69*, 381–392.

Commission Regulation (EC) No. 2073/2005 (2005). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union, L338*, 1–26.

Coppola, S., Mauriello, G., Aponte, M., Moschetti, G., & Villani, F. (2000). Microbial succession during ripening of Naples-type salami, a southern Italian fermented sausage. *Meat Science, 56*, 321–329.

Corbière Morot-Bizot, S., Leroy, S., & Talon, R. (2006). Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. *International Journal of Food Microbiology, 108*(2), 210–217.

Demeyer, D. (2004). Meat fermentation: principles and applications – Chapter 20. In Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J. Josephsen, W.-K. Nip, & P. S. Stanfield, et al. (Eds.), *Handbook of food and beverage fermentation technology* (pp. 353–368). New York: Marcel Decker Inc..

Dordet-Frisoni, E., Talon, R., & Leroy, S. (2007). Physical and genetic map of the *Staphylococcus xylosus* C2a chromosome. *FEMS Microbiology Letters, 266*(2), 184–193.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F., & Metaxopoulos, J. (2005). Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science, 69*, 307–317.

Dudez, A. M., Chaillou, S., Hissler, L., Stentz, R., Champomier-Vergès, M., Alpert, C. A., et al. (2002). Physical and genetic map of the *Lactobacillus sakei* 23K chromosome. *Microbiology, 148*(Pt. 2), 421–431.

Egeter, O., & Brückner, R. (1996). Catabolite repression mediated by the catabolite control protein CcpA in *Staphylococcus xylosus*. *Molecular Microbiology, 21*(4), 739–749.

Fiegler, H., Bassias, J., Jankovic, I., & Brückner, R. (1999). Identification of a gene in *Staphylococcus xylosus* encoding a novel glucose uptake protein. *Journal of Bacteriology, 181*(16), 4929–4936.

Fontana, C., Cocconcelli, P. S., & Vignolo, G. (2005). Monitoring the bacterial population dynamics during fermentation of artisanal Argentinean sausages. *International Journal of Food Microbiology, 103*(2), 131–142.

Furet, J. P., Quenee, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology, 97*(2), 197–207.

Garcia-Varona, M., Santos, E. M., Jaime, I., & Rovira, J. (2000). Characterisation of micrococcaceae isolated from different varieties of chorizo. *International Journal of Food Microbiology, 54*(3), 189–195.

Giammarinaro, P., Leroy, S., Chacornac, J.-P., Delmas, J., & Talon, R. (2005). Development of a new oligonucleotide array to identify staphylococcal strains at species level. *Journal of Clinical Microbiology, 43*(8), 3673–3680.

Giovannacci, I., Queguiner, S., Ragimbeau, C., Salvat, G., Vendeuvre, J. L., Carlier, V., et al. (2001). Tracing of *Salmonella* spp. in two pork slaughter and cutting plants using serotyping and macrorestriction genotyping. *Journal of Applied Bacteriology, 90*(1), 131–147.

Giraffa, G. (2002). Enterococci from foods. *FEMS Microbiology Reviews, 26*(2), 163–171.

Greco, M., Mazette, R., De Santis, E. P. L., Corona, A., & Cosseddu, A. M. (2005). Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. *Meat Science, 69*, 733–739.

Hugas, M., Garriga, M., & Aymerich, M. T. (2003). Functionality of enterococci in meat products. *International Journal of Food Microbiology, 88*(2–3), 223–233.

Iacumin, L., Comi, G., Cantoni, C., & Cocolin, L. (2006a). Ecology and dynamics of coagulase-negative cocci isolated from naturally fermented Italian sausages. *Systematic and Applied Microbiology, 29*, 480–486.

Iacumin, L., Comi, G., Cantoni, C., & Cocolin, L. (2006b). Molecular and technological characterisation of *Staphylococcus xylosus* isolated from naturally fermented Italian sausages by RAPD, Rep-PCR and Sau-PCR analysis. *Meat Science, 74*, 281–288.

Lebert, I., Leroy, S., Lebecque, A., Chacornac, J. P., & Talon, R. (2006). Saucissons secs fermiers du Massif central – Développement d'un ferment indigène pour améliorer la sécurité et maintenir la typicité des saucissons. *Viandes et Produits Carnés, 25*(5), 177–180.

Lebert, I., Leroy, S., Giammarinaro, P., Lebert, A., Chacornac, J. P., Bover-Cid, S., et al. (2007). Diversity of micro-organisms in environments and dry fermented sausages of French traditional small units. *Meat Science, 76*, 112–122.

Lebert, I., Leroy, S., & Talon, R. (2007). Microorganisms in traditional fermented meats – Chapter 11. In F. Toldra, Y. H. Hui, I. Astiasaran, W. K. Nip, J. G. Sebranek, E. T. F. Silveira, L. H. Stahnke, & R. Talon (Eds.), *Handbook of fermented meat and poultry* (pp. 113–1124). Blackwell publishing.

Leroy, S., Chevallier, I., Lebert, I., Chacornac, J. P., & Talon, R. (2006). Saucissons secs fermiers du Massif central – Identification et caractérisation de la flore d'intérȩt technologique : bactéries lactiques et staphylocoques à coagulase négative. *Viandes et Produits Carnés, 25*(5), 171–175.

Madsen, S. M., Beck, H. C., Ravn, P., Vrang, A., Hansen, A. M., & Israelsen, H. (2002). Cloning and inactivation of a branched-chain-amino-acid aminotransferase gene from *Staphylococcus carnosus* and characterization of the enzyme. *Applied and Environmental Microbiology, 68*(8), 4007–4014.

Marceau, A., Mera, T., Zagorec, M., & Champomier-Vergès, M. C. (2001). Protein expression under uracil privation in *Lactobacillus sakei*. *FEMS Microbiology Letters, 200*(1), 49–52.

Martín, B., Garriga, M., Hugas, M., & Aymerich, T. (2005). Genetic diversity and safety aspects of enterococci from slightly fermented sausages. *Journal of Applied Microbiology, 98*(5), 1177–1190.

Martín, B., Garriga, M., Hugas, M., Bover-Cid, S., Veciana-Nogues, M. T., & Aymerich, T. (2006). Molecular, technological and safety characterization of Gram-positive catalase-positive cocci from slightly fermented sausages. *International Journal of Food Microbiology, 107*(2), 148–158.

Mauriello, G., Casaburi, A., Blaiotta, G., & Villani, F. (2004). Isolation and technological properties of coagulase negative staphylococci from fermented sausages of southern Italy. *Meat Science, 67*(1), 149–158.

Morot-Bizot, S., Talon, R., & Leroy-Sétrin, S. (2003). Development of specific PCR primers for a rapid and accurate identification of *Staphylococcus xylosus*, a species used in food fermentation. *Journal of Microbiological Methods, 55*(1), 279–286.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science, 65*(2), 859–867.

Planchon, S. (2006). *Aptitude de Staphylococcus carnosus et Staphylococcus xylosus à former des biofilms. Etude d'une souche "biofilm positif" par une approche protéomique*. Thèse de Docteur d'Université – Spécialité Sciences des Aliments, Option Microbiologie. Université Blaise Pascal, Clermont-Ferrand II, France.

Rantsiou, K., Drosinos, E. H., Gialitaki, M., Urso, R., Krommer, J., Gasparik-Reichardt, J., et al. (2005). Molecular characterization of *Lactobacillus* species isolated from naturally fermented sausages produced in Greece, Hungary and Italy. *Food Microbiology, 22*, 19–28.

Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P., Comi, G., et al. (2005). Culture-dependent and -independent methods to investigate the microbial ecology of Italian fermented sausages. *Applied and Environmental Microbiology, 71*(4), 1977–1986.

Rebecchi, A., Crivori, S., Sarra, P. G., & Cocconcelli, P. S. (1998). Physiological and molecular techniques for study of bacterial community development in sausage fermentation. *Journal of Applied Microbiology, 84*, 1043–1049.

Reij, M. W., & Den Aantrekker, E. D. (2004). Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology, 91*(1), 1–11.

Rosenstein, R., Nerz, C., Resch, A., & Götz, F. (2005). Comparative genome analyses of staphylococcal species. In *Proceedings of the second european conference on prokaryotic genomics*.

Rossi, F., Tofalo, R., Torriani, S., & Suzzi, G. (2001). Identification by 16S-23S rDNA intergenic region amplification, genotypic and phenotypic clustering of *Staphylococcus xylosus* strains from dry sausages. *Journal of Applied Microbiology, 90*, 365–371.

Samelis, J., Metaxopoulos, J., Vlassi, M., & Pappa, A. (1998). Stability and safety of traditional Greek salami – A microbiological ecology study. *International Journal of Food Microbiology, 44*(1–2), 69–82.

Talon, R. (2006). Saucissons secs fermiers du Massif central – Principaux résultats du projet européen. *Viandes et Produits Carnés, 25*(5), 187–188.

Talon, R., Lebert, I., Lebert, A., Leroy, S., Garriga, M., Aymerich, T., Drosinos, E. H., Zanardi, E., Ianeri, A., Fraqueza, M. J., Patarata, L., & Laukova, A. (2007). Traditional dry fermented sausages produced in small-scale processing units in Mediterranean countries and Slovakia. 1. Microbial ecosystems of processing environments. *Meat Sciences*, doi:10.1016/j.meatsci.2007.05.006.

Talon, R., & Leroy, S. (2006). Latest developments in meat bacterial starters – Chapter 16. In L. M. L. Nollet & F. Toldra (Eds.), *Advanced technologies for meat processing* (pp. 401–418). New York: CRC Press/ Taylor and Francis Group.

Talon, R., Leroy-Sétrin, S., & Fadda, S. (2004). Dry fermented sausages – Chapter 23. In Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J. Josephsen, W.-K. Nip, & P. S. Stanfield, et al. (Eds.), *Handbook of food and beverage fermentation technology* (pp. 397–416). New York: Marcel Dekker Inc..

Urso, R., Comi, G., & Cocolin, L. (2006). Ecology of lactic acid bacteria in Italian fermented sausages: Isolation, identification and molecular characterization. *Systematic and Applied Microbiology, 29*, 671–680.

Wagner, E., Doskar, J., & Götz, F. (1998). Physical and genetic map of the genome of *Staphylococcus carnosus* TM300. *Microbiology, 144*(Pt. 2), 509–517.