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Mini Review

Ecology of lactic acid bacteria and coagulase negative cocci in fermented dry sausages manufactured in Italy and other Mediterranean countries: an overview

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Abstract

Most European fermented dry sausages still follow the traditional procedures in which fermentation and ripening depend on the activities of heterogeneous microbial communities, largely dominated by lactic acid bacteria (LAB) whose contribution primarily relies on the production of organic acids and volatile compounds, and coagulase negative cocci (CNC), which are responsible for colour development and stabilisation, proteolysis, lipolysis, and decomposition of free amino acids and peroxides. This review summarises the studies concerning the structure of these two microbial communities in Mediterranean traditional fermented sausages. Overall a high microbial complexity was highlighted, although several common features emerged: LAB are more numerous than CNC during fermentation and ripening, remaining more stable in the ripened products. Within LAB, facultatively heterofermentative lactobacilli generally prevail and, among them, the two psychrotrophic species *Lactobacillus sakei* and *Lactobacillus curvatus* are dominant. Within CNC, *Staphylococcus xylosus* neatly dominates, except in the Greek and French sausages.

Introduction

Fermentation, combined with salting, drying, and sometimes smoking, is a strategy for the preservation of meat which dates back to very ancient times, with evidence of the remains of a salami-like food in the tomb of Ramses III (1166 b.C.), and the description of a method for the preservation of leg ham trim found in De Agricoltura (Cato the Censor, 234–149 b.C). According to Lucke (2000), the manufacturing of fermented sausages probably originated in the temperate regions of the Mediterranean area, because of their climate which is particularly favourable for the ripening process, and it is still most widespread in these countries. In fact, Europe is today the major producer and consumer of fermented sausages, which are generally manufactured from pork or, more rarely, from beef, veal, or other meats (Talon *et al*., 2004). The production starts with the meat and fat which are cut into small pieces or minced and added with salt and spices, and, in some cases, with sugar, herbs and/or other ingredients; the carefully homogenised mixture is then stuffed into casings, and undergoes fermentation and ripening (with contemporary drying). The use of preservatives (nitrate and nitrite) is generally adopted according to the pertinent legislation (Reg. EC 1333/2008 and subsequent modifications), unless subject to other regulations for protected denomination products.

Fermented sausages can be classified using different criteria, for instance on the basis of their final water activity and/or pH, or on the basis of the process conditions applied, such as ripening duration or the application of smoke (Lucke, 2000). Using these criteria, two main categories of fermented meat products have been identified, grouping together those manufactured in the North and the South of Europe, respectively (Talon *et al*., 2007). The former products, such as German and Hungarian salami, are fast-fermented to a pH value below 5, and are usually smoked, whereas those manufactured in the main countries of the Mediterranean area (Italy, France, Greece, Spain) are dry (with ripening > 4 weeks and a_w < 0.90) or semi-dry (with ripening < 4 weeks and a_w between 0.90 and 0.95), not smoked, and with a surface microbiota including moulds, yeasts and gram positive cocci (Lucke, 2000). The fermentation rate may vary (especially with temperature) and the final pH can be close to 5, or comprised between about 5.3 and 6.2 (Garcìa-Varona *et al*., 2000; Rantsiou *et al*., 2005a; Aymerich *et al*., 2006; Lebert *et al*., 2007). In fact, as traditions and people's preferences

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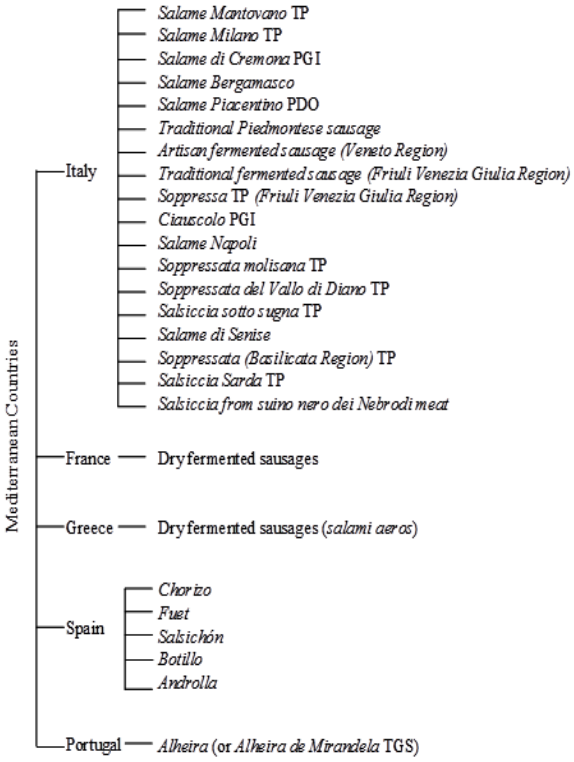
vary greatly in different countries and regions, the ingredients used and the manufacturing techniques adopted also differ considerably within each of these categories (Talon, 2006). Furthermore, most of the numerous products manufactured today in Europe are made following traditional procedures in which fermentation and ripening rely on the activities of extremely heterogeneous communities of indigenous microorganisms derived from both the raw material and the manufacturing environment (Chevallier *et al.*, 2006). Two wide groups of bacteria largely predominate: lactic acid bacteria (LAB) whose contribution primarily relies on acidification and the production of volatile compounds through the fermentation of carbohydrates, and the group known as either coagulase–negative cocci (CNC) or (gram-positive)-catalase-positive cocci (GCC^{+}/CPC), which includes both micrococci and coagulase–negative staphylococci (CNS). In fermented sausages, CNC are mainly responsible for colour development and stabilisation, proteolysis, lipolysis, and the decomposition of free amino acids and peroxides (Talon *et al.*, 2004; Iacumin *et al*., 2006; Rantsiou and Cocolin, 2006; Talon and Leroy, 2006; Urso *et al*., 2006). Yeasts and moulds also play a minor, though relevant, role, through the formation of a superficial film, which exerts a protective action against both excessive dehydration and the oxidation of the lipid fraction due to oxygen and light (Gardini *et al*., 2001, Cocolin *et al*., 2006).

The aim of this review, whose flow sheet is shown in Figure 1, is to summarise the studies carried out on the indigenous microbiota of the traditional Italian fermented meat products, most of which have received the PDO (Protected Designation of Origin) or PGI (Protected Geographical Indication) status in accordance with EU regulations (Reg EC 510/06 and 628/08), or are certified as traditional products (TP), being included in the official list of traditional products published by the Italian Ministry of Agriculture and Forestry (G.U. Repubblica Italiana no. 147, 27/06/2013 Suppl. Ord. no. 52). For the purpose of comparison, investigations carried out on traditional products manufactured in other Mediterranean countries are also discussed. To help the reader to follow the article, a summary table (Table 1) has been included.

The microbiota of fermented meat products manufactured in Italy

Fermented meat products manufactured in Southern Italy commonly have a stronger flavour than those produced in Northern and Central Italy, due to the addition of different types and quantities



PDO Protected Designation of Origin
PGI Protected Geographical Indication
TGS Traditional Guaranteed Speciality
TP certified as traditional products (included in the official list of traditional products published by the Italian Ministry of Agriculture and Forestry).

Figure 1. The article flow sheet

of ingredients (such as particular meat cuts and spices, like chilli pepper). On the other hand, the use of different recipes is likely to determine differences in the composition and dynamics of the microbiota, which in turn affect the final features of the products. Therefore salami from Northern-Central and Southern Italy have been treated separately in the following paragraphs.

Northern and Central Italy

Many fermented meat products manufactured in the Northern and Central regions of Italy, such as *salame Milano, salame mantovano, Salame di Cremona* PGI, *salame bergamasco,* and *Salame Piacentino* PDO have denominations which include the name of the place where the production is (mainly) located, as evidence of their ancient tradition, irrespective of their PDO, PGI, or TP status.

In an early attempt to characterise the lactobacilli and staphylococci communities of *salame Milano*, a combined approach including physiological tests and molecular analyses was used on samples collected during two months of ripening (Rebecchi *et al*., 1998). After 15 days and up to the end of the ripening period, *Lactobacillus sake* (an orthographically incorrect

Table 1. Non-exhaustive overview of the species diversity of lactic acid bacteria (LAB) and/or coagulase negative cocci (CNC) in the main fermented dry sausages manufactured in Italy and other Mediterranean countries

Country	Salami	Identification methods	LAB		CNC		Reference(s)
			Counts (log cfu g^{-1})	Species	Counts (log cfu g^{-1})	Species	
Italy	*Salame Milano*	Physiological tests and molecular analyses.	8.0	*Lb. sake, Lb. plantarum.*	6.9	*Staph. xylosus, Staph. sciuri.*	Rebecchi *et al.*, 1998.
	Soppressa (Veneto Region)	RAPD and species-specific PCR analyses.	n.a.	*Lb. sakei, Lb. curvatus.*	n.a.	n.a.	Andrighetto *et al.*, 2001.
	Traditional *Piedmontese sausage*	Reverse transcription RT-PCR-DGGE coupled with RNA-based pyrosequencing	8.0	*Lb. curvatus, Lb. sakei, Lb. piscium, Lb. algidus, Leu. carnosum, Leu. gelidum, Leu. mesenteroides,*	7.0	*Staph. xylosus, Staph. succinus, Staph. equorum,*	Greppi *et al.*, 2015.
	Traditionally fermented sausages (Friuli Venezia Giulia)	Physiological tests (API 50 CHL), PCR-DGGE, RAPD-PCR.	4.0-9.0	*Lc. lactis* ssp. *lactis*, *Lb. sakei, Lb. curvatus, Lb. plantarum, Leuconostoc* spp., *Pediococcus* spp.	5.2-8.8	*Staph. xylosus, Staph. warneri, Staph. pasteuri, Staph. saprophyticus, Staph. epidermidis, Staph. cohnii, Staph. equorum, Staph. carnosus, M. caseolyticu.*	Cocolin *et al.*, 2001a; Cocolin *et al.*, 2001b ; Comi *et al.*, 2005; Rantsiou *et al.*, 2005b; Urso *et al.*, 2006; Iacumin *et al.*, 2006; Kozačinski *et al.*, 2008.
	Salame mantovano Salame cremonese Salame bergamasco	PCR-DGGE, RAPD-PCR.	8.0	*Lb. sakei, Lb. curvatus, Lb. plantarum, Lb. paraplantarum, Leu. citreum, W. hellenica, Lb. algidus, Leu. mesenteroides/pseudomesenteroides.*	4.0-6.0	n.a.	Cocolin *et al.*, 2009.
	Salame mantovano	RAPD-PCR.	8.0-9.0	*Lb. curvatus, Lb. paracasei, Lb. plantarum, Lb. salivarius, Lb. fermentum.*	6.1-4.4	*Staph. xylosus, Staph. saprophyticus, Staph. epidermidis, K. varians.*	Pisacane *et al.*, 2015.
	Salame Piacentino	Physiological tests and molecular analyses, RAPD-PCR, ARDRA, High-throughput sequencing.	8.2	*Lb. acidophilus, Lb. helveticus, Lb. sakei, Lb. antri, Lb. oris, Lb. vaginalis, Lb. brevis, Lb. panis, Lb. versmoldensis, Lb. coryniformis, Lb. paracasei, Lb. zeae, Lb. curvatus, Lb. paralimentarius, Lb. frumenti, Lb. plantarum, Lb. graminis, Lb. reuteri.*	4.0-7.5	*Staph. auricularis, Staph. arlettae, Staph. caseolyticus, Staph. carnosus, Staph. gallinarum, Staph. warneri, Staph. condimenti, Staph. nepalensis, Staph. lentus, Staph. sciuri, Staph. saprophyticus, Staph. equorum, Staph. kloosii, Staph. simulans, Staph. succinus, Staph. epidermidis, Staph. cohnii, Staph. hominis, Staph. pasteuri, Staph. xylosus.*	Di Cagno *et al.*, 2008; Polka et al., 2015; Rebecchi *et al.*, 2015.
	Salame Piacentino		n.a.		n.a.		
	Ciauscolo	PCR-DGGE.	7.5	*Lb. sakei, Lb. curvatus, Lb. plantarum, P. pentosaceus.*	5.0	*Staph. xylosus, Staph. equorum, Staph. saprophyticus.*	Silvestri *et al.*, 2007; Aquilanti *et al.*, 2007; Santarelli *et al.*, 2007; Petruzzelli *et al.*, 2010; Federici *et al.*, 2014.
	Salame Napoli	Phenotypic and physiological methods, antibiotyping and genotyping.	8.0-8.8	*Lb. sakei, Lb. curvatus, Lb. alimentarius, Lb. casei* ssp. *tolerans, Lb. plantarum.*	6.2-7.0	*Staph. saprophyticus, Staph. chromogenes, Staph. hominis, Staph. warneri, Staph. lugdunensis, Staph. epidermidis, Staph. capitis, Staph. cohnii, Staph. simulans.*	Coppola *et al.* 1995; Coppola *et al.* 2000; Moschetti *et al.*, 1997; Mauriello *et al.*, 2000.
	Soppressata molisana	Phenotypic and physiological methods.	8.0	*Lb. plantarum, Lb. paracasei* ssp. *paracasei, Lb. viridescens, Lb. coryniformis* ssp. *torquens, Lb. paralimentarius, Lb. brevis, Lb. graminis, Lb. curvatus.*	4.0-5.0	*Staph. xylosus, Staph. equorum, Staph. kloosii, Staph. simulans, M. varians, M. kristinae, M. roseus.*	Coppola *et al.*, 1997; Coppola *et al.*, 1998.
	Soppressata del Vallo di Diano	PCR-analysis.	6.7-9.3	*Lb. sakei, Lb. curvatus.*	4.2-7-3	*Staph. xylosus, Staph. equorum , Staph. succinus.*	Villani *et al.*, 2007.
	Salame di Senise	RAPD-PCR, Partial rDNA sequencing.	7.0	*Lb. sakei, Lb. curvatus, Lb. casei, Lc. lactis* subsp. *lactis.*	6.0	*Staph. saprophyticus, Staph. equorum, Staph. succinus.*	Baruzzi *et al.*, 2006.

	Salsiccia and *soppressata* (Basilicata Region)	Phenotype-based tests and molecular methods.	8.0	*Lb. plantarum, Lb. curvatus, Leu. carnosum , Leu. gelidum, Leu. pseudomesenteroides.*	6.0	*Staph. pulvereri, Staph. vitulus, Staph. saprophyticus , Staph. equorum, Staph. pasteuri, Staph. succinus, Staph. intermedius, Staph. epidermidis, Staph. warneri , Staph. lentus, Macr. caseolyticus.*	Parente *et al.*, 2001; Bonomo *et al.*, 2008; Blaiotta *et al.*, 2004; Bonomo *et al.*, 2009.
	Salsiccia sotto sugna	RAPD-PCR.	n.a.	n.a.	n.a.	*Staph. xylosus, Staph. simulans, K. varians, K. roseus, K. kristinae.*	Rossi *et al.*, 2001.
	Salsiccia sarda	Phenotype-based tests and molecular methods.	8.2-10.2	*Lb. sakei, Lb. plantarum, Lb. curvatus.*	5.4-9.1	*Staph. xylosus, K. varians, Micrococcus* spp.	Mangia *et al.*, 2007; Mangia *et al.*, 2008; Greco *et al.* 2005.
	Salsiccia from *suino nero dei Nebrodi* meat	Phenotype-based tests and molecular methods.	7.0	*Lb. sakei.*	6.9	n.a.	Francesca *et al.*, 2013.
France	Dry fermented sausages	Phenotype-based tests and molecular methods.	6.5-7.7	*Lb. sakei, Leuconostoc* spp., *Lactococcus* spp.	6.2-7.9	*Staph. equorum, Staph. saprophyticus, Staph. succinus, Staph. vitulinus, Staph. pasteuri, Staph. carnosus, Kocuria* spp.	Chevallier *et al.*, 2006; Ammor *et al.*, 2005; Talon, 2006; Leroy *et al.*, 2010; Corbière Morot-Bizot *et al.*, 2006; Coton *et al.*, 2010.
Greece	Dry fermented sausages	Phenotype-based tests and molecular methods.	7.8-8.2	*Lb. sakei, Lb. plantarum, Lb. curvatus, Lb. pentosus, Lb. rhamnosus, Lb. brevis, Lc. lactis* subsp. *lactis, W. hellenica, W. paramesenteroides, W. viridescens, W. minor.*	5.0-7.7	*Staph. saprophyticus, Staph. xylosus, Staph. carnosus, Staph. simulans, Staph. cohnii Staph. haemolyticus.*	Samelis *et al.*, 1994 ; Samelis *et al.*, 1998 ; Papamanoli *et al.*, 2002; Papamanoli *et al.*, 2003; Drosinos *et al.*, 2005; Rantsiou *et al.*, 2005a; Kozačinski *et al.*, 2008.
Spain	*Chorizo*	Phenotype-based tests, Real-time PCR.	6.5-7.6	*Lb. brevis, Lb. curvatus, Lb. sakei, Lc. lactis, P. acidilactici, P. pentosaceus, Leu. mesenteroides.*	6.3	*Staph. xylosus, Staph. intermedius, Staph. equorum, Staph. saprophyticus.*	Garcia-Varona *et al.*, 2000; Benito *et al.*, 2007; Aymerich *et al.*, 2003; Aymerich *et al.*, 2006; Martin *et al.*, 2006; Fonseca et al., 2013.
	Salsichòn	Physiological tests (API CHL 50), RAPD-PCR.	6,5-7.6	*Lb. brevis, Lb. curvatus, Lb. plantarum, Lb. sakei, Lc. lactis, P. acidilactici, P. pentosaceus, Leu. mesenteroides.*	6.3	*Staph. epidermidis, Staph. xylosus, Staph. warneri, K. varians.*	Benito *et al.*, 2007; Aymerich *et al.*, 2006 ; Martin *et al.*, 2006.
	Fuet	Species-specific PCR, RAPD-PCR.	8.13	*Lb. sakei, Lb. plantarum, Lb. curvatus.*	6.7	*Staph. carnosus, Staph. epidermidis, Staph. xylosus, Staph. warneri, K. varians.*	Aymerich *et al.*, 2003; Aymerich *et al.*, 2006; Martin *et al.*, 2006.
	Botillo		8.8	*Lb. alimentarius, Lb. curvatus, Lb. plantarum, Lb. farciminis, Leu. mesenteroides* subsp. *mesenteroides.*	6.5	*Staph. xylosus, Staph. lentus, Staph. cohnii, Staph. epidermidis, Staph. sciuri , Staph. capitis, Micrococcus* spp.	Garcia Fontan *et al.*, 2007a.
	Androlla	Physiological tests.	9.1	*Lb. sakei, Lb. plantarum, Lb. curvatus, Lb. alimentarius.*	3.6	*Staph. xylosus, Staph. epidermidis, Staph. equorum, Staph. capitis. Staph.*	Garcia Fontan *et al.*, 2007b.
Portugal	*Alheira* (or *Alheira de Mirandela*	PCR-DGGE.	7.0-8.0	*Lb. sakei, Lb. curvatus, Lb. brevis, Lc. lactis, Leu. mesenteroides, Leu. lactis.*	4.4-6.5	*Macr. caseolyticus.*	Albano *et al.* 2008; Ferreira *et al.*, 2006; Esteves *et al.*, 2008.

cfu colony forming units
Lb. *Lactobacillus*; Lc. *Lactococcus*; Leu. *Leuconostoc*; K. *Kocuria*; Macr. *Macrococcus*; M. *Micrococcus*; P. Pediococcus; Staph. *Staphylococcus*; W. *Weissella*
PCR-DGGE Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis
RAPD Random Amplified Polymorphic DNA
ARDRA Amplified Ribosomal DNA Restriction Analysis
n.a. not available

synonym for *Lactobacillus sakei*) and *Lactobacillus plantarum* were found as the two dominant species, whereas *Staphylococcus xylosus* and *Staphylococcus sciuri* prevailed within the CNS population, with values of 30 and 25%, respectively. LAB counts increased from about 2 to 8 log cfu g^{-1} after 15 days and remained stable, whereas micrococci and non-pathogenic staphylococci reached their maximum (6.9 log cfu g^{-1}) after 30 days.

A few years later, in a study on artisan sausages produced in the Veneto region (soprèssa and other traditional salami) 36 isolates of lactobacilli were collected from the products at different ripening times (1 week, 2 and 6 months) and 17 others from the cold room environments (Andrighetto *et al.*, 2001). The results of RAPD and species-specific PCR analyses on the isolates proved that 39.1 and 34.8 % of those from the traditional salami were *Lb. sakei* and *Lactobacillus curvatus*, respectively, while 92.3% of the isolates from the long-ripened soprèssa belonged to the former species. The finding that all the 17 isolates from the cold room environment as well as from the 6 month-ripened soprèssa belonged to *Lb. sakei* suggested that the high adaptability of this species to unfavourable conditions of temperatures and aw is one of the causes of its dominance in fermented dry sausages.

The CNC occurring in traditionally fermented

sausages produced in the Friuli Venezia Giulia region were identified by PCR-DGGE (Cocolin *et al*., 2001a): more than 86% of the 90 isolates from meat samples (collected just before stuffing and at 3, 10, 20 and 45 days of ripening) were *Staph. xylosus* and the percentage rose to 100% in the samples from 10 to 45 days. In the same work, LAB counts increased from 4.92 to more than 9 log cfu g^{-1} in the first 10 days, then decreased slightly, whereas CNC constantly increased (from about 4 to 8.8 log cfu g^{-1}).

The microbiota of fermented sausages from the Friuli Venezia Giulia region were investigated again during manufacturing (at 0, 3, 10, 20 and 45 days) through a culturedependent and -independent approach (Cocolin *et al*., 2001b); once more, LAB counts higher than 8 log cfu g^{-1} were detected early on and remained until the end of ripening, while for CNC lower values (about 6 log cfu g^{-1}), decreasing after 20 days, were recorded. Accordingly, multiple bands (most of which referred to *Staphylococcus* spp.) were visible in the PCR-DGGE profiles corresponding to the early fermentation phase, whereas only LAB bands were detected after 10 days of fermentation. After 3 days, *Staph. xylosus* was the only *Staphylococcus* species occurring in the DGGE profiles whereas *Lb. sakei* and *Lb. curvatus* were the only two species of lactobacilli (this evidence also being confirmed by the culturedependent method). These results were basically substantiated when the dynamics of the LAB and CNC communities were further investigated at three different factories, during fermentation and ripening, lasting for different periods (Comi *et al*., 2005; Rantsiou *et al*., 2005b; Urso *et al*., 2006; Iacumin *et al.*, 2006). The dynamics of LAB and CNC counts were close to those reported by Cocolin *et al*. (2001a); *Lb. sakei* and *Lb. curvatus* were again found as the dominant species, accounting for 353 and 67 out of 465 isolates, respectively, followed by *Lb. plantarum,* which was the only other species found with an appreciable frequency (7 and 10 isolates, respectively) in two of the three plants (Urso *et al*., 2006). The RAPD-PCR profiles of the cultures belonging to the two main species were grouped into clusters, some of which included strains from different fermentations while others included only strains from a specific fermentation. Concerning the ecology and dynamics of CNC populations, *Staph. xylosus* was confirmed as the main species in all three plants, although in one of them *Staphylococcus warneri* and *Staphylococcus pasteuri* participated in ripening to the same extent as the former species; other species (*Staphylococcus saprophyticus*, *Staphylococcus epidermidis, Staphylococcus cohnii, Staphylococcus equorum, Staphylococcus carnosus* and *Micrococcus caseolyticus*) were found more sporadically in the three productions (Iacumin *et al.*, 2006). Quite different results for the LAB community during sausages manufacturing (from day 0 to 28) in the same Italian region were obtained in a comparative study on sausages produced in different Southern-Eastern European countries (Kozačinski *et al*., 2008); in this case the 150 LAB isolates were identified using API 50 CHL (Biomerieux) and were ascribed to: *Lactococcus. lactis* ssp. *lactis* (39 isolates), *Lactobacillus fermentum* (21), *Lb. plantaurum* (17), *Lb. curvatus* (12) and 8 minority species including leuconostocs and pediococci. Among CNC, *Staph. xylosus* was confirmed as dominating throughout the production process (as was found from the identification of cultures with the API Staph method); 111 out of 150 CNC isolates were indeed ascribed to this species, whereas *Staph. warneri* and *Staph. saprophyticus* were only marginally found, accounting for 11 and 5 isolates respectively, together with other minority species. As far as the loads of LAB and CNC are concerned, viable counts ranging from 4.64 and 4.02 to 8.44 and 5.22 were recorded, respectively. More recently, the possible influence of the breed of pork on the composition of CPC in traditional fermented sausages manufactured in North-Eastern Italy was investigated (Iacumin *et al.*, 2012). A significant increase in CPC during the first few days of fermentation has always been recorded with viable counts of 5-6 log cfu g^{-1} within 3 days. Semi-quantitative molecular methods were used to determine the CPC ecology: *Staph. xylosus* and *Staph. equorum* were predominant in all the monitored fermentations. *Staphylococcus haemolyticus, Staphylococcus lentus, Micrococcus luteus, M. caseolyticus* and *Staphylococcus succinus* were also present, but their load was variable under the different experimental conditions. The results of cluster analysis allowed a plant-specific CPC ecology to be revealed, as well as the influence of the breed on the occurrence of some CPC species.

The LAB communities of the three traditional sausages *salame mantovano, salame cremonese,* and *salame bergamasco* produced in eleven plants where starter cultures had never been used, were also comparatively investigated during ripening, using culture-dependent and -independent methods (Cocolin *et al.*, 2009). The viable counts always revealed the dominance of LAB (above 8 log cfu g^{-1}) and only in two of the eleven plants this microbial group underwent a descending tendency right from the beginning of ripening. CNC viable counts were stable during the monitoring period at about 4-6 log cfu g^{-1}. In the isolation campaign, *Lb. sakei* and *Lb.*

curvatus together accounted for more than 90% of the isolates collected from the three products at 5, 30, and 60 days of ripening. In 8 plants, more than 60% of the isolates belonged to *Lb. sakei*, whereas in the remaining three plants, *Lb. curvatus* accounted for 80% of the collected cultures. In all the plants, *Lb. plantarum, Lactobacillus paraplantarum, Leuconostoc citreum* and *Weissella hellenica* were isolated with clearly lower frequencies. The DGGE profiles from the analysis of the DNA extracted directly from the salami samples confirmed the dominance of *Lb. sakei* and *Lb. curvatus*, whereas other bands attributable to the minority species *Lactobacillus algidus, Leuconostoc mesenteroides/ pseudomesenteroides,* and *Lb. paraplantarum* were sporadically found. The cluster analysis of the Randomly Amplified Polymorphic DNA (RAPD) patterns of the 293 *Lb. sakei* and the 177 *Lb. curvatus* isolates revealed a high intra–specific biodiversity; in particular, the results obtained suggested the existence of a plant-specific population, whereas a geographic characterisation of the products was not identified. A more recent study on *salame mantovano* produced using two different casing types (derived from two different portions of the pig's large intestine) was especially aimed at investigating the contribution of casing microbiota to the ecology of the sausage fermentation/ripening process (Pisacane *et al.*, 2015). As commonly observed in salami fermentation, the counts of lactobacilli reached values between 8 and 9 log cfu g^{-1} within 8 days and remained quite constant until the end of ripening, independently from the type of casing used. The predominant species also resulted unaffected by the type of casing, being *Lb. sakei* in both cases, whereas the minority species (*Lb. curvatus*, *Lb. paracasei, Lb. plantarum, Lb. salivarius* and *Lb. fermentum* were found in different percentages in the two types of productions, the latter species being isolated only in the salami made with Gentile casing. Also for the population of Gram positive-Catalase positive (GPCP) cocci, the most representative species (*Staph. xylosus, Staph. saprophyticus, Staph. epidermidis* and *Kokuria varians*) were found in both types of production, though some differences were again detected for the minority species; also the GPCP counts seemed to be affected by the type of casing used. Independently from the casing type used, LAB showed a higher biodiversity than GPCP cocci (with 28 clusters vs. 16 and 14 single strains vs. 10) .

The bacterial diversity in *Salame Piacentino* PDO was first studied by Di Cagno and co-workers (2008) and the prevalence of *Lb. sakei* and *Lb. curvatus* among LAB isolates and of *Staph. xylosus* among CNC was once again found together with values of the two autochthonous communities (8.2 and 7.5 cfu g^{-1}, respectively after 10 days of ripening) within the ranges commonly observed. More recently, *Salame Piacentino* samples withdrawn during ripening from 6 different local factories were analysed by using high-throughput sequencing (HTS) coupled to the PCR amplification of the 16S rRNA subunit (Polka *et al*., 2015). Thirty-two different *Staphylococcus* and 33 *Lactobacillus* species where identified in the salami from different producers, while the whole data set obtained accounted for 13 main families and 98 rare ones, 23 of which were present in at least 10% of the investigated samples, with casings being the major sources of the observed diversity. Multivariate analyses also showed that batches from 6 local producers tend to cluster altogether after 21 days of ripening, thus indicating that HTS has a great potential for fine scale differentiation of local fermented foods. Rebecchi and colleagues (2015) lately reported the results of the first investigation carried out on antibiotic resistant CNS isolated from *Salame Piacentino* production chain. Among the isolates, *Staph. warneri, Staph. condimenti, Staph. nepalensis, Staph. lentus, Staph. sciuri, Staph. saprophyticus, Staph. equorum, Staph. simulans, Staph. epidermidis, Staph. cohnii, Staph. hominis, Staph. pasteuri*, and *Staph. xylosus* were discovered. The results highlighted that the CNS isolated during food processing contained AR genes, but the technological process involved in the *Salame Piacentino* under study was able to reduce the extent of antibiotic resistant strains.

Traditional Piedmontese sausage samples from a local meat factory in the area of Torino (Italy) were analyzed by Greppi *et al*. (2015) together with swab samples collected in the production plant. Reverse transcription RT-PCR-DGGE coupled with RNA-based pyrosequencing of the 16S rRNA gene were used to study the diversity of metabolically active microbiota occurring during the natural fermentation of salami. Microbial counts of LAB attested at about 8 log cfu g^{-1}, while CNC counts were about 7 log cfu g^{-1}. The most frequently isolated species during maturation time were *Staph. succinus*, *Staph xylosus* and *Lb. sakei*; moreover, repetitive extragenic palindromic PCR (rep-PCR) analysis showed that *Staph. succinus* and *Staph. xylosus* isolated from swabs and salami samples clustered together, suggesting possible contamination during the production process. RT-PCR-DGGE and rRNA-based pyrosequencing showed a metabolically active populations dominated by *Staph. succinus, Lb. sakei* and *Leuconostoc carnosum.*

Ciauscolo is the only protected (PGI) salami manufactured in Central Italy; its microbial ecology has already been investigated in a study concerning both the bacterial communities (LAB and CNC) and the eumycetes (yeasts and moulds) in ready-for-sale salami, purchased from 22 plants located in the Marche region (Silvestri *et al.*, 2007). The results of viable counts of LAB and CNC showed a certain variability (higher for CNC), probably reflecting differences in the manufacturing techniques and environmental conditions. Notwithstanding such heterogeneity, a clear dominance of LAB over CNC was always seen, with counts mostly higher than 7.5 log cfu g^{-1} for LAB and lower than 5.0 log cfu g^{-1} for CNC. In the DGGE profiles obtained by analysing the DNA extracted directly from the salami samples, two bands (attributed to *Lb. sakei* and *Lb. curvatus*) were found with the highest frequency of about 30% each. From cluster analysis of the DGGE profiles, bacterial diversity did not seem to depend either on plant location or on production scale or technology. On the basis of calculated diversity indices, higher heterogeneity was found for bacteria than for yeasts and moulds, thus confirming the secondary role of the latter two microbial groups in the ripening process of salami. One of the 22 *Ciauscolo* productions considered was then subjected to a polyphasic analytical approach aimed at assessing the microbial dynamics throughout the manufacturing process (Aquilanti *et al*., 2007; Santarelli *et al*., 2007; Petruzzelli *et al*., 2010). As expected, high loads of LAB were again found from the beginning of the ripening period: in fact from the 3rd day to the end of ripening, the values of log cfu g^{-1} were comprised between 7 and 8 (or little more) for LAB, as required by the PGI production scheme, and between 5 and 6 (or little more) for CNC. The identification of 58 bacterial isolates allowed 35 of them to be identified as *Staph. xylosus*, 18 as *Lb. plantarum* and 5 as *Lb. curvatus*, whereas in the DGGE profiles (from the DNA extracted either directly from the samples, or from the cells bulk), six bands (attributed to *Lb. sakei, Lb. curvatus, Lb. plantarum, Staph. xylosus, Staph. equorum, Staph. saprohyticus*) were detected from the 3rd to the 45th day of ripening, albeit with different intensities. Interestingly, a band corresponding to the closest relative of *Lc. lactis* ssp. *lactis* was found in the DGGE profiles until the 3rd day (from the DNA obtained with both methods). A subsequent study (Federici *et al*., 2014) was aimed at characterising the LAB population of *Ciauscolo* salami from samples withdrawn in 5 different factories. Fourty-two isolates were identified through ARDRA profiling coupled with sequencing of the 16S rRNA gene; 14 different profiles were obtained, encompassing 5 clusters and 9 single strains with the two major groups (15 and 9 isolates, respectively) displaying more than 99% identity with the 16S sequence of *Lb. sakei* and *Pediococcus pentosaceus*, respectively.

Southern Italy

Fermented meat products manufactured in Southern Italy include many different types, called with the generic names of *salame, soppressata,* or *salsiccia*. They are produced following the ancient traditions of the different regions, and the name of the location where the production takes place is often included in their denomination, although most of these products do not have protected denominations at EU level.

In the earliest studies, the microbial ecology of products manufactured in the South of Italy was generally investigated through the isolation of a large number of cultures followed by the study of their main physiological features. This approach was used by Coppola *et al*. (1995) to investigate the microbiota of *salame Napoli*, a traditional product from the Campania region produced without any addition of sugar to the minced meat, and smoked for up to 10 days. In general, a change in the microbiota was seen during ripening, with a rapid increase in mesophilic lactobacilli counts to 8 log cfu g^{-1} during the first 7 days of ripening followed by a progressive decrease in the further maturation phase. By contrast, a slow increase in the load of the CNC was seen from initial counts of about 5 log cfu g^{-1} to about 7 log cfu g^{-1} after 30 days of ripening. The isolation campaign revealed a clear predominance of the species *Lb. sakei* and *Lb. curvatus*, accounting for 63 and 29% of the isolates, respectively, as well as the occurrence of minority species, namely *Lactobacillus alimentarius, Lactobacillus casei* ssp. *tolerans*, and *Lactobacillus bavaricus* (reclassified to *Lactobacillus sake* in the year 1995).

A few years later, the dynamics of the microbial populations during the spontaneous fermentation and curing of *salame Napoli* were again investigated by Coppola *et al*. (2000) who sampled five batches of salami immediately after stuffing and at 2, 7, 14, 23 and 41 days of ripening. In accordance with the previous investigation, lactobacilli predominated over CNC and yeasts, both on the surface and in the interior, throughout the ripening period. The maximum values were 8.8 and 6.2 log cfu g^{-1}, for LAB and CNC, respectively, which remained quite stable until the end of ripening. The isolation campaign produced 327 isolates, which underwent phenotype-based tests: most of the 191 phenotypically identified

lactobacilli were ascribed to *Lb. sakei* (55 isolates), *Lb. sake* former *Lb. bavaricus* (65) (today both classified as *Lb. sakei*), whereas *Lb. curvatus* (16), *Lb. plantarum* (10) and other species were found with much lower frequencies; leuconostocs (18 isolates) prevailed among coccus–shaped LAB. As far as the 136 isolates of CNC are concerned, *Staph. xylosus* was the species most frequently found, accounting for 18 isolates, followed by *Staph. saprophyticus* (6 isolates), *Staphylococcus chromogenes* (4 isolates) and other minority species, namely *Staphylococcus hominis, Staph. warneri, Staphylococcus lugdunensis*, and *Staph. epidermidis*. It is worth noticing that as much as 82% of the CNS isolates failed to be identified at species level using the API Staph method. Notwithstanding this drawback, the dominance of *Staph. xylosus* in *salame Napoli* was confirmed through antibiotyping and genotyping of 42 staphylococcal isolates, 30 of which belonged to the above cited species and the remaining ones to the minority species *Staphylococcus capitis, Staph. saprophyticus, Staph. hominis, Staph. cohnii* and *Staphylococcus simulans* (1 isolate each) or remained unidentified at species level (7 isolates) (Moschetti *et al.*, 1997; Mauriello *et al.*, 2000). The same culture-dependent approach was used in two studies concerning the CNC/CNS and LAB communities of *soppressata molisana* (Coppola *et al.*, 1997; Coppola *et al.*, 1998). *Soppressata*, a traditional fermented meat product from the Molise region, was characterised by counts of lactic acid bacteria above 8 log cfu g^{-1} from the first week to the end of ripening, and of CNC from about 4 to 5 log cfu g^{-1}. Among the 138 cultures of CNC and CNS, 80 were ascribed to the genus *Staphylococcus*, in particular *Staph. xylosus* (60 isolates), whereas *Staph. simulans, Staph. equorum* and *Staphylococcus kloosii* were found as less frequent species. A further 58 isolates belonged to the genus *Micrococcus* (today invalid taxon), with a prevalence of *Micrococcus kristinae*, followed by *Micrococcus roseus*, and *Micrococcus varians*. Once again, the lactobacilli community was dominated by *Lb. sakei* (125 out of 183 isolates), followed by *Lb. plantarum* (13), *Lactobacillus paracasei* ssp. *paracasei* (11), *Lactobacillus viridescens* (today classified as *Weissella viridescens*) (10), *Lactobacillus coryniformis* ssp. *torquens* (6), *Lactobacillus paralimentarius* (6), *Lactobacillus brevis* (6), *Lactobacillus graminis* (4) and *Lb. curvatus* (2).

More recently, the microbial ecology of *soppressata del Vallo di Diano*, a traditional product from the Campania region was investigated by Villani *et al*., (2007) with the twofold aim of identifying the species involved in this production and of selecting LAB and CNS strains suitable for use as starter cultures. The LAB and staphylococci viable counts, as determined in the fully ripened samples purchased from ten different factories, were extremely heterogeneous, ranging from 6.7 to 9.3 and from 4.2 to 7.3 log cfu g^{-1}, respectively, most likely reflecting differences in the manufacturing procedures. When the variable V1 region of the 16S rRNA gene was analysed and the bands identified at species level, *Lb. sakei* and *Lb. curvatus* were found as the main LAB species, in five and six samples, respectively, whereas *Staph. xylosus, Staph. succinus*, and *Staph. equorum* were the prevailing CNS (each species found in five samples).

In Basilicata, a region in Southern Italy, different kinds of traditional fermented sausages are produced without microbial starters. Among them, the *Salame di Senise* (a traditional dry sausage manufactured in some small-scale sausage factories throughout the Sinni Valley region) has been investigated with respect to the naturally associated microbiota (Baruzzi *et al.*, 2006). The results obtained revealed that lactobacilli were the dominant microbiota at the end of ripening with viable counts on MRS- and MSA-agar about 7.0 log cfu g^{-1} and 6.0 log cfu g^{-1}, respectively, in the 25 days ripened sausages; isolates were genotypically identified as belonging to *Lb. sakei, Lb. curvatus* and *Lb. casei* species and two strains of *Lb. sakei* and *Lb. curvatus* were found at both the beginning and the end of ripening.

The LAB community of traditional *salsiccia* and *soppressata* manufactured in the Basilicata region, was investigated by Parente *et al.* (2001) by comparing artisan and industrial products during manufacturing and ripening (lasting 20-25, or up to 40 days, for salsiccia and *soppressata*, respectively). The results of this study are included in the present review with the exception of those concerning some of the industrial productions that included the addition of starter cultures. As far as the other productions are concerned, with a few exceptions, the LAB population at the end of ripening was close to or higher than 8.0 log cfu g^{-1}; it was dominated by facultatively heterofermentative lactobacilli and *Lb. sakei* accounted for 62% of the isolates, whereas other lactobacilli (*Lb. plantarum* and *Lb. curvatus*) and leuconostocs (*Leu. carnosum, Leuconostoc gelidum* and *Leu. pseudomesenteroides*) were found in much lower percentages. These identification results, obtained on the basis of 28 phenotype-based tests and further cluster analysis, were then confirmed (with few exceptions) using molecular methods (Bonomo *et al*., 2008). Further research work was carried out on the same productions of *salsiccia* and *soppressata* from the Basilicata

region to assess the entity of the CNS community, its diversity and its dynamics (Blaiotta *et al*., 2004). Only the artisan productions obtained without any starter culture had staphylococci loads which, although quite variable, usually reached values > 6.0 log cfu g^{-1} at the end of ripening. A total of 306 isolates were collected from the artisan plants and identified using a set of 18 phenotypic tests followed by cluster analysis: *Staph. xylosus* was isolated throughout the ripening process at all the plants and from almost all the samples, together with some species *(Staphylococcus pulvereri, Staphylococcus vitulus, Staph. saprophyticus, Staph. equorum)* found with appreciable frequencies and others (*Staph. pasteuri, Staph. succinus, Staphylococcus intermedius, Staph. epidermidis, Staph. warneri, Staph. lentus* and *Macrococcus caseolyticus*) isolated to a lesser extent. In general, the composition of the CNS community varied significantly with sausage type, ripening time and plant; for example, *Staph. saprophyticus* was isolated with the highest frequency in one of the plants and *Staph. equorum* in another, whereas these two species were found more sporadically in other productions. A succession of species and even biotypes was highlighted in some samples and *Staph. xylosus* often tended to replace the other species during ripening. Thirty-five isolates from this work (plus other two), were subsequently screened on the basis of their technological properties to elucidate their possible role during meat fermentation process (Bonomo *et al*., 2009).

Staph. xylosus was also found as the dominant species among 51 isolates collected during the ripening of six batches of *salsiccia sotto sugna*, another artisan sausage, typically manufactured in the Basilicata region (Rossi *et al*., 2001).

The production of fermented meat products is also an ancient tradition in the two main islands of Southern Italy: Sardinia and Sicily. The evolution of the autochthonous microbiota of *salsiccia sarda* was monitored throughout the manufacturing process (Mangia *et al*., 2007). At the end of ripening (21 days), the viable counts of LAB and CNC reached values of 10 and 5.4 log cfu g^{-1} , respectively; overall, 36 bacterial isolates collected during ripening were identified on the basis of biochemical traits, as: *Lb. curvatus* (13 isolates), *Lb. plantarum* (10), *Staph. xylosus* (5), *Staphylococcus* spp. (7), *Kocuria varians* (6), and *Micrococcus* spp. (1), the latter two isolates until day 12 of ripening. One year later, a similar study was carried out on *salsiccia sarda* manufactured with lean sheep meats (Mangia *et al*., 2008). At the end of ripening (23 days) viable counts of LAB and CNC were 10.2 and 9.1 log cfu g^{-1}, respectively; overall, 103 bacterial isolates collected during ripening were identified on the basis of phenotype-based assays, as follows: *Lb. plantarum* (43 isolates), *Lactobacillus* spp. (18), *Staph. xylosus* (20), *Staphylococcus* spp. (15), *Micrococcus* spp. (7). Once more, among CNC and CNS, those ascribed to the genus *Micrococcus* were isolated with higher frequency during the early maturation stage, being later replaced by the staphylococci. Interestingly no isolates were identified as *Lb. sakei* in the sausages made either from pork or from sheep meat, although it must be taken into consideration that, unfortunately, the use of phenotype-based methods does not always allow reliable identification. In both types of products, made from pork or sheep meat, LAB viable counts increased rapidly and at the end of ripening reached loads (> 10 log cfu g^{-1}) which are higher than those usually reported in literature; by contrast, viable counts of micro-staphylococci were considerably different in the sausages made with pork or sheep meat, with values around 5 and 9 log cfu g^{-1}, respectively, the latter being, again, higher than the values usually reported for this microbial group in fully ripened sausages.

The dominance of LAB (mainly facultatively heterofermentative mesophilic rods) during the ripening of Sardinian sausages was also reported by Greco *et al*. (2005). In more detail, *Lb. sakei* accounted for 43% of the 112 isolates, *Lb. plantarum* for 16.6% and *Lb. curvatus* for 13.3%. In this case, LAB viable counts (8.2 log cfu g^{-1}) were closer to the average values reported in literature.

More recently, the microbiota of *salsiccia* and *salame* manufactured from the meat of the black Sicilian swine known as *Suino nero dei Nebrodi* was investigated during ripening that lasted two and six weeks, respectively (Francesca *et al*., 2013). Regarding LAB, rods and cocci showed a similar trend during the production of *salsiccia*, whereas they behaved differently during the (longer) ripening of salami. However, in both cases, LAB reached count values higher than 7 log cfu g^{-1} and, interestingly, CNC counts attained almost the same levels (6.9 log cfu g^{-1}). *Lb. sakei* was the only LAB species identified during ripening from the first week onwards, whereas *Lc. lactis* was isolated only in minced meat or soon after stuffing.

The microbiota of fermented meat products manufactured in other Mediterranean countries

France

Manufacturing of dry sausages in France, mostly concentrated in the Rhone-Alpes and Auvergne

regions, is still largely performed following traditional procedures (Chevallier *et al*., 2006). The microbial ecosystem of these productions has been investigated in a small-scale facility considering both the processing environment and the products during and at the end of the manufacturing process. As far as the meat samples are concerned, viable counts of indigenous LAB and CNC reached levels of 6.5-7.7 and 6.2-7.3 log cfu g^{-1}, respectively (Chevallier *et al*., 2006); in the same products, *Lb. sakei* was predominant after one week and at the end of ripening. Quite surprisingly the remaining isolates were enterococci, lactococci and leuconostocs (Ammor *et al*., 2005). The EU project *Tradisausage* (Talon, 2006) has led to the identification of the structure, in terms of entity and composition, of the microbial communities characterising the environment, the meat samples during production, and the final products at nine artisan French production units where commercial starters had never been used: the viable counts of CNC and LAB in the ripened products were 6.5 and 7.9 log cfu g^{-1}, respectively (Lebert *et al.*, 2006). When the composition of the LAB and CNC communities in the products from two of these nine production units was investigated using phenotype- plus genotype-based analyses and fluorescence spectroscopy, *Lb. sakei* was again found as the prevalent species in one case, whereas members of the genus *Enterococcus* were largely prevalent in the other; with regard to the CNC, all the 69 isolates from ripened sausages in one factory were staphylococci, whereas in the products from the other factory the isolates were all staphylococci except one which was identified as *Kocuria* spp. The species *Staph. equorum* was by far the most frequent, accounting for 42 and 51 of the 69 isolates from sausages manufactured in each of the two factories, respectively; *Staph. saprophyticus* and *Staph. xylosus* accounted for 16 and 10 isolates, respectively, in the products from the former factory, whereas 15 isolates of *Staph. succinus* were found in those from the latter (Leroy *et al.*, 2006). The same authors (Leroy *et al.*, 2010) extended the study of the staphylococcal community to the environments and the products of all the nine small-scale processing units. Their results suggested that some strains, probably introduced by the meat, persisted in the manufacturing environment, while others were more specifically adapted to the meat products. A total of 409 GCC^+ isolates were collected from casings, minced meat mixtures, fermented and ripened sausages, 388 of which were ascribed to the genus *Staphylococcus* and identified at species level through molecular methods. Taking into account all the nine production units, the largest species diversity was found in the raw materials, since 10 different species were found in the meat mixtures and 7 in the casings, whereas the number of species found in the samples collected during fermentation and ripening decreased to 6 and 5, respectively. Overall, 11 different species were found in the meat products with the following percentages (on total meat isolates): *Staph. equorum* (58.2%), *Staph. saprophyticus* (11.9%), *Staph. xylosus* (11.3%), *Staph. succinus* (7.7%), *Staph. carnosus* (3.6%), *Staph. pasteuri* (2.3%), *Staphylococcus vitulinus* (2.1%), and *Staph. xylosus, Staph. sciuri* (reclassified to *Staph. sciuri* ssp. *sciuri*), *Staphylococcus arlettae* (0.3%, each). *Staph. equorum* was isolated in all the final products, *Staph. saprophyticus* was the second species detected in these samples, while *Staph. succinus, Staph. vitulinus*, and *Staph. xylosus* were found only episodically; however all these five species were found at each step of sausage manufacturing, and hence they all seemed to be well-adapted to the meat and process ecosystems. The diversity of the strains did not decrease during the process as 9, 7 and 9 PFGE profiles were found for the meat mixture, and the fermented and the ripened sausages, respectively, whereas only two profiles were stably detected at these different stages of manufacturing. The staphylococcal community occurring in the environment and in the meat samples (during and at the end of the manufacturing process) was also investigated in another small unit producing traditional dry fermented sausages (Corbière Morot-Bizot *et al.*, 2006). The counts of GCC^+, in fully ripened sausages were dominated by staphylococci (94%) and reached about 6 and 8 log cfu g^{-1} in winter and spring, respectively. The dominant species *Staph. equorum* and *Staph. succinus* were isolated from both the environment and the meat samples all through the production. Quite surprisingly, the two species *Staph. xylosus* and *Staph. carnosus* were sporadically found only in spring samples and never in the fully ripened sausages.

The biodiversity of coagulase negative staphylococci (CNS) isolated from traditional fermented sausages produced in 23 small units in three different regions of France was further investigated in comparison with that of CNS isolated from traditional French cheeses and clinical samples (Coton *et al.*, 2010). The 204 sausages isolates exhibited a lower biodiversity than cheese isolates, with 8 identified species, 5 of which occurred also in the sausages production environment. *Staph. equorum* was dominant in the environmental samples (42.1% of the isolates), whereas *Staph. xylosus* dominated both in the raw/fermented meat (39.5%) and the

final products (41.4%). On the basis of the PFGE profiles the intraspecific biodiversity was higher for the isolates belonging to the species *Staph. equorum* than for those ascribed to *Staph. xylosus*, feasibly due to the major adaptation of the latter species to the fermented products under study, or to the possible contamination from the commercial starter cultures occasionally used.

Greece

Dry fermented sausage *(salami aeros)* is a relevant production in the Greek meat industry (Samelis *et al*., 1998). Similar to the products previously discussed, the earliest studies on Greek fermented meat products, were also carried out using the conventional approach relying on the isolation of a large number of cultures followed by their biochemical characterisation. With this approach, Samelis *et al*. (1994) isolated 348 LAB cultures from a naturally fermented Greek dry salami; among the lactobacilli, *Lb. curvatus* was the dominant species (88 isolates), followed by *Lb. sakei* (76) and *Lb. plantarum* (34). The majority of the remaining isolates (120) were ascribed to the genus *Weissella*; most of them (86) were leuconostoc-like bacteria, 60 of which were not attributed to any species, 11 were identified as *W. hellenica*, 4 as *W. viridescenens*, and 11 (only tentatively) as *Weissella paramesenteroides,* whereas among the other *Weissella* isolates, 31 were identified as *Weissella minor*. Other species were found with minor frequencies.

The same approach was used to investigate the ecology of LAB and CNC in four batches of Greek dry salami (Samelis *et a*l., 1998): viable counts of the two microbial groups reached values higher than 8.0 and equal to 5-6 log cfu g^{-1}, respectively. Out of the 240 LAB isolates, 84% were presumptively identified as members of the facultatively heterofermentative *Lb. sakei/curvatus* group, whereas staphylococci were largely prevalent among the 224 CNC isolates; most of them (82.4%) were novobiocin-resistant members of the *Staph. saprophyticus* group (with a great dominance of *Staph. saprophyticus* over *Staph. xylosus*), whereas 15.6% were ascribed to the novobiocin-susceptible *Staph. epidermidis* group. Phenotype-based methods were also used to identify 100 CNC and 147 LAB cultures isolated from two types of Greek dry sausage at four different stages of ripening (Papamanoli *et al*., 2002; 2003): in more detail, 91% of the micrococci belonged to the genus *Staphylococcus*, whereas 5% were identified as *Kocuria varians*; the species most frequently isolated was *Staph. saprophyticus* (22%), followed by *Staph. carnosus* (20%) and *Staph. xylosus* (10%); LAB dominated the microbiota during fermentation and ripening with viable counts as high as 8.38 log cfu g^{-1}; furthermore, 90% of the isolates were lactobacilli with *Lb. sakei* prevailing (49 isolates), followed by *Lb. curvatus* (24) and *Lb. plantarum* (7). The dominance of LAB over other bacterial groups was also found by investigating the microbiota of three batches of Greek dry sausages (Drosinos *et al.*, 2005): in all the 3 batches, LAB viable counts at the end of ripening were about 8 log cfu g^{-1}, whereas those of catalase-positive cocci decreased from about 5 to 2-4 log cfu g^{-1}. On the basis of the phenotype-based characterisation, the majority of the 288 LAB isolates were assigned to the genus *Lactobacillus: Lb. plantarum, Lactobacillus pentosus* and *Lb. plantarum/pentosus* together accounted for 68.1% of the isolates, whereas *Lb. curvatus* accounted for 7.3% and *Lb. sakei* for 3.5% (the same percentage as *Lactobacillus rhamnosus*); other minority lactobacilli were *Lb. paracasei* subsp. *paracasei*, *Lactobacillus salivarius* and *Lb. brevis.* Among coccus-shaped LAB, *Lc. lactis* subsp. *lactis* was the most frequent species detected, accounting for 4.9% of the total number of LAB isolates. All the 219 isolates of catalase-positive cocci were assigned to the genus *Staphylococcus* with *Staph. saprophyticus* (31.1%) as the predominant species, followed by *Staph. xylosus* (19.2%) and *Staph. simulans* (11.4%). The same authors (Drosinos *et al*., 2007) investigated the physico-chemical and microbiological characteristics of spontaneously fermented sausages during production (from day 0 to 28), in two medium-sized enterprises located in Southern Greece. Viable counts of LAB and CNC increased from 4.43 to 8.28 and from 4.33 to 5.21 (average) log cfu g^{-1}, respectively. A total of 300 LAB and 300 staphylococci isolates were collected during fermentation and ripening, and further identified on the basis of their phenotypic features. In this case, the majority of LAB were identified as *Lb. plantarum* (45.6%) and *Lb. sakei* (43.4%). The remaining 11% of the isolates were identified as *Lb. curvatus* (4.3%), *Lb. rhamnosus* (2.7%) and *Lb. brevis* (0.7%), whereas 3.3% could not be assigned to any species. Most staphylococci were identified as *Staph. saprophyticus*, 15.3% as *Staph. simulans,* 12.6% as *Staph. xylosus*, while several other strains were present in lower percentages ranging from 1.0% (*Staph. equorum*) to 6.0% (*Staph. cohnii* ssp. *cohnii*).

Molecular methods were used to study the ecology of LAB in naturally fermented sausages produced in Greece, in comparison with those from Italy and Hungary (Rantsiou *et al*., 2005a): the isolates were identified by 16S rRNA gene sequencing, after grouping them on the basis of their DGGE profiles. Greek sausages were characterised by the presence

of a high number of *Lb. curvatus* (48.2 % of the isolates), followed by *Lb. sakei* (19.2%) and *Lb. plantarum* (14.9%). After 28 days of ripening, LAB counts reached almost 8 log cfu g^{-1}.

Another comparative study concerning the microbiota of traditionally fermented sausages manufactured in Greece, Italy and other countries in South-East Europe was carried out a few years later (Kozačinski *et al*., 2008), investigating LAB and CNC communities in Greek sausages at day 0 and after 2, 4, 7, 14 and 28 days. The viable counts of LAB and CNC ranged from 4.45 and 4.42 to 7.78 and 2.97 log cfu g^{-1}, respectively. The identification of the 150 LAB isolates with API 50 CHL yielded results which were quite different from those reported previously; in fact, *Lb. plantarum* was found to prevail (43%) followed by *Lb. pentosus* and *Lb. curvatus* (10.7%, each), whereas *Lb. sakei* accounted for only 4% of the isolates collected until day 7. On the other hand, among the 150 CNC isolates identified with API Staph, the species most commonly isolated in previous studies were found until the end of ripening, namely *Staph. saprophyticus* (34.7%), *Staph. xylosus* (14.7%) and *Staph. simulans* (11.3%); the species *Staph. haemolyticus* (11.3%) was mostly isolated in the early stages and until days 7 and 10 other minority species were also identified.

Spain and Portugal

An early investigation on the microbiota of Spanish dry fermented sausages was carried out with the aim of characterising the lactobacilli community. Among the 194 cultures identified on the basis of phenotype-based assays, *Lb. sakei* and *Lb. plantarum* were the predominant species (Rovira *et al*., 1997). Most investigations carried out on Spanish artisan fermented meat products considered three different types of sausage, namely chorizo, *fuet* and *salsichon*, classified as slightly fermented and low acid products, with a final pH comprised between 5.3 and 6.2 (Aymerich *et al*., 2006). The CNC community of *chorizo* produced in six factories located in three different regions of Castilla and Leòn was characterised on the basis of phenotype-based analyses: all the 426 isolates collected at three stages of ripening were staphylococci, with *Staph. xylosus* as the largely predominant species (95%), whereas *Staph. intermedius* and *Staph. saprophyticus* were found in much lower percentages of 9 and 6%, respectively (Garcìa-Varona *et al*., 2000).

The LAB communities occurring in four naturally fermented dry sausage productions (*salsichòn* and *chorizo*) were investigated, at two different factories, in the North and in the South of the Extremadura region, respectively (Benito *et al*., 2007). Samples were collected at 0, 60, and 120 days of ripening, from 12 batches (three for each type of sausage at each factory) and a total of 192 LAB isolates were collected. On the basis of carbohydrate fermentation, as determined by API CHL 50, 31.2% of the isolates were identified as *Pediococcus pentosaceus*, 26.9% as *Lc. lactis,* 18.6% as *Pediococcus acidilactici,* and 17% as *Lb. brevis*. *Leuc. mesenteroides, Lb. plantarum* and *Lb. curvatus* were found only sporadically. However, the phenotypic identification was only partially confirmed when the identity of nine *Pediococcus* isolates, pre-selected as possible candidates for use as starter cultures, was assessed through RAPD-PCR. All the isolates were in fact identified as *P. acidilactici* although previous tests had assigned four of them to *P. pentosaceus.*

Molecular methods were used to study the LAB and CNC communities of *fuet* and *chorizo* (Aymerich *et al.*, 2003); when isolation was carried out without any enrichment step, *Lb. sakei* and *Lb. curvatus* were detected in 11.8% of the samples, whereas *Lb. plantarum* and *Staph. xylosus* were found in 17.6%; interestingly, after enrichment, *Lb. sakei* and *Staph. xylosus* were isolated from all the samples. The percentages of *Lb. plantarum, Lb. curvatus, Staph. carnosus* and *Staph. epidermidis* varied depending on the sausage type; the viable counts of LAB and CNC, on the contrary, were not significantly different between the two products, with mean values of 8.13 and 6.74 log cfu g^{-1}, respectively. The same authors used species-specific PCR to characterise 250 LAB cultures isolated from 21 samples of *chorizo, fuet* and *salchicón* purchased from local butchers and supermarkets (Aymerich *et al*., 2006). *Lb. sakei* was once again the predominant species (74%) followed by *Lb. curvatus* (21.2%) and *Leuc. mesenteroides* (4.8%). By using plasmid profiling and RAPD-PCR, a high biodiversity was observed among the isolates, with a total of 144 different strains (112 within *Lb. sakei*, 23 within *Lb. curvatus* and 9 within *Leuc. mesenteroides*). The GCC^{+} population in *chorizo, fuet* and *salchicón* was also characterised at species and strain level (Martin *et al*., 2006): the distribution of the species was similar in the three products studied. *Staph. xylosus* was the predominant species in all sausage types (72.9–83.3%), whereas other species were found at much lower average percentages, namely *Staph. warneri* (8.3%); *Staph. epidermidis* (5.8%), *Staph. carnosus* (4.6%), and *K. varians* (0.4%). The combination of RAPD-PCR and plasmid profiling allowed the discrimination of 208 different profiles among the 240 Gram-positive isolates, indicating a great intraspecific diversity. Different results

with respect to the staphylococcal population were obtained in a more recent study carried out during the ripening of Galician *chorizo* (Fonseca *et al*., 2013). The data obtained both by molecular identification of plate isolates and by real-time PCR, showed that the dominant species among staphylococci was *Staph. equorum* and the authors suggested that this species could have been underestimated in other studies because of the difficulties of discriminating it from *Staph. xylosus*, especially when phenotypical methods of identification are used. As the population of lactobacilli are concerned, the most representative species was confirmed to be *Lb. sakei*.

The microbiological characteristics of *Botillo*, a traditional dry-fermented sausage made in North-Western Spain (Galicia and Castilla y Leòn Regions), were investigated by Garcìa Fontan *et al*., (2007a). The viable counts for LAB and salt tolerant bacteria were 8.87 and 6.56 log cfu g^{-1}, respectively. A total of 150 isolates per growth medium used were identified through phenotype-based methods. *Lb. sakei* predominated among the isolates collected from MRS agar, accounting for 23.3% of the isolates, followed by *Lb. alimentarius* (17.3%), *Lb. curvatus* (15.3%), *Lb. plantarum* (12%), *Lactobacillus farciminis* (10%), and *Leuc. mesenteroides* subsp. *mesenteroides* (2.66%). Only 34 (22.6%) out of the 150 isolates collected from SPC agar added with 7.5% NaCl belonged to the CNC family. Among the staphylococci, *Staphylococcus saprophyticus* (8.6% of the isolates) was the most frequently isolated, followed by *Staph. xylosus* (4%), *Staph. lentus* (2%) and *Staph. cohnii* (1.3%); *Staph. epidermidis, Staph. sciuri* and *Staph. capitis* were isolated with very low frequency, whereas the isolates ascribed to the genus *Micrococcus* could not be identified at species level. Similar results were obtained when the same authors used the same experimental approach to investigate the microbiota of *Androlla*, a further traditional dry-fermented sausage manufactured in North-Western Spain (Garcìa Fontan *et al*., 2007b). The microbial ecology of 20 samples, manufactured by 20 different producers, was analysed through a culture-dependent approach. Viable counts of LAB and salt-tolerant bacteria were of the same order of magnitude of those recorded in *Botillo*, and a same species diversity was found in both products. In more details, the average values of viable counts were 9.11 and 6.87 log cfu g^{-1} for LAB and salt-tolerant bacteria, respectively. Isolates collected from MRS agar were prevalently ascribed to *Lb. sakei* (43.5%), and with lower frequency to *Lb. curvatus* (25%), *Lb. alimentarius* (20.5%) and *Lb. plantarum* (4%). Only 56 out of the 200 salt-tolerant bacterial isolates belonged to the CNC group. *Staph. xylosus* was the main species (44.6%), followed by *M. luteus* (33.9%), whereas *Staph. epidermidis; Staph. equorum, Staph. capitis, Staph. saprophyticus, Micrococcus lylae, K. varians* and *K. kristinae* were isolated with neatly lower frequencies.

Though little information exists about fermented sausages manufactured in Portugal, several microbiological studies have been focussed on Alheira (or *Alheira de Mirandela*) a Portuguese Traditional Guaranteed Speciality (TGS) deriving from the ancient Jews tradition, that is manufactured on the large scale (more than 500 tons per year) in the Northern area of Trás-os-Montes (Ferreira *et al*., 2006; 2007; Esteves *et al*., 2008; Albano *et al*., 2008). This horse-shoe shaped fermented sausage, that is always eaten after cooking, has unique characteristics, largely deriving from the peculiar ingredients and manufacturing process; indeed, the meat mixture including poultry, pork, and occasionally beef, is boiled in a spiced broth together with bread, stuffed into casings, and then smoked for 2-8 days. Albano *et al.* (2008) investigated the microbial community of *Alheira* samples collected from six different producers using traditional methods and PCR-DGGE-analysis. Identification of the DGGE bands allowed only a few LAB species to be identified, namely *Lc. lactis, Lb. curvatus*, and *Lb. sakei, Lb. brevis, Leuc. mesenteroides,* and *Leuconostoc lactis,* whereas the species *Macr. caseolyticus* was the sole belonging to the CNC group. The dominance of LAB over CNC was also revealed by viable counts of these microbial group, which in most cases were higher than 7.5 log cfu g^{-1}, whereas those of CNC varied between 4.4 and 6.2 log cfu g^{-1}. The key role played by LAB during fermentation of *Alheira* emerges also from other two studies concerning the microbiological characteristics of this product, with particular reference to food safety (Ferreira *et al*., 2006; Esteves *et al*., 2008). In the first study carried out on 96 *Alheira* samples collected from four different factories, LAB average viable count was ~8 log cfu g^{-1}, whereas in the second investigation, performed onto samples collected from 12 different producers, LAB counts were often higher than 7.5 log cfu g^{-1}, whereas those of CNC were higher than 6.5 log cfu g^{-1}. In both studies significant differences were found among *Alheira* samples from different producers.

Conclusions

In the last two decades, a really conspicuous amount of information has emerged from

the extraordinary research effort dedicated to the identification of the microbial ecology of spontaneously fermented dry sausages manufactured in the Mediterranean area. This review has focussed on those data (the majority) concerning the two key communities of LAB and CNC, in an attempt to give a comprehensive, albeit not exhaustive, overview of their structure, in terms of both entity and composition, and their dynamics in these productions. Given that, the studies here reviewed concern products manufactured without the addition of commercial starter cultures and hence fermented and ripened thanks to the activities of indigenous microorganisms, a high complexity of these communities has always been evidenced, especially when more and more powerful molecular methods of analysis are used. Several peculiar traits have been highlighted for each production studied, as was foreseeable, given that the structure and dynamics of the indigenous microbiota are strictly dependent on the process environment and conditions and that the latter, in turn, influence the values of the fundamental physico-chemical parameters, such as pH, Rh, and a_w, during and at the end of manufacturing. Accordingly, Rantsiou and colleagues (2005a), in their comparative study on naturally fermented sausages produced in Greece, Hungary and Italy, affirmed that a definite microbiota is spontaneously selected and so-called country-specific strains can been found as specifically associated to certain products. Nonetheless, a series of similar features shared by the different productions discussed here, implies that they are connected by a sort of common thread. As far as the loads of the two main bacterial communities are concerned, it appears evident that LAB are more numerous than CNC during fermentation and ripening and tend to remain more stable in the fully ripened products. Within the LAB population, facultatively heterofermentative lactobacilli generally prevail and, among them, the two psychrotrophic species *Lb. sakei* and *Lb. curvatus* were found to be dominant in most studies. The former species was often isolated with the highest frequency, although sometimes the opposite occurred, or the two species were found at similar levels; *Lb. plantarum* was generally isolated with less frequency, but even in this case exceptions were found, probably due to particular processing conditions (such as those found for the Italian products *salame Milano* and *Ciauscolo*). The same is also true for the members of other LAB genera, such as *Weissella, Leuconostoc, Lactococcus,* and *Pediococcus* since they were in general found as minority species, except in one study on Spanish sausages and one on the Portuguese *Alheira*. As regards the CNC community, the dominance of *Staph. xylosus* emerged with even greater evidence, with the exception of the sausage productions in Greece (*Staph. saprophyticus* prevalent) and France (*Staph. equorum* prevalent with either *Staph. saprophyticus* or *Staph. succinus* as co-dominant species and *Staph. xylosus* sometimes only marginally found). In fact, the diversity among CNC was generally higher than that recorded for LAB, since a larger number of species played a secondary or marginal role (different in the various productions studied); they include members of the genera *Staphylococcus* or *Kocuria* although staphylococci largely prevailed within CNC, in all the studies reviewed.

References

Albano, H., Henriques, I., Correia, A., Hogg, T. and Teixeira P. 2008. Characterization of microbial population of 'Alheira' (a traditional Portuguese fermented sausage) by PCR-DGGE and traditional cultural microbiological methods. Journal of Applied Microbiology 105(6): 2187–2194.

Ammor, S., Rachman, C., Chaillou, S., Prévost, H., Dousset, X., Zagorec, M., Dufour, E. and Chevallier, I. 2005. Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. Food Microbiology 22(5): 373-382.

Andrighetto, C., Zampese, L. and Lombardi, A. 2001. RAPD-PCR characterization of lactobacilli isolated from artisanal meat plants and traditional fermented sausages of Veneto region (Italy). Letters in Applied Microbiology 33(1): 26-30.

Aquilanti, L., Santarelli, S., Silvestri, G., Osimani, A., Petruzzelli, A. and Clementi, F. 2007. The microbial ecology of a typical Italian salami during its natural fermentation. International Journal of Food Microbiology 120(1-2): 136-145.

Aymerich, T., Martìn, B., Garriga, M., Vidal-Carou, M. C., Bover-Cid, S. and Hugas, M. 2006. Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. Journal of Applied Microbiology 100(1): 40-49.

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

Baruzzi, F., Matarante, A., Caputo, L. and Morea, M. 2006. Molecular and physiological characterization of natural microbial communities isolated from a traditional Southern Italian processed sausage. Meat Science 72(2): 261-269.

Benito, M. J., Martin, A., Aranda, A., Perez-Nevado, F., Ruiz-Moyano, S. and Cordoba, M.G. 2007. Characterization and selection of autochthonous lactic acid bacteria isolated from traditional Iberian dry-fermented salchichón and chorizo sausages. Journal

of Food Science 72(6): 193-201.

Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R. and Parente, E. 2004. Diversity and dynamics of communities of coagulase–negative staphylococci in traditional fermented sausages. Journal of Applied Microbiology 97(2): 271-284.

Bonomo, M. G., Ricciardi, A., Zotta, T., Parente, E. and Salzano, G. 2008. Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). Meat Science 80(4): 1238-1248.

Bonomo M. G., Ricciardi, A., Zotta, T., Sico, M. A. and Salzano, G. 2009. Technological and safety characterization of coagulase-negative staphylococci from traditionally fermented sausages of Basilicata region (Southern Italy). Meat Science 83(1): 15-23

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

Cocolin, L., Dolci, P., Rantsiou, K., Urso, R., Cantoni, C. and Comi, G. 2009. Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular methods. Meat Science 82(1): 125-132.

Cocolin, L., Manzano, M., Aggio, D., Cantoni, C. and Comi, G. 2001a. 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(1): 59-64.

Cocolin, L., Manzano, M., Cantoni, C. and Comi, G. 2001b. 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., Urso, R., Rantsiou, K., Cantoni, C. and Comi, G. 2006. Dynamics and characterization of yeasts during natural fermentation of Italian sausages. FEMS Yeast Research 6(5): 692-701.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C. and Cocolin, L. 2005. Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Science 69(3): 381-392.

Coppola, R., Giagnacovo, B., Iorizzo, M. and Grazia, L. 1998. Characterization of lactobacilli involved in the ripening of *soppressata molisana*, a typical southern Italy fermented sausage. Food Microbiology 15(3):47-353.

Coppola, R., Iorizzo, M., Saotta, R., Sorrentino, E. and Grazia, L. 1997. Characterization of micrococci and staphylococci isolated from *soppressata* molisana, a Southern Italy fermented sausage. Food Microbiology 14(1): 47-53.

Coppola, R., Marconi, E., Rossi, F. and Dellaglio, F. 1995. Produzioni artigianali di *salame* tipo Napoli: aspetti chimici e microbiologici. Italian Journal of Food Science 7:5 7-62.

Coppola, S., Mauriello, G., Aponte, M., Moschetti, G. and Villani, F. 2000. Microbial succession during ripening of Naples–type salami, a southern Italian fermented sausage. Meat Science 56(4): 321-329.

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

Coton, E., Desmonts, M. H., Leroy, S., Coton, M., Jamet, E., Christieans, S., Donnio, P. Y., Lebert, I. and Talon, R. 2010. Biodiversity of Coagulase-Negative Staphylococci in French cheeses, dry fermented sausages, processing environments and clinical samples. International Journal of Food Microbiology 137(2-3): 221-229.

Di Cagno, R., Chaves Lopez, C., Tofalo, R., Gallo, G., De Angelis, M., Paparella, A., Hammes, W. and Gobbetti, M. 2008. Comparison of the compositional, microbiological, biochemical and volatile profile characteristics of three Italian PDO fermented sausages. Meat Science 79(2): 224-235.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F. and Metaxopoulos, J. 2005. Characterization of the microbial flora from a traditional Greek fermented sausage. Meat Science 69(2): 307-317.

Drosinos, E. H., Paramithiotis, S., Kolovos, G., Tsikouras, I. and Metaxopoulos, I. 2007. Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. Food Microbiology 24(3): 260–270.

Esteves, A., Patarata, L., Saraiva, C. and Martins, C. 2008. Assessment of the microbiological characteristics of industrially produced Alheira, with particular reference to foodborne pathogens. Journal of Food Safety 28(1): 88–102.

Federici, S., Ciarrocchi, F., Campana, R., Ciandrini, E., Blasi, G. and Baffone, W. 2014. Identification and functional traits of lactic acid bacteria isolated from Ciauscolo salami produced in Central Italy. Meat Science 98: 575-584.

Fonseca, S., Cachaldora, A., Gómez, M., Franco, I. and Carballo, J. 2013. Monitoring the bacterial population dynamics during the ripening of Galician chorizo, a traditional dry fermented Spanish sausage. Food Microbiology 33: 77-84.

Ferreira, V., Barbosa, J., Vendeiro, S., Mota, A., Silva, F., Monteiro, M. J., Hogg, T., Gibbs, P. and Teixeira, P. 2006. Chemical and microbiological characterization of alheira: A typical Portuguese fermented sausage with particular reference to factors relating to food safety. Meat Science 73(4): 570-575.

Ferreira, V., Barbosa, J., Silva, J., Felício, M. T., Mena, C., Hogg, T., Gibbs, P. and Teixeira, P. 2007. Characterisation of alheiras, traditional sausages produced in the North of Portugal, with respect to their microbiological safety. Food Control 18(5): 436–440.

Francesca, N., Sannino, C., Moschetti, G. and Settanni, L. 2013. Microbial characterisation of fermented meat

products from the Sicilian swine breed "Suino nero dei Nebrodi". Annals of Microbiology 63(1): 53-62.

Garcìa Fontan, M. C., Lorenzo, J. M., Martinez, S., Franco, I. and Carballo, J. 2007a. Microbiological characteristics of Botillo, a Spanish traditional pork sausage. LWT Food Science and Technology 40(9): 610-1622.

Garcìa Fontan, M. C., Lorenzo, J. M., Parada, A., Franco, I. and Carballo, J. 2007b. Microbiological characteristics of "androlla", a Spanish traditional pork sausage. Food Microbiology 24(1): 52-58.

Garcìa-Varona, M., Santos, E. M., Jaime, I. and Rovira, J. 2000. Characterisation of Micrococcaceae isolated from different varieties of chorizo. International Journal of Food Microbiology 54(3): 189-195.

Gardini, F., Suzzi, G., Lombardi, A., Galgano, F., Crudele, M. A., Andrighetto, C., Schirone, M. and Tofalo, R. 2001. A survey of yeasts in traditional sausages of southern Italy. FEMS Yeast Research 1(2): 161-167.

Greco, M., Mazzette, R., De Santis, E. P. L., Corona, A. and Cosseddu, A. M. 2005. Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. Meat Science 69(4): 733-739.

Greppi, A., Ferrocino, I., La Storia, A., Rantsiou, K., Ercolini, D. and Cocolin, L. 2015. Monitoring of the microbiota of fermented sausages by culture independent rRNA-based approaches. International Journal of Food Microbiology *http://dx.doi.org/10.1016/j.ijfoodmicro.*2015.01.016.

Iacumin, L., Comi, G., Cantoni, C. and Cocolin, L. 2006. Ecology and dynamics of coagulase–negative cocci isolated from naturally fermented Italian sausages. Systematic and Applied Microbiology 29(6): 480-486.

Iacumin, L., Manzano, M. and Comi, G. 2012. Catalase-positive cocci in fermented sausage: Variability due to different pork breeds, breeding systems and sausage production technology. Food Microbiology 29(2): 178-186.

Kozačinski, L., Drosinos, E., Čaklovica, F., Cocolin, L., Gasparik-Reichardt, J. and Veskovi, S. 2008. Investigation of microbial association of traditionally fermented sausages. Food Technology and Biotechnology 46(1): 93-106.

Lebert, I., Leroy, S., Giammarinaro, P., Chacornac, J. P. and Talon, R. 2006. Saucissons secs fermiers du Massif central: ecosystèmes microbiens des saucissons et de l'environnement. Viandes et Produits Carnés 25(5): 165-170.

Lebert, I., Leroy, S., Giammarinaro, P., Lebert, A., Chacornac, J. P., Bover-Cid, S., Vidal-Carou, M.C. and Talon, R. 2007. Diversity of micro-organisms in environments and dry fermented sausages of small traditional French processing units. Meat Science 76(1): 112–122.

Leroy, S., Giammarinaro, P., Chacornac, J. P., Lebert, I. and Talon R. 2010. Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units. Food Microbiology 27(2): 294-301.

Lucke, F.K. 2000. Fermented Meats. In: Microbiological safety and quality of food, Lund, B., Baird-Parker, A. C., Gould, G.W. (Eds.), pp.420-444.

Mangia, N.P., Murgia, M.A., Garau, G. and Deiana, P. 2007. Microbiologia e valutazione igienico sanitaria della salsiccia sarda. Industrie Alimentari 46: 533-536.

Mangia, N.P., Murgia, M.A., Garau, G., Merella, R. and Deiana, P. 2008. Sardinian fermented sheep sausage: microbial biodiversity resource for quality improvement. Options Méditerranéennes Series A 78: 273-277.

Martin, B., Garriga, M., Hugas, M., Bover-Cid, S., Veciana-Nogués, M.T. and 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., Moschetti, G., Villani, F., Blaiotta, G. and Coppola, S. 2000. Antibiotic resistance of coagulase–negative staphylococci isolated from artisanal Naples-type salami. International Journal of Food Science and Nutrition 51(1): 19-24.

Moschetti, G., Mauriello, G. and Villani, F. 1997. Differentiation of Staphylococcus xylosus strains from Italian sausages by antibiotyping and low frequency restriction fragment analysis of genomic DNA. Systematic and Applied Microbiology 20(3): 432-438.

Papamanoli, E., Kotzekidou, P., Tzanetakis, N. and Litopoulou-Tzanetaki, E. 2002. Characterization of *Micrococcaceae* isolated from dry fermented sausage. Food Microbiology 19(5): 441-449.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetakis, E. and 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.

Parente, E., Grieco, S. and Crudele, M. A. 2001. Phenotypic diversity of lactic acid bacteria isolated from fermented sausages produced in Basilicata (Southern Italy). Journal of Applied Microbiology 90(6): 943-952.

Petruzzelli, A., Blasi, G., Masini, L., Calza, L., Duranti, A., Santarelli, S., Fisichella, S., Pezzotti, G., Aquilanti, L., Osimani, A. and Tonucci, F. 2010. Occurrence of *Listeria monocytogenes* in salami manufactured in the Marche region (Central Italy). The Journal of Veterinary Medical Science 72(4): 499-502.

Pisacane, V., Callegari, M.L., Puglisi, E., Dallolio, G. and Rebecchi A. 2015. Microbial analyses of traditional Italian salami reveal microorganisms transfer from the natural casing to the meat matrix. International Journal of Food Microbiology 207: 57-65.

Polka, J., Rebecchi, A., Pisacane, V. and Morelli, L. 2015. Bacterial diversity in typical Italian salami at different ripening stages as revealed by high-throughput sequencing of 16s rRNA amplicons. Food Microbiology 46: 342-356.

Rantsiou, K. and Cocolin, L. 2006. New developments in the study of the microbiota of naturally fermented sausages as determined by molecular methods: a

review. International Journal of Food Microbiology 108(2): 255-267.

Rantsiou, K., Drosinos, E. H., Gialitaki, M., Urso, R., Krommer, J., Gasparik-Reichard, J., Tòth, S., Metaxopoulos, I., Comi, G. and Cocolin, L. 2005a. Molecular characterization of *Lactobacillus* species isolated from naturally fermented sausages produced in Greece, Hungary and Italy. Food Microbiology 22(1): 19-28.

Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P., Comi, G. and Cocolin, L. 2005b. 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. and Cocconcelli, P.S. 1998. Physiological and molecular techniques for the study of bacterial community development in sausage fermentation. Journal of Applied Microbiology 84(6): 1043-1049.

Rebecchi, A., Pisacane, V., Callegari, M. L., Pugliesi, E. and Morelli, L. 2015. Ecology of antibiotic resistant coagulase-negative staphylococci isolated from the production chain of a typical Italian salami. Food Control 53: 14-22.

Rossi, F., Tofalo, R., Torriani, S. and 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(3): 365-371.

Rovira, A., Nieto, J.C., Rodriguez, A., Reguera, J.I. and Gonzàles, Z. 1997. Characterization and selection of lactobacilli isolated from Spanish fermented sausages. Microbiologia 13(2): 201-208.

Samelis, J., Maurogenakis, F. and Metaxopoulos, J. 1994. Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. International Journal of Food Microbiology 23(2): 179-196.

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

Santarelli, S., Aquilanti, L., Silvestri, G., Osimani, A., Petruzzelli, A. and Clementi, F. 2007. Evoluzione delle comunità microbiche nel corso della maturazione del Ciauscolo. Industrie Alimentari 46: 744-748.

Silvestri, G., Santarelli, S., Aquilanti, L., Beccaceci, A., Osimani, A., Tonucci, F. and Clementi, F. 2007. Investigation of the microbial ecology of Ciauscolo, a traditional Italian salami, by culture–dependent techniques and PCR-DGGE. Meat Science 77(3): 413-423.

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

Talon, R. and 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, S. and Lebert, I. 2007. Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters. Meat Science 77(1): 55-62.

Talon, R., Leroy-Setrin, S. and 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. and Cocolin, L. 2006. Ecology of lactic acid bacteria in Italian fermented sausages: isolation, identification and molecular characterization. Systematic and Applied Microbiology 29(8): 671-680.

Villani, F., Casaburi, A., Pennacchia, C., Filosa, L., Russo, F. and Ercolini, D. 2007. Microbial ecology of the *Soppressata* of Vallo di Diano, a traditional dry fermented sausage from Southern Italy, and *in vitro* and *in situ* selection of autochthonous starter cultures. Applied and Environmental Microbiology 73(17): 5453-5463.

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