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MICROBIOTA OF THE FERMENTED SAUSAGES: INFLUENCE TO PRODUCT QUALITY AND SAFETY

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Abstract

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Nowadays a great variety of traditional dry fermented sausages is present on the market. Differences in their sensorial characteristics depend on their composition, as well as complex biochemical reactions which occur during the fermentation process. The production of fermented sausages is greatly influenced by the changes in the composition of microbiota. It is generally agreed that microbiota of the fermented sausages is mainly constituted of lactic acid bacteria and coagulase-negative staphylococci. Fermentation of different sausages can, also, involve other microorganisms as yeasts and moulds. Development of different microbial groups or even specific species can significantly affect the characteristics of the final product. The influence of the microbiota can, also, be seen in the contribution to the product safety especially by the synthesis of lactic acid and bacteriocins. On the other hand, the use of these species can have potential hazards related to biogenic amines and enterotoxin production, as well as antibiotic resistance. The review deals with the composition of microbiota of different traditional fermented sausages and the impact of microbiota to product safety and potential hazards.

Key words: fermented sausages, lactic acid bacteria, coagulase-negative staphylococci, safety, quality

Introduction

Dry fermented sausages are made from chopped ground meat, usually pork or beef, and pork fat. Ingredients are mixed and stuffed into casings, afterwards sausages are conserved by drying with or without smoking. Traditional fermentation relies on natural microbiota, while modern production demands the use of starter cultures (Petaja-Kannninen and Puolanne, 2007).

Due to the great variety of products in the world it is difficult to make the classification of fermented sausages. The basic classification of the sausages is on semi-dry and dry sausages according to the level of moisture in the final product. Semi dry sausages have the loss of weight during fermentation less than 20%, the water activity (a_w) from 0.90 to 0.95 and they are most often heated during smoking. Dry sausages have the loss of weight during fermentation more than 30%, have a_w value less than 0.90 and are not heated during smoking (Toldrà, 2002).

Complex biochemical and physical reactions that occurs during the process of the fermentation of the sausages induce significant changes in the characteristics of the sausages. They can be summarized as follows: a decrease in pH, changes in the initial microbiota, the reduction of nitrates to nitrites and the latter to nitric oxide, the formation of nitrosomyoglobin, solubilization and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena, and dehydration (Casaburi et al., 2008).

Microbiota of fermented sausages consists of lactic acid bacteria (LAB), coagulase-negative staphylococci (CNS), yeasts and molds. The development of desired microbiota, and inhibition of the harmful microorganisms is affected by temperature, pH, a_w, O_2, metabolites and additives (Toldrà, 2002).

The aim of this paper is to review the composition of microbiota in the fermented sausages, as well as the impact of bacteria to the product safety. Also, the risk of the use of LAB and CNS will be discussed.

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Lactic Acid Bacteria

Lactic acid bacteria have an important and crucial role in the production of fermented sausages. The products of their metabolism affect the process of ripening, development of desired sensory and nutritive characteristics of the products and, at the same time, inhibit the development of undesired microbiota (Veskovic–Morachanin, 2010). LAB isolated from fermented sausages belong to genera *Lactobacillus, Weisella, Leuconostoc, Pediococcus, Enterococcus* and Lactococcus (Comi et al., 2005; Di Cagno et al., 2008; Rantsiou et al., 2006; Urso et al., 2006). LAB that are the most frequently used in meat starters belong to the species: *Lactobacillus casei, Lactobacillus curvatus, Lactobacillus pentosus, Lactobacillus sakei, Lactobacillus rhamnosus, Pediococcus acidilactici* and *Pediococcus pentosaceus* (Veskovic–Morachanin, 2010). The frequency of isolation of LAB species from different types of dry sausages is shown in Table 1.

Lactobacilli affect flavor, texture and nutritive value of the products due to their acidification abilities, the synthesis of bacteriocins and egzopolisaccharides and proteolytic characteristics. This enables their wide application in the production of fermented sausages (Leroy and De Vuyst, 2004). The number of lactobacilli in the final product varies in the range from 8 to 9 log CFU/g (Hammes et al., 2008). *Lb. sakei, Lb. plantarum* and *Lb. curvatus* (Comi et al., 2005; Urso et al., 2006; Kozachinski et al., 2008; Rantsiou et al., 2005; Drosinos et al., 2007; Aquilanti et al., 2007; Cocolin et al., 2009; Fontana et al., 2005; Danilovic et al., 2011) are usually the most frequently isolated from fermented sausages. *Lb. sakei* is usually dominant in different types of the sausages as „sudžuk" (Kesmen et al., 2012), Bolougne sausages

Table 1
LAB strains isolated from different traditional fermented sausages

Origin	Traditional product	LAB isolated from the product	Frequency of isolation, %	Reference
Serbia	Petrovac sausages	*Lb. sakei*	48.1	Danilovic, 2012
		Lb. curvatus	1.3	
		Ln. mesenteroides	36.2	
		P. pentosaceus	10	
		E. casseliflavus	3	
		E. durans	1.4	
	Sremska sausages	*Lb. fermentum*	24	Kozachinski et al., 2008
		Lb. curvatus	7.3	
		Lb. brevis	9.3	
		Lb. plantarum	6	
		Lb. delbrueckii ssp. *delbrueckii*	9.3	
		Lb. delbrueckii ssp. *bulgaricus*	2.6	
		Lc. lactis ssp. *lactis*	6.6	
		Lb. cellobiosus	4.6	
		Lb. collinoides	4.6	
		Lb. acidophilus	0.6	
		Lb. paracasei ssp. *paracasei*	0.6	
		Ln. mesenteroides ssp. *cremoris*	2.6	
		Ln. mesenteroides ssp. *mesenteroides*	12.6	
		E. faecalis	6.6	
		E. faecium	2	
Croatia	n.d.	*Lb. plantarum*	34	Kozachinski et al., 2008
		Lb. curvatus	18	
		Lb. pentosus	6.7	
		Lb. plantarum	5.3	
		Lb. fermentum	4	
		Lb. brevis	20.7	
		Ln. mesenteroides ssp. *mesenteroides*	5.9	
		Lc. lactis ssp. *lactis*	2	
		P. pentosaceus	3.3	

Table 1 continued

Hungary	n.d.	*Lb. curvatus*	7.3	Rantsiou et al., 2005
		Lb. paraplantarum/plantarum[a]	2.4	
		Lb. plantarum	1.6	
		Lb. sakei	70.8	
		Ln. citreum	0.8	
		Ln. mesenteroides	4.0	
		W. paramesenteroides/hellenica[a]	5.8	
		W. viridescens	7.3	
	n.d.	*Lb. sakei*	28.7	Kozachinski et al., 2008
		Lb. plantarum	3.4	
		Lb. curvatus	3.4	
		Lb. delbrueckii	3.4	
		Lb. alimentarius	3.4	
		Lb. amylophilus	2.7	
		Lb. bavaricus	2	
		Lb. salivarius	0.7	
		Lb. acidophilus	0.7	
		Lb. maltoromicus	0.7	
		Lb. yamanashiensis	0.7	
		Ln. mesenteroides ssp. *mesenteroides*	16.7	
		Ln. mesenteroides dextranicum	4.7	
		Lb. sanfrancisco	4.7	
		W. wiridescens	2	
		Lb. cofosus	1.4	
		Lb. halotolerans	0.7	
		Lb. fructivorans	0.7	
		Ln. citreum	0.7	
		Ln. eonos	0.7	
		unidentified	17.4	
Greece	n.d.	*E. faecium/durans*[a]	4.4	Rantsiou et al., 2005
		Lb. alimentarius	1.8	
		Lb. casei/paracasei[a]	5.3	
		Lb. curvatus	48.2	
		Lb. paraplantarum	5.3	
		Lb. paraplantarum/plantarum[a]	0.9	
		Lb. plantarum	14.9	
		Lb. sakei	19.2	
	n.d.	*Lb. curvatus* ssp. *curvatus*	16.3	Papamanoli et al., 2003
		Lb. buchneri	15.6	
		Lb. plantarum	4.8	
		Lb. paracasei ssp. *paracasei*	15	
		Lb. paracasei ssp. *tolerans*	2.7	
		Lb. sakei	33.3	
		Lb. casei	1.4	
		Lb. coryniformis ssp. *coryniformis*	0.7	
		Lb. paraplantarum	0.7	
		Lb. rhamnosus	0.7	
		W. viridescens	0.7	
		Ln. pseudomesenteroides	1.4	
		Leuconostoc spp.	0.7	
		Pediococcus spp.	2	
		E. faecalis	0.7	
		E. faecium	3.4	

Table 1 continued

Greece	n.d.	*Lb. plantarum*	43.3	Kozachinski et al., 2008
		Lb. curvatus	10.7	
		Lb. pentosus	10.7	
		Lb. rhamnosus	3.3	
		Lb. sakei	4	
		Lb. paracasei ssp. *paracasei*	1.3	
		Lb. salivarius	0.7	
		Lb. brevis	8.7	
		Ln. mesenteroides ssp. *mesenteroides*	6	
		Ln. lactis	4	
		Lc. lactis ssp. *lactis*	6.7	
		E. faecium	0.7	
	n.d.	*Lb. sakei*	43.3	Drosinos et al., 2007
		Lb. plantarum	45.6	
		Lb. brevis	0.7	
		Lb. curvatus	4.3	
		Lb. rhamnosus	2.7	
Italy	n.d.	*E. pseudoavium*	0.7	Comi et al., 2005
		Lc. lactis ssp. *lactis*	0.7	
		Lb. brevis	0.7	
		Lb. curvatus	36	
		Lb. paraplantarum	4.7	
		Lb. paraplantarum/pentosus[a]	2.7	
		Lb. plantarum	6	
		Lb. sakei	42.7	
		Ln. citreum	0.7	
		Ln. mesenteroides	2.7	
		W. paramesenteroides/hellenica[a]	2.7	
	n.d.	*E. pseudoavium*	0.8	Rantsiou et al., 2005
		Lc. lactis ssp. *lactis*	0.8	
		Lb. curvatus	29.8	
		Lb. paraplantarum	5.8	
		Lb. paraplantarum/pentosus[a]	3.3	
		Lb. plantarum	6.6	
		Lb. sakei	48.8	
		Ln. citreum	0.8	
		Weissella paramesenteroides/hellenica[a]	3.3	
	n.d	*Lc. garvieae*	0.4	Urso et al., 2006
		Lc. lactis	0.2	
		Lb. brevis	0.2	
		Lb. casei	2.1	
		Lb. curvatus	14.4	
		Lb. paraplantarum	0.2	
		Lb. plantarum	3.6	
		Lb. sakei	75.9	
		Ln. carnosum	0.6	
		Ln. mesenteroides	0.2	
		W. hellenica	1.7	
		W. paramesenteroides	0.2	
	n.d.	*Lb. sakei*	67	Bonomo et al., 2008
		Lb. plantarum	4	
		Lb. brevis	2	
		P. pentosaceus	16	
		Ln. carnosum	8	
		Ln. pseudomesenteroides	2	

Table 1 continued

Italy	„Filzetta"	*Lb. sakei*	51.1	Conter et al., 2005
		Lb. fermentum	15.5	
		Lb. brevis	2.2	
		Ln. mesenteroides	10	
		unidentified	21.1	
	„Ciauscolo"	*Lb. plantarum*	78.3	Aquilanti et al., 2007
		Lb. curvatus	21.7	
	„Salame bergamasco"	*Lb. sakei*	34.4	Cocolin et al., 2009
		Lb. curvatus	58.9	
		Lb. paraplantarum	1.1	
		Ln. mesenteroides	2.2	
	„Salame cremonese"	*Lb. sakei*	68.8	Cocolin et al., 2009
		Lb. curvatus	30.7	
		W. hellenica	0.4	
	„Salame mantovano"	*Lb. sakei*	61.2	Cocolin et al., 2009
		Lb. curvatus	32	
		Lb. plantarum	1.1	
		Ln. mesenteroides	3.4	
		Ln. citreum	2.2	
	„Varzi"	*Lb. sakei*	20	Di Cagno et al., 2008
		Lb. curvatus	80	
	„Brianza"	*Lb. sakei*	73.3	Di Cagno et al., 2008
		Lb. curvatus	13.3	
		P. pentosaceus	13.3	
	„Piacentino"	*Lb. sakei*	73.3	Di Cagno et al., 2008
		Lb. curvatus	13.3	
		Lb. coryniformis	13.3	
Argentina	n.d	*Lb. sakei,*	55	Fontana et al., 2005
		Lb. plantarum	40	
		Lb. curvatus	5	
	„Alheira"	*Lb. paraplantarum*	1.1	Albano et al., 2009
		Lb. plantarum	26.5	
		Lb. sakei	1.1	
		Weissella spp.	2.1	
		W. cibaria	1.1	
		W. viridescens	0.4	
		Lb. zeae	0.7	
		Lb. paracasei	1.4	
		Lb. ramnosus	2.1	
		Ln. mesenteroides	1.4	
		Lb. brevis	2.5	
		P. pentosaceus	3.2	
		P. acidilactici	1.1	
		Enterococcus spp.	11.3	
		E. faecalis	30.7	
		E. faecium	13.4	
Bosnia and Hercegovina	„Sudžuk"	*Lb. plantarum*	40.7	Kozachinski et al., 2008
		Lb. pentosus	18	
		Lb. curvatus	16.7	
		Lb. sakei	8.7	
		Lb. brevis	7.3	
		P. pentosaceus	4.7	
		Lc. lactis	3.3	
		Lb. salivarius	0.6	

Table 1 continued

Turkey	„Sudžuk”	*Lb. sakei*	37.5	Kesmen et al., 2012
		Lb. plantarum	16.7	
		Lb. curvatus	13.5	
		Lb. brevis	10.4	
		Lb. farciminis	5.2	
		Lb. alimentarius	1	
		Ln. citreum	2.1	
		Ln. mesenteroides	5.2	
		W.viridescens	6.2	
		unidentified	2.1	
Spain	„Botillo”	*Lb. sakei*	23.3	Garcia Fontan et al., 2007
		Lb. alimentarius	17.3	
		Lb. curvatus	15.3	
		Lb. plantarum	12	
		Lb. farciminis	10	
	„Salchichón” and „chorizo”	*Lb. sakei*	34	Casquete et al., 2012
		Lb. curvatus	11.5	
		Lb. lactis	7	
		Lb. plantarum	10	
		P. acidilactici	15	
		P. pentosaceus	22.5	
	„Salchichón”	*P. acidilactici*	59.2	Benito et al., 2008
		Lb. curvatus	2	
		Lb. plantarum	38.8	
	„Chorizo”	*P. acidilactici*	44.7	Benito et al., 2008
		Lb. sakei	2.1	
		Lb. curvatus	2.1	
		Lb. plantarum	10.6	
		Lb. brevis	40.4	
Belgium	Boulogne sausages	*Lb. sakei*	95.6	Janssens et al., 2012
		Lc. carnosum	0.6	
		Ln. mesenteroides	3.1	
		Lb. paralimentarus/mindensis/crustorum[a]	0.6	
	Belgian-type salami	*Lb. sakei*	100%	Janssens et al., 2012
Himalay	„Lang kargyong”	*Lb. sakei*	12.5	Rai et al., 2010
		Lb. curvatus	4.2	
		Lb. divergens	8.3	
		Lb. carnis	20.8	
		Lb. sanfrancisco	25	
		E. faecium	12.5	
		Ln. mesenteroides	16.7	
	„Faak kargyong ”	*Lb. brevis*	5.6	Rai et al., 2010
		Lb. plantarum	11.1	
		Lb. carnis	16.7	
		Ln. mesenteroides	44.4	
		E. faecium	22.2	

Table 1 continued

	„Yaak kargyong”	*Lb. sakei* *Lb. plantarum* *Lb. casei* *Lb. curvatus* *Lb. carnis* *Lb. divergens* *Lb. sanfrancisco* *Ln. mesenteroides* *E. faecium*	16.7 16.7 11.1 5.6 11.1 5.6 11.1 11.1 11.1	Rai et al., 2010
	„Lang satchu”	*Lb. casei* *Lb. carnis* *P. pentosaceus* *E. faecium*	5.3 5.3 26.3 63.1	Rai et al., 2010
	„Yaak satchu”	*P. pentosaceus*	100	Rai et al., 2010
	„Suka ko masu”	*Lb. plantarum* *Lb. carnis* *E. faecium*	20 13.3 66.7	Rai et al., 2010
Bali	„ urutan”	*Lb. plantarum* *Lb. farciminis* *Lb. fermentum* *Lb. hildardii* *P. acidilactici* *P. pentosaceus*	52.1 21.1 1.4 15.5 2.8 7.0	Antara et al., 2002

and Belgian-type salami (Janssens et al., 2012), „Botillo" sausages (Garcia Fontan et al., 2007), „salame cremonese" and „salame mantovano" (Cocolin et al., 2009), „Filzetta" sausages (Conter et al., 2005) and dry fermented sausages made in Italy (Comi et al., 2005; Urso et al., 2006; Rantsiou et al., 2005), Argentina (Fontana et al., 2005), Greece (Papamanoli et al., 2003, Drosinos et al., 2007) and Hungary (Kozachinski et al., 2008; Rantsiou et al., 2005). Such high presence of *Lb. sakei* can be explained by their ability to grow and proliferate on low temperatures and in the presence of high concentrations of NaCl (up to 9%) (Hufner et al., 2007). Other representatives of lactobacilli as *Lb. paracasei*, *Lb. paracasei* ssp. *paracasei, Lb. paracasei* ssp. *tolerans, Lb. casei*, *Lb. paraplantarum, Lb. brevis, Lb. pentosus, Lb. alimentarius, Lb. buchneri, Lb. plantarum, Lb. coryniformis* ssp. *coryniformis* and *Lb. rhamnosus* (Comi et al., 2005; Rantsiou et al., 2006; Papamanoli et al., 2003; Albano et al., 2009; Hugas et al., 1993 Pennacchia et al., 2004; Drosinos et al., 2005) can also be isolated from fermented sausages, but in much lower percent (Table 1).

The presence of leuconostocs may cause the formation of holes in the product so they can be considered undesirable in the production of fermented sausages (Ammor and Mayo, 2007). However, metabolic products as acetic acid, acetaldehyde, diacetil and ethanol, participate in the development of flavor of the fermented sausages (Lee et al., 2006). Additionally, these microorganisms may have antilisterial activity (Rantsiou et al., 2005), and can reduce the level of toxins (Hemme and Foucaud-Scheunemann, 2004; Aro Aro et al., 2010). The presence of leuconostocs in fermented sausages is usually very low, with a few exceptions (table 1). For example, in a Himalayas sausages "Lang kargyong" and "Faak kargyong" the content of the *Leuconostoc mesenteroides* was 16.7% and 44.4%, respectively (Rai et al., 2010). It was also present in the Turkish sausage "sudžuk" with 5.2% (Kesmen et al., 2012) while it was not detected in the same type of sausage from Bosnia and Hercegovina (Kozachinski et al., 2008).

Pediococci can contribute to the quality of the fermented foods and affect the reduction of the presence of undesired microbiota as *Listeria, Pseudomonas, Enterobacteriae*, etc. (Semjonovs and Zikmanis, 2008). Pediococci are often used as a starter culture in the production of fermented sausages in the USA (Leroy et al., 2006). On the other hand, the frequency of isolation of pediococci from the fermented sausages produced in European countries is usually very low (Table 1).

Enterococci may also participate in the fermentation of sausages. They can improve flavor, but they can, also, induce the transfer of the genes responsible for the antibiotic resistance (Franz et al., 1999; De Vuyst et al., 2003). Their number during the production process can achieve the value of 2-5 log CFU/g in the final product (Papamanoli et al., 2003; Franz et al., 2003; Samelis et al., 1998; Aymerich et al., 2003). *Enterococcus faecalis* and *Enterococcus facium* are the species that are the most frequently isolated from different types of fermented sausages (Table 1).

Coagulase-Negative Staphylococci

Differences in production technology of (traditional) fermented sausages, raw materials, additives, climate etc. significantly influence the affirmation of specific species and strains of coagulase-negative staphylococci (CNS), bacterial population which could contribute to products safety and quality. Many studies showed a huge differences in succession of CNS during the ripening of fermented sausages, reaching a final number of 3 to 9 $\log_{10}$ CFU/g depending on sausage type, competitiveness with LAB, pH, salt and other factors (Comi et al., 2005; Urso et al., 2006; Kozachinski et al., 2008; Drosinos et al., 2005; Samelis et al., 1998; Coppola et al., 2000; Cocolin et al., 2000; Zdolec et al., 2008). The most frequently isolated CNS species in fermented sausages are *Staphylococcus xylosus*, *S. eqourum, S. saprophyticus* and *S. carnosus* (Table 2). *S. equorum, S. succinuss, S. warneri, S. vitulinus, S. pasteuri, S. epidermidis, S. lentus, S. haemoliticus S. intermedius, S. saprophyticus, S. hominis, S.auricularis* etc. can also be isolated from fermented sausages, but with lower frequency (Coppola et al., 2000; Cocolin et al., 2001; Mauriello et al., 2004; Rebecchi et al., 1998; Babic et al., 2011).

Degree of proteolytic changes during the sausage maturation is related mainly to CNS proteases (Hammes and Hertel, 1998), in addition to some LAB (Casaburi, 2008). Further, CNS influences a course of change and intensity of lipid degradation (Hadzhiosmanovic, 1978). Products of lipolysis and proteolysis such as peptides, amino acids, carbonyls and volatile compounds contribute to specific flavor and texture of fermented meat products (Casaburi et al., 2008; Hughes et al., 2002; Toldrá, 2008). The importance of CNS in fermented sausage technology is also manifested in catalase activity (peroxide degradation) and nitrite reduction (Cocconcelli and Fontana, 2008). Comprehension on biochemical activity of CNS in meat matrix leaded to selection of technologically/safety acceptable strains as autochthonous starter cultures in order to standardize sensorial features and overall products quality (Zdolec et al., 2013).

Yeasts and Molds

The activity of yeasts and molds affects the development of the characteristic taste of the sausages, as well as their characteristic appearance. Their development is usually limited to the surface of the product and during 4 weeks of fermentation their number reaches the value of 5-7 log CFU/cm^2 (Hammes et al., 2008).

Yeasts found in the fermented sausages usually originate from the raw meat. They grow by forming white film on the surface resembling a kind of wrapper, which controls the speed of water loss and is considered to be quality criteria. The population of yeasts during the process of making the fermented sausage is affected by type of the product, the production itself (industrial or traditional), and the degree of smoking, the use of spices and the diameter of the sausages (Encinas et al., 2000). Coppola et al. (2000) have isolated 79 different strains of the yeasts from fermented sausages, belonging to genera *Debaryomyces, Candida, Trichosporon* and *Cryptococcus.* The dominant species was *Debaryomyces hansenni.* This species is the most frequently used in commercial starters (Toldrà, 2002). Flavor changes in the product are the consequence of the forming of the volatiles. The role of the yeasts is the consumption of the oxygen and the production of catalase, resulting in decomposition of peroxide (Lucke and Hechelmann, 1987).

At the beginning of the fermentation process yeasts are dominant microorganisms on the surface and make 95% of microbiota. After two weeks of fermentation yeasts and molds are present in equal amounts, while at the end of the fermentation process, from the fourth to the eighth week, molds are predominant in microbiota, while yeasts reduce logarithmically. The development of the molds is associated with the products with a longer period of fermentation. The main role of the molds in the fermentation of the sausages is the increase of ammonia, reduction of the lactic acid and proteolytic activity, which further contributes to sensor characteristics of fermented sausages (Lucke and Hechelmann, 1987).

The molds form the mycelium which reduces the quantity of oxygen on the surface of the product, improves the color of the meat, and inhibits the oxidation of fat and rancidity. Physical and chemical parameters that can affect the forming of the molds are: composition of the sausages, relative humidity, temperature, a_w etc. (Toldrà, 2002). The most common species in the fermented sausages are *Penicillium, Aspergillus, Mucor* and *Cladosporium* (Hierro et al., 1999). *Penicillium nalgiovense, Penicillium chrysogenum*, *Penicillium camemberti* and *Penicillium gladioli* are commonly used as starter cultures. The fermentation of the sausages in the south of Europe involves species such as *P. nalgiovense* and *P. chrysogenium*. It was found that *P. gladioli* have very good lipolytic and proteolytic activity, while *P. nalgiovense* has only proteolytic activity (Toldrà, 2002).

Table 2
CNC species isolated from different traditional fermented sausages

Origin	Traditional product	CNC isolated from the product	Frequency of isolation, %	Reference
Serbia	Sremska sausages	*S.saprophyticus*	21.1	Kozachinski et al., 2008
		S. auricularis	12.2	
		S. xylosus	21	
		S. capitis	12.2	
		S. hominis	18.8	
		S. warneri	6.7	
		S. cohnii	1.1	
		S. aureus	6.7	
Croatia	n.d.	*S. xylosus*	29.2	Kozachinski et al., 2008
		S. capitis	25	
		S. carnosus	25	
		S. saprophyticus	20.8	
	Kulen and traditional Slavonian sausages	*S.epidermidis*	69.2	Zdolec et al., 2013
		S.capitis	5.1	
		S.warneri	3.9	
		S. spp.	25.6	
Hungary	n.d.	*S. xylosus*	4.3	Kozachinski et al., 2008
		S. hominis	16	
		S. lentus	10	
		S. warneri	6	
		S. capitis	4	
		S. epidermidis	2	
		S. haemoliticus	1	
		S. auricularis	1	
		S. saprophyticus	1	
		S. cohnii	1	
Greece	n.d.	*S. saprophyticus*	31.1	Corbiere Morot-Bizot et al., 2006
		S.xylosus	19.2	
		S. simulans	11.4	
		S. capitis	8.2	
		S. haemoliticus	7.8	
		S. sciuri	7.8	
		S. caprae	5.9	
		Other species: *S. aureus/intermedius*[a], *S. hominis, S. warnieri, S. cohnii, S. epidermidis, S. carnosus*	<5.0	
	n.d.	*S. saprophyticus*	27.6	Papamanoli et al., 2002
		S. carnosus	25.9	
		S. cohnii	13.8	
		S. xylosus	5.2	
		S. epidermidis	5.2	
		S. hominis	5.2	
		K. varians	5.2	
		Other species: *S. capitis, S. warnieri, S. auricularis*	<5.0	

Table 2 continued

Greece	n.d.	*S. saprophyticus*	34.7	Kozachinski et al., 2008
		S. xylosus	14.7	
		S. simulans	11.3	
		S. haemoliticus	11.3	
		S. capitis	6	
		S. aureus/intermedius[a]	5.3	
		S.sciuri	3.3	
		S. hominis	2	
		S. auricularis	0.7	
		S. warneri	0.7	
		S. cohnii cohnii	0.7	
		S. epidermidis	0.7	
		S. caprae	8	
	n.d.	*S. cohnii cohnii*	6	Drosinos et al., 2007
		S. equorum	1	
		S. saprophyticus	50.3	
		S. simulans	15.3	
		S. xylosus	12.6	
Italy	n.d.	*S.xylosus*	127	Iacumin et al.,2006
		S. pasteuri	25	
		S. warneri	27	
		S. caseolitics	12	
		S. epidermidis	5	
		S. saprophyticus	4	
		S. equorum	2	
		S. carnosus	11	
		S. cohnii	1	
	n.d.	*S. equorum*	23.5	Rantsiou et al., 2005
		S. haemoliticus	2.4	
		S. pasteuri	12.9	
		S. saprophyticus	1.2	
		S. succinus	2.4	
		S. warneri	9.4	
		S. xylosus	48.2	
	n.d	*S. saprophyticus*	3.3	Kozachinski et al., 2008
		S. saprophyticus/simulans[a]	0.7	
		S. hominis	6.7	
		S.hominis/warneri[a]	4	
		S. warneri	7.3	
		S. xylosus	74	
		S. epidermidis	1.7	
		S. simulans	0.7	
		S. lentus	2	
	n.d.	*S. xylosus*	45.9	Bonomo et al., 2009
		S. equorum	18.9	
		S. pulvereri/vitulus[a]	10.8	
		S. caseoliticus	2.7	

Table 2 continued

Italy	n. d.	*S. xylosus* *S. saprophyticus* *S. equorum* *S. succinus* *S. warnieri* *S. lentus* *S. vitulus* *S. pasteuri* *S. epidermidis* *S. haemoliticus*	25.4 24.8 20.9 14.7 3.9 2.8 2.2 2.2 1.7 0.6	Mauriello et al., 2004
	„Soppressata Molisana”	*S. xylosus* *S. equorum* *S. simulans* *S. kloosii*	75 7.5 7.5 8.7	Coppola et al., 1997
	„Naples ”	*S. aureus* *S. chromogenes* *S. epidermidis* *S. hominis* *S. lugdunensis* *S. saprophyticus* *S. warneri* *S. xylosus*	1.4 2.9 0.7 0.7 0.7 4.4 0.7 13.2	Coppola et al., 2000
	„Naples ”	*S. xylosus* *S. saprophyticus* *S. lentus* *S. warneri* *S. succinus*	44.8 17.2 17.2 13.8 <5.0	Mauriello et al., 2004
	„Soppressata Ricigliano”	*S. saprophyticus* *S. xylosus* *S. succinus* *S. equorum* *S. vitulus* Other species: *S. pasteuri, S. warneri, S. haemoliticus*	32 29.5 14.1 11.5 5.1 <5.0	Cocolin et al., 2001
	„Soppressata Gia”	*S. xylosus* *S. saprophyticus* *S. equorum* *S. succinus* Other species: *S. warneri, S. lentus, S. vitulus, S. pasteuri*	25.4 24.8 20.9 14.7 <5.0	Blaiotta et al., 2004
	„Salame Milano”	*S. xylosus* *S. sciuri*	65 35	Corbiere Morot-Bizot et al., 2004
	„Sausages from Basilica”	*S. xylosus* *S. pulvereri* *S. equorum* *S. saprophyticus* Other species: *S. pasteuri, S. succinus, S. epidermidis*	51.2 13.4 10.2 10 <5.0	Corbiere Morot-Bizot et al., 2004

Table 2 continued

France	n.d	*S. equorum* *S. succinuss* Other species: *S. warneri, S. saprophyticus*	82 12 <5.0	Corbiere Morot-Bizot et al., 2004
Spain	„Botillo”	*S. saprophyticus* *S. xylosus* *S. lentus* *S. cohnii cohnii*	8.6 4 2 1.3	Garcia Fontan, et al., 2007
	„Fuet”	*S. xylosus* *S. warneri* *S. epidermidis* *K. varians*	72.9 12.5 12.5 <5.0	Martin et al., 2007
	„Salchichón” i „chorizo”	*S. xylosus* *S. aureus* *S. lugdunensis* *S. chromogenes* *S. sciuri* *S. saprophyticus* *S. capitis* *Kocuria* spp.	60.2 22.9 3.6 2.4 2.4 2.4 1.2 4.8	Martin et al., 2007
	„Salchichón”	*S. aureus* *S. epidermidis* *S. xylosus* *S. saprophyticus* *S. vitulus* *S. equorum*	26 0 23 36.9 4.7 4.3	Benito et al., 2008
	„Chorizo”	*S. aureus* *S. epidermidis* *S. xylosus* *S. saprophyticus* *S. vitulus* *S. equorum*	51.7 29.4 0 10.9 0 15.8	Benito et al., 2008
	„Chorizo”	*S. xylosus* *S. warneri* *S. epidermidis* *S. carnosus*	80.8 8.3 5.8 <5.0	Martin et al., 2007
	„Chorizo”	*S. xylosus* Other species: *S. intermedius, S. saprophyticus, S. hominis, S. epidermidis, S. aureus*	94.6 <5.0	Rebecchi et al., 1998
Switzerland	n.d.	*S. xylosus* *S. warneri* *S. aureus* *S. vitulinus* *S. chromogenes* *S. fleuretti* *S. saprophyticus* *S. succinus*	11.4 16.5 6.8 2.5 2.1 0.4 15.3 3.8	Marty et al., 2011
Slovakia	n.d.	*S. xylosus* *S. carnosus* Not identified	63.6 10.7 25.7	Iacumin et al., 2006

n.d.-not defined; [a]- identification could not be performed

The Effect of Microbiota on the Production Process of Fermented Sausages

The main physical, biochemical and microbiological transformations during the process of fermentation of the sausages are: acidification, lipolysis and proteolysis, synthesis of the aroma components and the reduction of the nitrate to nitrite (Hammes et al., 2008). Acidification occurs as the result of the development of LAB population during the production process. LAB synthesizes the lactic and acetic acid which cause the reduction of pH value resulting in the transformation of proteins to gel. The molar ratio of lactic and acetic acid is in the range of 7 to 20 (Krockel, 1995; Demeyer et al., 2000). The forming of lactate and the increased presence of NaCl in a product contributes to the characteristic flavor of the sausages. The importance of the acidification is in the inhibition of the development of pathogenic microorganisms and their inactivation during the process of ripening (Leistner, 1995).

Aromatic compounds are made by lipolytic and proteolytic degradation of phospholipids, microbial activity, smoking or the addition of spices (Hammes et al., 2008). Accumulation of amino acids and peptides to the level of 1% of dry matter leads the formation of the aroma and flavor of the fermented sausages (Hierro et al., 1999). Microorganisms are involved in the decomposition of the meat proteins to peptides affecting the sensor characteristics of products. LAB population reduces pH value of the environment and thus increases the activity of the muscle proteases which participate in the decomposition of the meat proteins (Kato et al., 1994). Lactobacilli transform proteins into oligopeptides, peptides and free amino acids (Molly et al., 1997). *Lb. sakei* has proteolytic activity against sarcoplasmatic and myofibrillar proteins (Sanz et al., 1999). *Lb. sakei* and *Lb. plantarum* increase the accumulation of glutamate which is associated to the piquant flavor of food (Hammes et al., 2008). Proteolytic activity was observed for some representatives of *S. carnosus* and *S. simulans* (Casaburi et al., 2008).

Analyzing of the sausages produced with and without starter cultures has shown that the lipolysis during ripening depends mainly on the endogenous enzymes of the meat. *Kocuria various* and *S. carnosus* or *S. xylosus* have lipolytic activity which is, however, inhibited with low pH values. Enzymes and biochemical reactions of the CNS contribute to the development of desired aroma, taste, texture and quality of the final product (Molly et al., 1997). The presence of lipase was confirmed for the species *S. xylosus* (Kenneally et al., 1998; Papamanoli et al., 2002), *S. carnosus, K. varians* (Papamanoli et al., 2002) and *S. warneri* (Talon et al., 1995).

Although LAB has poor proteolytic and lipolytic activity, some strains of *Lb. sakei, Lb. curvatus* and *Lb. plantarum* may have peptidases (Fadda et al., 1999; Fadda et al., 1999). Leucine and valine aminopeptidases can play the role in the formation of the aroma of fermented sausages (Ammor et al., 2005; Papamanoli et al., 2003). During mold fermentation lipolyctic activity of the surface microbiota helps the release of long chains amino acids (Selgas et al., 1999). Proteolytic and lipolytic activities have been stated in the yeasts belonging to genera *Debaryomyces*, *Pichia*, *Trichosporon* and *Cryptococcus* (Flores and Toldra, 2011).

Another very important process which occurs during the production of fermented sausages is the reduction of nitrates to nitrites. This process is related to the activity of CNS and leads to the formation of the characteristic red colour of the fermented sausages. The activity of nitrat reductase was stated in *S. xylosus*, *S. carnosus*, *S. epidermidis, S. equorum*, *S. lentus* and *S. simulans* (Talon et al., 1995; Mauriello et al., 2004).

The Effect of Microbiota to the Product Safety

The reduction of pH value is very important for the safety of the product since it reduces the growth of undesired microorganisms (Toldrà, 2002). Additionally, certain antimicrobial components, as bacteriocins, can eliminate unwanted acidotolerant microorganisms. Bacteriocins are extracellular peptides or protein molecules, which have certain bactericidal properties in regards to some microorganisms (Veskovic-Morachanin, 2010). Antimicrobial activity of these compounds in food depends on the quantity, number and the type of microorganisms, as well as the temperature of the product, pH of the environment and the reactions with the food components. Antimicrobial activity of the bacteriocins relies on the hydrophobic and electrostatic interactions with the cytoplasmatic cell membrane (Gänzle et al., 1999). LAB bacteriocins have higher activity against Gramm positive bacteria (Cleveland et al., 2001; Diep and Nes, 2002), while the activity against Gramm negative bacteria is possible after the disruption of the integrity of the outer membrane.

In general, the majority of lactobacilli species are able to produce bacteriocins. *Lb. sakei, Lb. curvatus* and *Lb. plantarum* are considered especially important since they can have significant antibacterial activity against *L. monocytogenes* (Veskovic- Morachanin, 2010). Antilisterial activity was also confirmed for some representatives of *E. caseliflavus* (Sabia et al., 2003), *E. faecium* (Hugas et al., 2003), *P. pentosaceus* (Albano et al., 2007) and *P. acidilactici* (Lucke, 2000).

Besides for LAB, bacteriocins were present in some strains of staphylococci. *S. equorum* has the ability to produce the macrocyclic peptide antibiotic, micrococcin P1, which inhibits the growth of *L. monocytogenes*. Additionally *S. xylosus* can produce heptapeptide RNAIII-inhibitory peptide (RIP), which inhibits the growth of *S. aureus* and the production of the toxins (Leroy et al., 2002).

The Risks of the Presence of Microbiota

Bacteria used in the production of fermented sausages can carry the genes for antibiotic resistance. The resistance can be either intrinsic, common for the whole species, or acquired, which is the result of mutation (Ammor et al., 2007). Antibiotic resistance can be achieved by many mechanisms that include the increased uptake of the antibiotic, increased export of the antibiotic, inactivation or modification of the target group, hydrolysis of the antibiotic, modification of the antibiotic and prevention of the antibiotic activity (Normark and Normark, 2002). It is confirmed that *Lactobacillus, Streptococcus, Lactococcus, Pediococcus* and *Leuconostoc* have intrinsic resistance to ciprofloxacin, gentamicin and streptomycin (Talon and Leroy, 2011). Some LAB is sensitive to antibiotics such as erythromycin, chloramphenicol, tetracycline or β-lactams, while *Lb. sakei* and *Lb. curvatus* are resistant to vankomicin (Gevers et al., 2003). Enterococci are the most resistant among LAB isolates. They are resistant to tetracycline, chloramphenicol, rifampicin, nitrofurantoine (Barbosa et al., 2009; Danilovic et al., 2012).

Some species of CNS are considered as opportunistic pathogens and major cause of nosocomial infections in humans and animals (Piette and Verschraegen, 2009). In food industry, the specific reasons for concern are biofilm-positive strains such as *S. epidermidis* (Schlegelová et al., 2008). Considering fermented sausages, Even et al. (2010) reported a low occurrence of safety hazards in CNS population. However, potential hazards are related to biogenic amine and enterotoxin producers (Dobranic et al., *in press*) as well as resistance genes carriers (Marty **et al., 2012**). Generally, the most relevant issue in food-related CNS is antimicrobial resistance mostly to erythromycin, tetracycline and penicillin (Even et al., 2010; Simeoni et al., 2008; Resch et al., 2008). Some recent studies showed a significant share of resistant strains in CNS population of spontaneously fermented sausages (Marty et al., 2012; Zdolec et al., 2013). Zdolec et al. (2013) reported a frequent presence of *tet*K and *tet*M genes in *S. epidermidis* as dominant species in naturally produced fermented sausages "Kulen" and "Homemade Slavonian sausage" from Croatia. These results could be of public-health significance due to gene transfer between different intrinsic bacterial species during dry sausage fermentation (Gazzola et al., 2012).

Biogenic amines (BA) can be considered as potential precursors for the forming of carcinogenic N-nitroso compounds (Karovicova and Kohaydova, 2005). The decarboxylation of the tyrosine, histidine and ornithine results in the forming of the tyramine, histamine and putrescine (Hammes et al., 2008). Decarboxylation of amino acids can play important energetic role in nutritive poor environment since decarboxylation system can lead to translocation of the electrons through the cytoplasmatic membrane (Konings et al., 1997). Biogenic amines can appear in meat under the influence of the bacteria *Enterobacteriaceae* and *Pseudomonas* (Santos et al., 1998). The conditions of the fermentation affect the synthesis of BA. Thus, the decarboxylase is more active in the acid environment where pH is from 4-5,5 (Halász et al., 1994) and temperature in the range of 20-37 ˚C (Roseiro et al., 2010). Great number of LAB isolated from meat also has the decarboxylation activity (Bover-Cid and Holzapfel, 1999). Lactobacilli belonging to the species *Lb. buchneri, Lb. alimentarius, Lb. plantarum, Lb. curvatus, Lb. farciminis, Lb. bavaricus, Lb.reuteri* and *Lb. sakei* usually have the ability to synthesize tyramine (Masson et al., 1996; Montel et al., 1999; Bover-Cid et al., 2001). Similar was observed for the representatives of leuconostoc, lactococci and enterococci (Suzi and Gardini, 2003). Besides LAB, CNS isolated from fermented sausages can also have decarboxylase activity. The ability to produce BA was observed for some strains *S. xylosus*, *S. carnosus* and *S. piscifermentans* (Silla, 1996; Montel et al., 1999). On the other hand, addition of some strains, like *Lb. plantarum,* as starter cultures can reduce the content of BA in the product (Tosukhowong et al., 2011). Decarboxilase negative starter cultures which induce rapid drop of pH value can reduce the development of the species which can synthesize BA (Bover-Cid et al., 2000; Suzi and Gardini, 2003). Another possibility of the reduction of BA in the final product is the use of the starter cultures with aminooxidase activity (Suzi and Gardini, 2003*;* Fadda et al., 2001).

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