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Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy

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Abstract

The aims of this study were to characterize the population of Micrococcaceae in different types of fermented sausages of Southern Italy and to determine the technological properties of *Staphylococcus* strains in order to evaluate the suitability of selected strains as starter cultures in the processing of dry fermented pork sausages. Ninety-six strains were studied to evaluate nitrate reductase, proteolytic, lipolytic and antioxidant activities as well as growth ability at different temperatures, pH's and NaCl concentrations. All the strains were classified as *Staphylococcus* except for one isolate assigned to *Kocuria* spp. The species most often isolated were *S. saprophyticus*, *S. xylosus* and *S. equorum*, although they were not equally distributed within the different sausages. Other species isolated were, in descending order of abundance, *S. succinus*, *S. warneri*, *S. lentus*, *S. vitulus*, *S. pasteuri*, *S. epidermidis*, and *S. haemolyticus*. In general, the *S. xylosus* strains exhibited the best technological properties that would make them eligible as good starter cultures for fermented meat products. However, strains belonging to other species also showed good technological properties. Finally, all strains grew at 10, 15 and 20 °C, in the presence of 10% and 15% of NaCl and at pH 5.0 and 5.5. The results showed that it is possible to formulate a broad variety of staphylococcal starter cultures, adaptable to different technological conditions and sausage manufacture practices.
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1. Introduction

Different microorganisms, derived from raw materials and the environment, naturally contaminate dry sausage mixtures (Larrouture, Ardaillon, Pépin M, & Montel, 2000). Among them, lactic acid bacteria (LAB) are responsible for the main event during dry sausage ripenings the fermentation of carbohydrates. This microbial activity leads to a decrease in pH, bringing muscle proteins below their pI with a consequent loss of water holding capacity (Miralles, Flores, & Perez-Martinez, 1996). Micrococcaceae, yeasts and moulds contribute to product quality by several metabolic processes. The moulds on sausage surfaces can lead to the

development of typical flavours and tastes through by lactate oxidation, proteolysis, degradation of amino acids and lipolysis (Sunesen & Stahnke, 2003). Yeasts, such as *Candida* spp. have been shown to improve flavour development during fermentation and ripening of meat products (Sorensen & Samuelsen, 1996). Micrococcaceae participate in development and stability of a good red colour through nitrate reductase activity that leads to the formation of nitrosomyoglobin. Furthermore, nitrate reduction produces nitrite that can limit lipid oxidation by three indirect mechanisms that may operate simultaneously. Nitrite may act by: (i) binding heme and preventing release of catalytic iron, (ii) binding non-heme iron thus inhibiting catalysis, (iii) and/or stabilizing olefinic lipids against oxidation (Talon, Walter, Chartier, Barrière, & Montel, 1999). Various aromatic substances and organic acids are released by protease and lipase activity of Micrococcaceae (Montel, Reitz, Talon, Berdagué, & Rousset, 1996). Proteolysis

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and lipolysis influence both texture and flavour development due to the formation of low molecular weight compounds, including peptides, amino acids, aldehydes, amines and free fatty acids, which are important flavour compounds, or precursors of flavour compounds. Finally, superoxide dismutase and catalase activity of Micrococcaceae prevent lipid oxidation (Barriére et al., 2001). In fact, peroxide radicals, involved in rancidity development, are detoxified by superoxide dismutase activity with consequent production of hydrogen peroxide, which is destroyed by catalase activity.

To obtain products with reproducible hygienic and organoleptic qualities in shorter ripening time, the industrial manufacture of dry fermented sausages uses starter cultures, mainly consisting a combination of LAB and Micrococcaceae. However, many fermented meat products in Southern Italy are still produced without the use of selected starters with consequent loss of product. The selection of autochthonous microflora for starter formulation could be a tool to preserve the typical characteristics of these products. Actually, Italian law permits the use of some species belonging to the genus *Staphylococcus* as starter for fermented sausage production (Repubblica Italiana, 1995). For these reasons, knowledge of the biochemical and technological properties of the strains involved in the ripening of typical fermented sausages is a matter of considerable interest (Coppola, Iorizzo, Saotta, Sorrentino, & Grazia, 1997).

The aim of this work was to assess the biochemical characteristics of 96 strains of six different *Staphylococcus* species, in order to select the most suitable as starters for fermented dry sausage production.

2. Materials and methods

2.1. Sausages, microbiological and physico-chemical analyses

Three types of high-quality fermented sausages were manufactured in three different factories without the use of starter cultures; denominations of the products and manufacture conditions are illustrated in Table 1.

Two independent 20 g samples (without casing) of each sausage type were collected aseptically, transferred to sterile plastic pouches, 10-fold diluted with sterile quarter-strength Ringers Solution (Oxoid Ltd, London, UK) and homogenised for 90 s using the laboratory blender Stomacher 400 (Seward, London, UK). Serial 10-fold dilutions were prepared from the same solution and inoculated in appropriate growth media.

Mesophilic LAB were enumerated on Rogosa agar (Oxoid) in anaerobic conditions after 48 h incubation at 30 °C; Micrococcaceae on Mannitol Salt Agar (MSA, Oxoid) after 48 h at 30 °C and yeasts and moulds on DRBC (Oxoid) after four days at 25 °C. The potentiometric measurement of pH was made by inserting the pin electrode of a pH-meter (Beckman PHI43) directly into each sample. Water activity (Aw) was measured using a 'Lufft aw-wert-Messer' supplied by PBI (Milan, Italy). Sausage types were sampled at different times:

- Naples-type salami at 0, 2, 7, 14, 23 and 33 days of ripening;
- soppressata of Ricigliano at 0, 6, 15, 29 and 40 days of ripening;
- soppressata of Gioi at 0, 13, 20, 29 and 43 days of ripening.

Table 1
Denominations and manufacture conditions of the three different types of fermented sausages

Manufacture condition	Three different types of fermented sausages		
	Naples type salami (NTS)	Soppressata of Ricigliano (SR)	Soppressata of Gioi (SG)
Origin of product	Naples province	Salerno province	Salerno province
Ingredients	70% lean pork, 12% pork belly, 2.4% NaCl, 0.1% black pepper, 2% powdered milk, 1.5% of a commercial mixture for salami consisting of pepper, ascorbic-acid, potassium nitrate, dextrose, lactose and sucrose	75% lean pork, 22.2% pork belly, 2.6% NaCl, 0.2% grain pepper	94% lean pork, 3.2% fat (one piece of parallelepiped shape in the core of product), 2.6% NaCl, 0.2% grain pepper
Casing	Natural	Natural	Natural
Filling	Mechanically under vacuum	Mechanically	Manual
Ripening condition	4–7 °C and 85–90% relative humidity for two h, 20–24 °C and 75–85% relative humidity for 24 h, 16–18 °C and 64–69% relative humidity for 33 days	The product was manufactured during winter and ripened in unconditioned rooms at temperatures (never below 10 °C) and humidities according to climatic events	The product was manufactured during winter and ripened in unconditioned rooms at temperatures (never below 10 °C) and humidities according to climatic events
Ripening time (days)	33	40	43

2.2. Strains of Micrococcaceae

Colonies from countable plates were purified by streaking on MSA and then tested for morphology, Gram-stain and catalase production. Gram-positive and catalase-positive cocci were maintained on P-agar (Phillips & Nash, 1985) slants stored at 4 °C and working cultures grown overnight at 37 °C in Tryptone Soya Broth (Oxoid). Isolates were subjected to the oxidation/fermentation test in OF medium and to anaerobic growth in semisolid thioglycollate medium (Evans & Kloos, 1972). Sensitivity to furazolidone, bacitracin and lysostaphin was determined as described by Kloos and Bannerman (1995). Production of pigment was observed on P-agar. Staphylococci were assayed for coagulase activity using the tube test with coagulase plasma (Becton, Dickinson and Company, NJ, USA) and for novobiocin sensitivity (Kloos,Tornabene, & Schleifer, 1974). One-hundred and seventy seven Micrococcaceae strains isolated during this study, reported in Table 2, were previously identified at species level (Blaiotta, 2002; Blaiotta et al., 2003; Blaiotta et al., in press) by applying phenotypic and genotypic methods.

Ninety-six strains (23 *S. xylosus*, 28 *S. saprophyticus*, 28 *S. equorum*, 6 *S. warneri*, 6 *S. succinus* and 5 *S. lentus*), chosen among isolates from samples at the end of ripening, were characterized by the tests described below.

2.3. Catalase assay

Catalase activity was measured on resting cells according to Aebi's method (Aebi, 1974). Five ml of each culture (resting cells with an $OD_{600} = 1.0$) were centrifuged at 13 000g for 5 min and the resulting pellet mixed with 1.5 ml 60 mM H_2O_2 in 20 mM phosphate buffer pH, 7.0. The activity was measured spectrophotometrically at 240 nm after 3 min of incubation at room temperature. Results are expressed in arbitrary units: μmoles of degraded H_2O_2/min/ml of cells with $OD_{600} = 1.0$.

2.4. Superoxide dismutase activity

The cell pellet of each strain was harvested by centrifugation of 2 ml of culture at 13000g for 5 min and washed once in 50 mM K_2HPO_4, pH 7.8. For SOD extraction the cell pellet (approximately 100 mg wet weight) was suspended in 1 ml of 50 mM K_2HPO_4 pH 7.8 and disrupted with glass beads (0.10–0.11 mm) on a vortex mixer for 5 min. After lysis, the suspension was centrifuged and the supernatant was used for determination of SOD activity as described below. Ten microlitre of cell extract were added to 1.0 ml of 150 μM nitroblue tetrazolium (NBT), 10 mM methionine, 1.2 μM riboflavin, 50 mM K_2HPO_4 pH 7.8 and incubated at room temperature in a light chamber with a 60 W bulb lamp for 8 min. Under this condition, riboflavin is excited by a photon and is able to oxidise the methionine. This donation of an electron results in the production of a superoxide molecule (O_2^-) that is able to reduce NBT resulting in a colour change which was measured spectrophotometrically at 560 nm against the appropriate solution. The presence of SOD leads to a reduction in the colour change. Results were expressed as percentage of optical density (OD) at 560 nm according to the following equation:

$$SOD\ activity = (1 - s/c) \cdot 100,$$

where s is the OD of the sample and c the OD of the control, consisting of a solution without adding cell extract and incubated under the same conditions.

Table 2
Species of Gram-positive and catalase-positive cocci isolated from the three different types of fermented sausage

Species[a]	Number of strains (%) in each sausage type[b]			
	NTS	SR	SG	All sausages
S. xylosus	13 (44.8)	23 (29.5)	9 (12.8)	45 (25.4)
S. saprophyticus	5 (17.2)	25 (32.0)	14 (20.0)	44 (24.8)
S. equorum	0	9 (11.5)	28 (40.0)	37 (20.9)
S. succinus	1 (3.4)	11 (14.1)	14 (20.0)	26 (14.7)
S. warneri	4 (13.8)	2 (2.6)	1 (1–4)	7 (3.9)
S. lentus	5 (17.2)	0	0	5 (2.8)
S. vitulus	0	4 (5.1)	0	4 (2.2)
S. pasteuri	0	3 (3.8)	1 (1.4)	4 (2.2)
S. epidermidis	0	0	3 (4.3)	3 (1.7)
S. haemolyticus	0	1 (1.3)	0	1 (0.6)
Kocuria spp.	1 (3.4)	0	0	1 (0.6)
Total	29 (100)	78 (100)	70 (100)	177 (100)

[a] Species were determined by biochemical and molecular tools (Blaiotta, 2002; Blaiotta, Pennacchia, Ercolini, Moschetti, & Villani, 2003; Blaiotta, Pennacchia, Parente, & Villani, in press).
[b] NTS, Naples Type Salami; SR, Soppressata of Ricigliano; SG, Soppressata of Gioi.

2.5. Nitrate reductase assay

Nitrate reductase activity was determined as described by Miralles et al. (1996) on YTA supplemented with 1 g l^{-1} KNO$_3$. The cell pellet of an overnight culture was resuspended in equal volume of 50 mM phosphate buffer pH 7.0 and 30 µl loaded into agar plates wells (6 mm diameter). After incubation at 30 °C for 7 h the plates were flooded with 1 ml of a 1:1 solution of NIT1 (0.8 g sulphanilic acid in 100 ml of acetic acid 5 N) and NIT2 (0.6 g *N-N*-dimethyl-1-Naphthylamine in 100 ml of acetic acid 5 N) for the detection of nitrite. The appearance of red haloes surrounding the wells indicated the presence of nitrate reductase activity.

2.6. Lipolytic activity

One ml of an overnight culture of each strain was inoculated into 10 ml of a broth containing 1% tryptone, 0.5% yeast extract, 3% NaCl, pH 7.0, supplemented with 4% (w/v) pork fat. For the preparation of media, pork fat was homogenised by vigorous shaking. After incubation at 30 °C for seven days, the samples were used for the determination of free fatty acids. The lipids were extracted into 10 ml of petroleum ether (Merck & Co. Inc., NJ, USA) by shaking for 1 min. The fatty acids of the upper phase (lipid extract) were titrated with NaOH (0.1 M) in ethanol using 1% phenolphthalein ethanol solution as indicator. Lipolytic activity was expressed as a percentage of oleic acid by the following equation:

$$\text{lipolytic activity} = (a \cdot N \cdot 28.2)/g,$$

where a is ml of NaOH used for titration, N is the normality of NaOH, 28.2 is the percent of equivalent weight of oleic acid and g the quantity of pork fat in the sample.

2.7. Proteolytic activity

Sarcoplasmic and myofibrillar proteins, extracted according to the method described by Mauriello, Casaburi, and Villani (2002), were added at a concentration of 0.57 mg/ml to an agar medium containing 0.5% tryptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar, pH 6.9. Sarcoplasmic and myofibrillar proteins were added to the medium after sterilization. The overnight culture of each strain was centrifuged at 13 000*g* for 5 min and the resulting pellet washed once with 20 mM phosphate buffer pH 7.0 and resuspended in an equal volume of the same buffer. Thirty microlitre of cell suspension were pipetted into wells (6 mm diameter) bored in the agar plates. After incubation at 37 °C for 48 h, the agar layers were removed from petri dishes and stained for 5 min in 0.05% (w/v) Brilliant Blue R (Sigma–Aldrich Srl, Milan, Italy) in methanol:acetic acid:water (50:40:10) and destained in methanol:acetic acid:water (25:5:70). A clear zone surrounding the inoculated wells indicated proteolytic activity and its diameter was measured in mm.

2.8. Effect of temperature and pH on microbial growth

The strains were tested for growth ability at different temperatures, pH's and NaCl concentrations. Growth was evaluated at 10, 15 and 20 °C in yeast tryptone agar (YTA, tryptone 1%, yeast extract 0.5%, NaCl 0.4%, agar 1.5%, pH 7.0). The effect of NaCl was determined in YTA supplemented with 10% and 15% NaCl. The effect of pH on growth in YTA adjusted to pH values of 5.0 and 5.5 by addition of HCl (0.1 M) was evaluated at 15 and 20 °C. Ten microlitre of an overnight culture of each strain were inoculated into the different media described above and the growth response, whether positive or negative, was registered every 12 h for a week.

3. Results

Changes in microbial contents and pH during ripening of each sausage are shown in Figs. 1–3. They show a decrease in pH during the first few days whereas in subsequent stages, except for Naples Type salami, there was an increase in pH.

During the first week of ripening, mesophilic LAB and Micrococcaceae increased quite rapidly to about 10^8–10^9 and 10^6 cfu/g, respectively. Yeasts showed slow growth during the first half of ripening and then decreased at different rates for each sausage. The distribution of the species in the three sausages is reported in Table 2. The technological properties of 96 of the 177
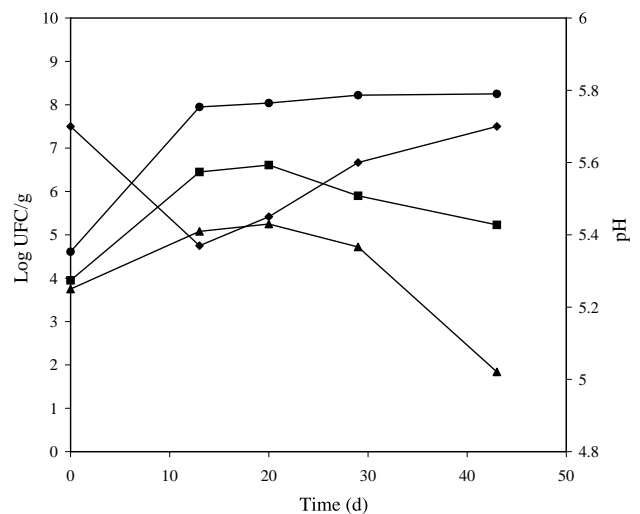

Fig. 1. Evolution of pH and microflora during ripening of Naples Type Salami. (◆) pH, (●) mesophilic lactic acid bacteria on Rogosa agar; (■) Micrococcaceae on mannitol salt agar; (▲) yeasts on DRBC.
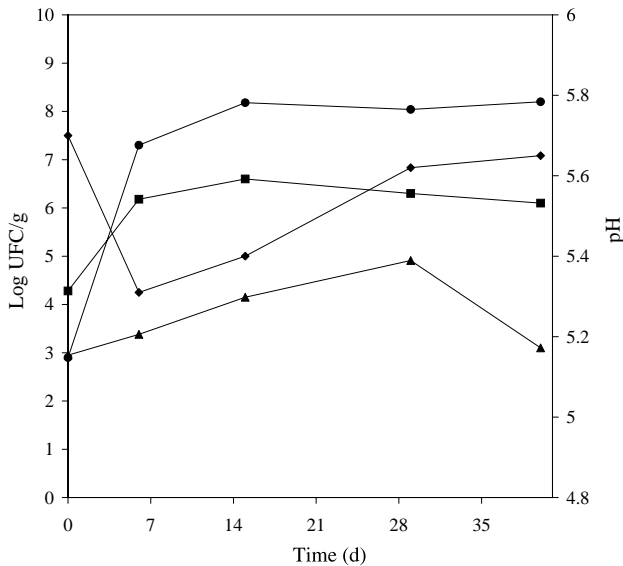
Fig. 2. Evolution of pH and microflora during ripening of Soppressata of Ricigliano. (◆) pH, (●) mesophilic lactic acid bacteria on Rogosa agar; (■) Micrococcaceae on mannitol salt agar; (▲) yeasts on DRBC.
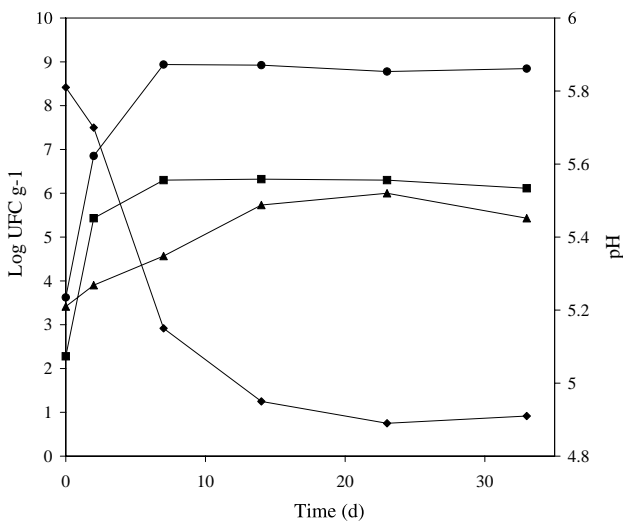

Fig. 3. Evolution of pH and microflora during ripening of Soppressata of Gioi. (◆) pH, (●) mesophilic lactic acid bacteria on Rogosa agar; (■) Micrococcaceae on mannitol salt agar; (▲) yeasts on DRBC.

strains, reported in Tables 3–8, showed high variability amongst strains. About 40% of the strains were unable either to reduce nitrate or to hydrolyse pork fat. Most of the strains unable to reduce nitrate belonged to *S. saprophyticus* species. On the contrary, higher nitrate reductase activities were registered among *S. xylosus* strains. *S. saprophyhticus* 5SE7 showed the highest lipolytic activity releasing 42.3% of the free fatty acids and no other strain was able to release more than 35% of free fatty acids from pork fat. All the species showed greater proteolysis against myofibrillar than sarcoplas-

mic proteins and the proteolytic activities against both type of proteins did not appear to be correlated ($r = 0.04$) when analysed by Pearson correlation with the Corr procedure of Systat software for Macintosh ver. 5.2.1. Only the strain *Staphylococcus equorum* SS8 showed high values of proteolytic activity on both protein fractions. Many strains exhibited no proteolytic capability against either protein fractions. All strains had antioxidative enzymes SOD and catalase. *S. lentus* and *S. saprophyticus* strains had the highest values of SOD and catalase activities while *S. equorum* and *S. warneri* strains had the lowest levels of catalase and SOD, respectively. *S. saprophyticus* 52k3 was the best producing 77.4% of SOD activity and 23.3 AU of catalase activity. All strains grew at temperatures usually used for meat fermentation (10 and 15 °C) in the presence of 10% and 15% of NaCl, as well as under all pH conditions (data not shown).

4. Discussion

The microbial changes during sausage ripening showed very good growth of mesophilic LAB in all samples, corresponding to a pH decrease. The latter was more marked in Naples Type salami, probably due the addition of milk powder to the sausage mixture, a source of sugar for microorganisms. In both soppressata types no sugar source was added and the pH reached a lower value (about 5.3) after the first period and then, at the end of ripening, rose to the initial value. As is well known, the final period of dry sausages ripening is characterized by the colonization on the sausages surface of abundant moulds, which oxidise lactate with consequent increase of pH (Grazia, Romano, Bagni, Roggiani, & Guglielmi, 1986). This phenomena was more evident in both soppressata types, probably due to the non-addition of sugar. In the NTS mesophilic LAB showed higher numbers in comparison with both soppressata types. In all sausages the Micrococcaceae showed a lower population of LAB (about 2–3 log units). The behaviour of Micrococcaceae reflected their poor competitiveness in the presence of actively growing aciduric bacteria (Samelis, Metaxopoulos, Vlassi, & Aristea, 1998). Although *S. carnosus* and *S. xylosus* are the most recommended staphylococcal starter culture for dry sausage production in Europe (Samelis et al., 1998), among our isolates none were identified as *S. carnosus* (Blaiotta, 2002; Blaiotta et al., 2003; Blaiotta et al., in press). Our results showed *S. saprophyticus*, *S. xylosus* and *S. equorum* were the most common species, in agreement with results of other authors (Coppola et al., 1997; Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000). The most abundant species was *S. xylosus* (44.8%) in NTS, *S. saprophyticus* (32.0%) in SR and *S. equorum* (40.0%) in SG. In a previous study

Table 3
Technological properties of *S. xylosus* strains[a]

Strains	Origin[b]	Nitrate reduction[c] (mm)	Proteolysis[d] (mm)	Lipolysis[e] (%)	SOD activity[f] (%)	Catalase activity[g] (AU)
AS5	NTS	0	2/8	0	20.8	16.2
AS6	NTS	3	0/2	4.9	36.9	20.3
AS9	NTS	11	2/2	14.8	5.4	12.4
AS15	NTS	17	24/2	20.4	44.8	14.3
AS26	NTS	18	0/8	14.8	56.7	15.7
BS3	NTS	9	24/8	20.4	29.3	14.8
BS4	NTS	15	0/9	17.6	63.2	16.2
BS20	NTS	18	2/8	20.4	13.9	21.3
CS6	NTS	11	9/0	9.87	66.9	18.4
CS7	NTS	15	18/0	17.6	60.0	8.8
DS18	NTS	18	2/0	16.9	12.0	16.7
DS20	NTS	3	2/0	0	40.2	13.6
DS26	NTS	13	2/14	16.9	21.2	14.8
5K07	SR	18	0/2	13.4	43.9	24.9
18K14	SR	19	0/2	9.16	2.0	15.2
34K35	SR	15	0/4	11.3	14.7	25.4
66K3	SR	15	0/0	9.16	38.7	25.3
41M06	SR	15	0/4	9.87	42.8	20.9
5SE14K	SG	9	0/0	11.3	47.2	11.6
5SE15K	SG	0	2/4	0	27.8	18.5
5SI3K	SG	3	0/0	4.9	59.3	16.1
5SI5K	SG	19	2/2	10.6	2.6	20.9
SS4	SG	17	0/12	0	48.3	15.8

[a] Values are means of three replicates, standard deviations are always lower than 20% of means.
[b] SG, Soppressata of Gioi; SR, Soppressata of Ricigliano; NTS, Naples Type Salami.
[c] Diameter of red haloes.
[d] Diameter of clear zone on myofibrillar/sarcoplasmic proteins.
[e] % of oleic acid.
[f] % of optical density.
[g] Arbitrary units: μmoles of degraded H_2O_2 min^{-1} ml^{-1} of cells with OD = 1.0.

(Coppola et al., 2000) *S. xylosus* was also the predominant species in other NTS sausages, in agreement with the results obtained in this work. Most *S. xylosus* strains showed technological properties that would make them eligible as starter cultures for fermented meat products. In particular, the strains AS15 and BS3, both from NTS, showed good activity of all the studied metabolic properties. Furthermore, our results showed *S. xylosus* to be the species with a good ability to reduce nitrate in agreement with results of other authors (Montel et al., 1996; Talon et al., 1999). Although *S. saprophyticus* is often isolated in large numbers from southern European salamis, it does not represent the principal species in fermented sausages, which is *S. xylosus*. Samelis et al. (1998) found in four samples of traditional Greek salami a dominance of *S. saprophyticus* with respect to *S. xylosus*. Recently, a similar result was reported by Papamanoli, Kotzekidou, Tzanetakis, and Litopoulou-Tzanetaki (2002), who found that *S. saprophyticus* represented 22% of the Micrococcaceae population on two types of Greek fermented sausages, while *S. xylosus* represent only 10%. Furthermore, according to Montel, Talon, Berdagué, and Cantonnet (1993) strains of *S. saprophyticus* and *S. warneri* have also been isolated from commercial French starter cultures. Subsequently,

in this study *S. saprophyticus* strains were evaluated for potential use as starter cultures. In agreement with the *S. saprophyticus* species characteristics, only two strains (AS1 and GB1) had the ability to reduce nitrate, the first criterion in the selection of strains to be used as starter cultures for sausage manufacture. However, it would be possible to use nitrate reductase non-producer strains in artisanal sausage manufactures where non-nitrate salts are added. Indeed, our results suggest that the use of *S. saprophyticus* strains, showing high values of SOD and catalase activity, could help to prevent off-flavours produced by lipid oxidation during sausage ripening. Samelis et al. (1998) reported that *S. saprophyticus* "should be selected and validated as starter culture in experimentally inoculated salamis" but this species is deemed to be potentially pathogenic and needs careful consideration (Hammes & Hertel, 1998).

S. equorum has rarely been described in meat products (Garcia, Zumalacárregui, & Diez, 1995; Coppola et al., 1997) although this species is often isolated from dairy products, especially smear cheeses (Bockelmann, 2002; Meugnier et al., 1996; Rozand et al., 1996). Furthermore, a high percentage of *S. equorum* was isolated from salt used for Spanish dry-cured ham (Cordero & Zumalacárregui, 2000). Sondergaard and Stahnke

Table 4
Technological properties of *S. saprophyticus* strains

Strains	Origin[a]	Nitrate reduction[b] (mm)	Proteolysis[c] (mm)	Lipolysis[d] (%)	SOD activity[e] (%)	Catalase activity[f] (AU)
AS1	NTS	12	0/6	0	30.7	22.3
AS41	NTS	0	0/6	0	52.5	21.1
GB1	NTS	9	0/6	3.5	60.9	19.8
5SE7	SR	0	0/0	42.3	11.9	20.2
0SI2	SR	0	0/0	9.2	4.4	20.3
50K3	SR	0	11/0	7.05	61.9	20.0
51K3	SR	0	29/0	0	44.1	24.2
52K3	SR	0	0/0	0	77.4	23.3
55K3	SR	0	0/0	7.05	64.0	18.1
56K3	SR	0	19/0	7.05	43.1	22.9
57K3	SR	0	14/0	7.05	74.4	17.3
58K3	SR	0	14/0	14.1	57.8	24.5
59K3	SR	0	14/6	0	62.7	23.9
60K3	SR	0	9/6	7.05	45.5	24.4
61K3	SR	0	0/0	7.05	20.6	21.8
62K3	SR	0	0/0	7.05	15.5	25.5
63K3	SR	0	0/0	0	54.2	20.0
64K3	SR	0	26/0	7.05	15.0	21.5
65K3	SR	0	0/0	7.05	14.6	22.1
67K3	SR	0	14/0	0	47.3	7.3
68K3	SR	0	0/0	7.05	55.6	26.9
69K3	SR	0	19/0	7.05	11.2	2.9
70K3	SR	0	0/0	7.05	56.9	25.8
71K3	SR	0	0/0	7.05	29.5	24.6
74K3	SR	0	14/0	0	72.8	21.2
75K3	SR	0	9/0	0	6.5	22.8
76K4	SR	0	9/0	0	41.8	22.2
79K4	SR	0	14/0	7.05	15.0	23.9

[a–g] See Table 3.

(2002) used two strains of *S. equorum* for a meat starter culture selection study. Most authors working on the characterization of Micrococcaceae from meat products identified the isolates by the API STAPH system. However, Sondergaard and Stahnke (2002) reported, for strains isolated from fermented meat products, a high identification probability for *S. xylosus* according to API STAPH results and identification as *S. equorum* by molecular analysis. Results obtained previously (Blaiotta, 2002; Blaiotta et al., 2003; Blaiotta et al., in press) suggest, using traditional identification methods only, on many cases strains of *S. equorum*, *S. lentus*, *S. warneri* and *S. saprophyticus* can be misidentified as *S. xylosus*; by contrast, strains of *S. xylosus* can be misidentified as *S. saprophyticus*, *S. sciuri*, *S. epidermidis* or *S. intermedius*. Hence, there is likely to be an underestimate of *S. equorum* species in meat products. However, whereas *S. equorum* strains made up 40.0% and 11.5% of the total isolates in SR and SG respectively (Table 2), no isolate of this species was found in NTS samples; the technological process (meat, ingredients, additives, ripening conditions, etc.) may well have an effect on the staphylococcal composition of the sausages. In fact, unlike NTS technology, SR and SG were manufactured without additives and ripened at temperatures

close to 10 °C (Table 1). This last condition may be favourable for *S. equorum* as strains of this species grow well at temperature <10 °C (Schleifer, Klipper-Balz, & Devriese, 1984).

Montel, Talon, Cantonnet, and Fournaud (1992) and Montel et al. (1996) isolated strains of *S. warneri* from French dry sausages and characterized them for technological properties as well as proteolytic and lipolytic activity and flavour production. They stressed the higher lipolytic activity of *S. warneri* with respect to other staphylococcal species. In agreement with this finding, our results showed that *S. warneri* strains were able to release between 15.9% and 35.2% of free fatty acids. High percentages of *S. warneri* and *S. lentus* were also found in NTS as well as *S. succinus* in the other sausages.

The meat substrates (e.g. muscle protein and pork fat), as used out in this study, could be more suitable for the detection of technological properties of a potential microbial meat starter than other non-meat substrates, such as powdered milk or gelatin and tributyrin or Tween 80.

In conclusion, our results showed that a broad variety of staphylococcal starter cultures might be formulated, which are adaptable to different technological

Table 5
Technological properties of *S. equorum* strains

Strains	Origin[a]	Nitrate reduction[b] (mm)	Proteolysis[c] (mm)	Lipolysis[d] (%)	SOD activity[e] (%)	Catalase activity[f] (AU)
SE2	SG	11	19/0	0	20.0	9.8
SE3	SG	9	9/2	0	14.3	16.0
SE4	SG	9	12/6	0	16.0	11.2
SE5	SG	11	9/0	0	8.1	15.3
SE6	SG	11	0/2	0	48.0	18.1
SE7	SG	14	0/0	0	4.9	7.1
SE8	SG	0	0/0	28.2	10.4	5.8
SE9	SG	7	0/0	7.05	40.9	11.1
SE10	SG	11	0/0	0	66.3	8.9
SE12	SG	14	0/0	0	32.1	14.0
SI1	SG	11	0/0	0	11.2	19.7
SI2	SG	7	12/4	0	34.7	11.9
SI3	SG	11	9/0	35.2	42.1	3.1
SI4	SG	7	9/0	35.2	60.2	8.4
SI5	SG	9	0/0	0	17.6	20.5
SI7	SG	0	0/6	28.2	9.3	1.9
SI8	SG	9	0/2	0	58.6	16.0
SI9	SG	9	0/4	0	46.4	14.5
SS1	SG	9	0/0	0	63.4	15.8
SS2	SG	0	6/0	0	9.8	13.5
SS3	SG	4	0/2	0	6.6	13.2
SS5	SG	17	0/4	0	31.4	18.2
SS6	SG	14	0/0	0	22.7	16.0
SS7	SG	17	0/0	35.2	5.5	2.0
SS8	SG	0	24/14	0	73.2	14.6
SS9	SG	0	9/6	28.2	44.4	8.1
SS10	SG	0	19/0	0	34.0	14.5
SS13	SG	11	9/0	7.05	71.0	15.1

[a–g] See Table 3.

Table 6
Technological properties of *S. succinus* strains

Strains	Origin[a]	Nitrate reduction[b] (mm)	Proteolysis[c] (mm)	Lipolysis[d] (%)	SOD activity[e] (%)	Catalase activity[f] (AU)
BS12	NTS	6	24/2	1.41	25.1	24.9
80K4	SR	0	0/0	7.05	21.8	11.8
77K3	SR	0	9/0	14.8	26.0	19.8
53K3	SR	0	24/2	0	40.2	21.9
72K3	SR	0	0/0	0	65.2	23.2
5SI10	SG	4	0/0	14.8	15.6	20.0

[a–g] See Table 3.

Table 7
Technological properties of *S. warneri* strains

Strains	Origin[a]	Nitrate reduction[b] (mm)	Proteolysis[c] (mm)	Lipolysis[d] (%)	SOD activity[e] (%)	Catalase activity[f] (AU)
ES2	NTS	0	19/2	25	13.5	4.1
BS5	NTS	0	0/0	15.9	16.3	12.1
CS10	NTS	9	0/0	19	4.4	14.5
ES1	NTS	0	19/4	25	9.9	4.6
1A5	SR	0	0/0	15.9	21.5	16.0
98M5	SR	0	14/0	35.2	24.2	1.4

[a–g] See Table 3.

Table 8
Technological properties of *S. lentus* strains

Strains	Origin[a]	Nitrate reduction[b] (mm)	Proteolysis[c] (mm)	Lipolysis[d] (%)	SOD activity[e] (%)	Catalase activity[f] (AU)
AS11	NTS	10	0/6	11.8	65.3	19.4
AS3	NTS	10	0/4	0	78.3	25.6
AS4	NTS	10	0/0	0	78.4	24.9
BS2	NTS	7	0/2	0.7	79.9	26.6
AS7	NTS	10	24/4	0.7	80.5	22.6

[a-g] See Table 3.

conditions and sausage type manufacture. Considering the reactions that could be taking in place in the meat mixture and the involvement of endogenous meat enzymes and complex microflora, further studies are necessary to evaluate the technological properties of microbial strains in sausage production and to evaluate their effects on product quality.

Acknowledgements

References

Aebi, H. (1974). Catalase. In H. U. Bergmeyer (Ed.), *Methods of analytic analysis* (pp. 673–684). New York and London: Academic Press.

Barriére, C., Centeno, D., Lebert, A., Leroy-Sétrin, S., Berdagué, J. L., & Talon, R. (2001). Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiology Letters, 201*, 181–185.

Bockelmann, W. (2002). Development of defined surface starter cultures for the ripening of smear cheeses. *International Dairy Journal, 12*, 123–131.

Blaiotta, G. (2002). Polyphasic molecular approach to identification of staphylococci in food ecosystems. Doctorate thesis. Department of Food science, University of Naples "Federico II".

Blaiotta, G., Pennacchia, C., Ercolini, D., Moschetti, G., & Villani, F. (2003). Combining denaturing gradient gel electrophoresis of 16S rDNA V3 region and 16S-23S rDNA spacer region polymorphism analyses for the identification of staphylococci from Italian fermented sausages. *Systematic and Applied Microbiology, 26*, 423–433.

Blaiotta, G., Pennacchia, C., Parente, E., & Villani, F. (in press). Design and evaluation of specific PCR primers for rapid and reliable identification of *Staphylococcus xylosus* strains isolated from dry fermented sausages. *Systematic and Applied Microbiology*.

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

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

Cordero, M. R., & Zumalacárregui, J. M. (2000). Characterization of Micrococcaceae isolated from salt used for Spanish dry-cured ham. *Letters in Applied Microbiology, 31*, 303–306.

Evans, J. B., & Kloos, W. E. (1972). Use of shake cultures in a semisolid thioglycollate medium for differentiating Staphylococci from Micrococci. *Applied Microbiology, 23*, 326–331.

Garcia, I., Zumalacárregui, J. M., & Diez, V. (1995). Microbial succession and identification of Micrococcaceae in dried beef cecina, an intermediate moisture meat product. *Food Microbiology, 12*, 309–315.

Grazia, L., Romano, P., Bagni, A., Roggiani, D., & Guglielmi, G. (1986). The role of moulds in the ripening process of salami. *Food Microbiology, 3*, 19–25.

Hammes, W. P., & Hertel, C. (1998). New developments in meat starter cultures. *Meat Science, 49*, 125–138.

Kloos, W. E., & Bannerman, T. L. (1995). Staphylococcus and micrococcus. In *Manual of clinical microbiology* (6th edn). Washington, DC: American Society for Microbiology.

Kloos, W. E., Tornabene, T. G., & Schleifer, K. H. (1974). Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and Micrococcus kristinae. *International Journal of Systematic Bacteriology, 24*, 79–101.

Larrouture, C., Ardaillon, V., Pépin M, M., & Montel, M. C. (2000). Ability of meat starter cultures to catabolize leucine and evaluation of the degradation products by using an HPLC method. *Food Microbiology, 17*, 563–570.

Mauriello, G., Casaburi, A., & Villani, F. (2002). Proteolytic activity of *Staphylococcus xylosus* strains on pork myfibrillar and sarcoplasmic proteins and use of selected strains in the production of 'Naples type' salami. *Journal of Applied Microbiology, 92*, 482–490.

Meugnier, H., Bes, M., VernozyRozand, C., Mazuy, C., Brun, Y., Freney, J., & Fleurette, J. (1996). Identification and ribotyping of *Staphylococcus xylosus* and *Staphylococcus equorum* strains isolated from goat milk and cheese. *International Journal of Food Microbiology, 31*, 325–331.

Miralles, M. C., Flores, J., & Perez-Martinez, G. (1996). Biochemical tests for the selection of *Staphylococcus* strains as potential meat starter cultures. *Food Microbiology, 13*, 227–236.

Montel, M. C., Talon, R., Cantonnet, M., & Fournaud, J. (1992). Identification of *Staphylococcus* from French dry sausage. *Letters Applied Microbiology, 15*, 73–77.

Montel, M. C., Talon, R., Berdagué, J. L., & Cantonnet, M. (1993). Effects of starter cultures on the biochemical characteristics of French dry sausages. *Meat Science, 35*, 229–240.

Montel, M. C., Reitz, J., Talon, R., Berdagué, J. L., & Rousset, S. (1996). Biochemical activities of Micrococcaceae and their effects on the aromatic profiles and odours of dry sausages model. *Food Microbiology, 13*, 489–499.

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

Phillips, E., & Nash, P. (1985). Culture media. In E. H. Lenette, A. Balows, W. J. Jr., Hausler, & H. L. Shadomy (Eds.), *Manual of

clinical microbiology (4th ed., pp. 1051–1092). Washington, DC: American Society for Microbiology.

Repubblica Italiana (1995). Decreto del 28/12/1994 del Ministero della Sanità. Autorizzazione all'impiego di colture di avviamento, "starter microbici" nella preparazione degli insaccati carnei la cui tecnologia produttiva non comporti trattamenti col calore. Gazzetta Ufficiale, Serie Generale, N. 89 del 15/4/1995.

Rozand, C. V., Mazuy, C., Perrin, G., Haond, F., Bes, M., Brun, Y., & Fleurette, J. (1996). Identification of Micrococcaceae isolated from goat's milk and cheese in the Poitou-Charentes region. *International Journal of Food Microbiology, 30*, 373–378.

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

Schleifer, K. H., Klipper-Balz, R., & Devriese, L. A. (1984). *Staphylococcus arlettae* sp. nov., *S. equorum* sp. nov., *S. kloosii* sp. nov.: three coagulase-negative novobiocin-resistant species from animals. *Systematic and Applied Microbiology, 5*, 501–509.

Sondergaard, A. K., & Stahnke, L. H. (2002). Growth and aroma production by *Staphylococcus xylosus, S. carnosus* and *S. equorum* – a comparative study in model systems. *International Journal of Food Microbiology, 75*, 99–109.

Sorensen, B. B., & Samuelsen, H. (1996). The combined effects of environmental conditions on lipolysis of pork fat by lipase of the meat starter culture organisms *Staphylococcus xylosus* and *Debaryomyces hansenii. International Journal of Food Microbiology, 35*, 59–71.

Sunesen, L. O, & Stahnke, L. H. (2003). Mould starter culture for dry sausages – selection, application and effect. *Meat Science, 65*, 935–948.

Talon, R., Walter, D., Chartier, S., Barrière, C., & Montel, M. C. (1999). Effect of nitrate and incubation conditions on the production of catalase and nitrate reductase by staphylococci. *International Journal of Food Microbiology, 52*, 47–56.