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ScienceDirect
International Journal of Food Microbiology 112 (2006) 223–229
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Protease and esterase activity of staphylococci☆

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Abstract

The aim of this work was to characterize protease and esterase activities of staphylococci in order to establish if they could contribute to the release of amino acids and short-chain fatty acids during ripening of fermented sausages. Eighteen *Staphylococcus* strains belonging to the species *Staphylococcus xylosus* (5), *S. saprophyticus* (3), *S. equorum* (4), *S. carnosus* (4) and *S. simulans* (2), previously isolated from different types of Southern Italian fermented sausages, were screened for proteinase, aminopeptidase and esterase activities. Most of the staphylococci strains lacked detectable levels of proteinase activity against casein-fluorescein isothiocynate. In the active strains, this activity was extracellular or cell-envelope associated. The studied staphylococci strains also showed low levels of aminopeptidase activities, which preferentially hydrolysed substrates containing L-methionine, L-leucine and L-phenylalanine as N-terminal residue. In contrast, all staphylococcal strains possessed significant activity against short-chain fatty acid esters. The maximum esterase activities were detected in whole-cell suspensions and cell-free extracts and to a lesser extent in the extracellular medium. The substrates preferentially hydrolysed were ρ-nitrophenyl (ρ-NP) butyrate and ρ-NP caprylate and, secondly, ρ-NP palmitate. The extracellular extracts of most of the strains were only active against ρ-NP butyrate except for those of *S. equorum* (SI3, SI4) and *S. simulans* (Ssm12, Ssm21), which also hydrolysed ρ-NP caprylate and ρ-NP palmitate. The cell-free extracts and whole cells were mainly active against ρ-NP butyrate and ρ-NP caprylate, showing activity levels from 1760 U/mg of proteins to 54 U/mg of proteins and from 12,200 U/mg of proteins to 133 U/mg of proteins respectively. These activities were especially high in the strains that belonged to *S. xylosus* and *S. equorum* species. The diversity of the studied metabolic properties and, especially, the esterase activities in different staphylococcal species and even strains of the same species emphasize the relevance of these in vitro characterization studies for a rational selection of new starter cultures.
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Keywords: Staphylococcus; Esterase; Protease; Aminopeptidase

1. Introduction

Staphylococcus species are used as starter cultures for dry-cured meat products such as fermented sausages and play important technological roles. These microorganisms primarily contribute to the development and stability of the desired red colour by means of their nitrate reductase activity (Miralles et al., 1996). In addition, they contribute to the development of other organoleptic properties such as texture and flavour (Hammes and Hertel, 1998). These functions are accomplished by specific enzymes involved in the metabolism of proteins and lipids. The activity of both muscle and microbial enzymes (protease and lipase) during the processing of dry fermented sausages contributes to the generation of peptides, free amino acids and free fatty acids (Montel et al., 1998; Toldrá et al., 2001). These compounds are known to be involved in the overall flavour of cured meat products by their taste and also as precursors of other volatile aroma compounds.

Previous studies have demonstrated that the aroma of fermented meat products can be modulated by the presence of different *Staphylococcus* spp. (Berdegué et al., 1993; Stahnke, 1995; Søndergaard and Stahnke, 2002). Particularly, strains of the species *Staphylococcus carnosus* and *S. xylosus* are able to produce low molecular weight compounds, including esters, amino acids, aldehydes, amines and free fatty acids, which have an impact in flavour. During the ripening of meat products the

☆ This paper was presented at the 19th International ICFMH Symposium, Food Micro 2004, Potorož Slovenia, 12-16 September 2004.

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doi:10.1016/j.ijfoodmicro.2006.04.008

relative contribution of the microbial flora is governed by the identity and the number of microorganisms, their intrinsic metabolic activities and the expression of these activities in the product environment. Therefore, the estimation of the role of the staphylococci in such processes requires preliminary studies of their metabolic activities in model conditions in order to identify the strains with higher potential in the catabolism of proteins, amino acids and lipids. Only limited research has been reported on these topics regarding strains of *Staphylococcus* spp. (Miralles et al., 1996; Papamanoli et al., 2002; Mauriello et al., 2004).

The aim of this work was to investigate in vitro the protease and esterase activities of 18 staphylococcal strains isolated from different Italian fermented sausages in order to understand whether they could contribute to the release of short-chain fatty acids, peptides and amino acids and, accordingly, select the most active and technologically adequate strains.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Eighteen *Staphylococcus* strains of the species *S. xylosus* (5), *S. saprophyticus* (3), *S. equorum* (4), *S. carnosus* (4) and *S. simulans* (2), isolated from different types of Southern Italian fermented sausages were included in this study (Table 1). Bacterial strains were routinely grown in BHI broth (Merck, Darmstadt, Germany) at 30 °C for 24 h and then maintained at −80 °C in 15% (v/v) glycerol. For enzymatic assays, the growth medium was inoculated (1%) with overnight culture of each strain, previously sub-cultured twice in BHI broth at 30 °C for 24 h and then incubated at 30 °C for 16 h.

2.2. Preparation of cell suspensions and extracts for enzymatic assays

The culture supernatants were separated by centrifugation (10,000 ×*g*, 10 min, 4 °C), constituting the extracellular extracts (EEs). Then, cell pellets were washed twice in 0.085% (w/v) NaCl, containing 20 mM $CaCl_2$, and suspended in a 1.5% initial volume of 50 mM Tris–HCl, pH 7.0, constituting the whole-cell suspensions (WCs). To prepare cell-free extracts (CFE), cells were collected as stated above, washed twice in 50 mM Tris–HCl pH 7.0 and suspended in a 1.5% initial volume of 50 mM Tris–HCl, pH 7.0. Then, an equivalent volume of glass beads (0.5-millimetre diameter; Sigma, St. Louis, MO) was added to the cell suspension and cell disruption was carried out in an mini-Bead-Beater (Biospec Products, Washington, N.C.) by six shakings for 30 s each with 1-minute intervals on ice. The unbroken cells and debris were removed by centrifugation (20,000 ×*g*, 10 min, 4 °C) and the supernatant obtained constituted the CFE.

2.3. Assay of proteinase and aminopeptidase activities

Proteinase activity was determined using casein-FITC type II (Sigma, St. Louis, MO) as the substrate, by a modification of the procedure described by Fadda et al. (1999a,b). The reaction mixture consisted of 70 μl of 50 mM Tris–HCl (pH 7.0) containing 0.4% casein-FITC, and 100 μl of enzyme sample (WC or EE). This mixture was incubated at 37 °C for up to 20 min. The resulting fluorescence was measured in a multiscan fluorimeter (Fluoroskan II; Labsystems, Helsinki, Finland) at 485 and 538 nm as excitation and emission wavelengths, respectively. One unit of activity was defined as the amount of enzyme hydrolysing 1 nmol of substrate per h at 37 °C. Proteinase activity was expressed as units per millilitre of culture supernatant or per milligram of protein.

Aminopeptidase activity was measured against different aminoacyl-7-amido-methyl coumarin (AMC) derivative (L-Met-, L-Arg-, L-Leu-, L-Lys-, L-Phe-, L-Ala- and L-Val-AMC; Sigma, St. Louis, MO), essentially as described by Sanz and Toldrá (2001). The reaction mixture consisted of 250 μl 50 mM Tris–HCl (pH 7.0), containing 0.1 mM aminoacyl-AMC, and 50 μl enzyme sample (WC or CFE). The resulting fluorescence was measured after 10 min of incubation at 37 °C in a multiscan fluorimeter at 360 and 440 nm of excitation and emission wavelengths, respectively. One unit of activity was defined as described above, and the aminopeptidase activity was expressed as units per milligram of protein.

2.4. Assay of esterase activity

Esterase activity was determined using three ρ-nitrophenyl esters (ρNP) (ρNP-butyrate, ρNP-caprylate and ρNP-palmitate; Sigma, St. Louis, MO) as substrates. Solutions of 0.8 mM ρNP substrates were prepared in isopropanol: 0.1 mM phosphate buffer, pH 7.0, (1:10) containing 2% (v/v) Triton-X-100. The reaction mixture consisted of 250 μl ρNP substrate solution and

Table 1
List of strains used in this study

Species	Strain	Origin [a]	Source [b]
S. xylosus	SS4	DSA	SoCG
	66K3	DSA	SoCR
	DS18	DSA	NTS
	BS1	DSA	NTS
	20266	DSM	
S. saprophyticus	51K3	DSA	SoCR
	ES1	DSA	NTS
	5SE7	DSA	SoCR
S. equorum	SI3	DSA	SoCG
	SI4	DSA	SoCG
	SE4	DSA	SoCG
	SS9	DSA	SoCG
S. carnosus	SC28	DSA	SaCVD
	SC31	DSA	SaCVD
	SC54	DSA	SaCVD
	SC55	DSA	SaCVD
S. simulans	Ssm12	DSA	SaCVD
	Ssm21	DSA	SaCVD

[a] DSM: Deutsche Sammlung von Mikroorganismen und Zelkulturen, Braunschweig, Germany; DSA: Dipartimento di Scienze degli Alimenti, Università degli Studi di Napoli "Federico II".

[b] NTS: Naples-Type Salami; SoCG: "Soppressata" from Campania Region (Gioi); SoCR: "Soppressata" from Campania Region (Ricigliano); SaCVD: "Sausage" from Campania Region (Vallo di Diano).

40 μl of enzyme sample (WC, EE and CFE). The mixtures were incubated at 30 °C for 15 min. The release of ρ-nitro-phenol was measured at 415 nm in a 550 Microplate Reader (Bio-Rad, Hercules, CA). One unit of activity was defined as the amount of enzyme releasing 1 nmol of ρ-nitro-phenol per h at 30 °C. Esterase activity was expressed as units per milligram of protein for WC and CFE or units of OD_{600} nm for EE.

2.5. Determination of protein concentration

The protein concentration was determined by the bicinchoninic acid (BCA) method with BCA protein assay reagent (Pierce, Rockford, Ill.). Bovine serum albumin was used as the standard.

Whole-cell suspensions of the *Staphylococcal* stains were treated with NaOH 1 N, incubated at 90 °C for 5 min and centrifuged at 10,000 ×*g* for 10 min at 4 °C. The supernatants obtained were used for protein quantification.

50 μl of each sample (CFEs and WC supernatants) was pipetted in replicate into microplate wells containing 200 μl of the BCA working reagent (50:1 (v/v); reagent A: reagent B) (Pierce, Rockford, Ill.). The plates were covered, incubated at 60 °C for 15 min and after incubation cooled to room temperature. The absorbance was measured at 560 nm on a plate reader.

3. Results

3.1. Proteinase and aminopeptidase activities

The proteinase and aminopeptidase activities of the studied *Staphylococcus* strains are shown in Tables 2 and 3, respectively. Only 6 out of the 18 assayed strains showed proteinase activity in their EEs and only 2 strains (Ssm12 and Ssm21) of *S. simulans* in WCs. The highest activities were found in culture supernatants (EEs) of *S. xylosus* SS4 and 66K3 and *S. carnosus* SC54 (Table 2). The CFEs of most of the staphylococci strains lacked detectable levels of aminopeptidase activity or showed very low activity (data not shown).

Instead, the aminopeptidase activity of WCs of the tested strains had broad substrate specificity, hydrolysing basic, hydrophobic and aromatic amino acids (Table 3). Only *S. saprophyticus* ES1 and *S. equorum* SI4 did not hydrolyse any of the tested substrates. The substrates containing L-methionine, L-leucine and L-phenylalanine were hydrolysed at the highest rates, while those containing L-arginine, L-lysine and L-valine were hydrolysed at the lowest rates. *S. saprophyticus* 51K3 was the only strain able to hydrolyse all the tested substrates except for L-lysine and L-valine and *S. carnosus* SC31 was the one that showed the highest exopeptidase activity against L-leucine-AMC (130.3 U/mg protein).

3.2. Esterase activities

The results of the esterase activities assayed in every subcellular fraction (EE, CFE and WC) are shown in Table 4. The hydrolysis rates of the esterase activities of the studied staphylococci varied according to the carbon chain length of the esterified fatty acid. The EEs, WCs and CFEs of strains of the different staphylococci species preferentially hydrolysed ρNP-butyrate.

Table 2
Proteinase activity of *Staphylococcus* strains against FITC-casein

Strains	Activity toward FITC-labeled casein	
	Extracellular extract (EE) (U/ml)[a]	Whole cell (WC) (U/mg of protein)[b]
S. xylosus		
SS4	9.22±0.60	NH[c]
66K3	3.78±0.10	NH
DS18	NH	NH
BS1	NH	NH
20266	NH	NH
S. saprophyticus		
51K3	NH	NH
ES1	NH	NH
5SE7K	0.96±0.05	NH
S. equorum		
SI3	NH	NH
SI4	NH	NH
SE4	NH	NH
SS9	1.90±0.1	NH
S. carnosus		
SC28	NH	NH
SC31	NH	NH
SC54	3.78±1.0	NH
SC55	NH	NH
S. simulans		
Ssm12	NH	0.22±0.01
Ssm21	1.73±0.2	1.82±0.1

The values are the means of results of three different assays±S.D.
[a] (U/ml): units per ml of culture supernatant.
[b] (U/mg): units per milligrams of proteins.
[c] NH: non-hydrolysed.

The hydrolysis rates of activities found in EEs of all strains varied from 54.7 to 678.7 U/O.D against ρNP-butyrate and from 26.7 to 800.0 against ρNP-caprylate. All the strains assayed showed esterase activities against ρNP-butyrate whereas most of staphylococci strains lacked detectable levels of extracellular activities against ρNP-caprylate. In fact, only eight strains (*S. xylosus* DS18, *S. saprophyticus* 5SE7k, *S. equoum* SI3, SI4, SS9, *S. carnosus* SC54 and *S. simulans* Ssm12, Ssm21) showed capability to hydrolyse this ester. In general the extracellular activity against ρNP-butyrate was higher than that found against ρNP-caprylate, except for the strains *S. simulans* Ssm21 and *S. equorum* SI4.

The highest activity against ρNP-butyrate was exhibited for EEs strains of *S. equorum*, in particular by *S. equorum* SI3 and SI4 and by two strains of *S. simulans*. The strain *S. simulans* Ssm12 exerted also higher activity against ρNP-caprylate.

All the strains studied showed WC and intracellular activities (CFEs) against ρNP-butyrate and caprylate, except for CFEs

Table 3
Aminopeptidase activities (U/mg of protein)[a] of *Staphylococcus* strains

Strains	Amino acid–AMC substrate						
	Met	Arg	Leuc	Lys	Phe	Ala	Val
S. xylosus							
SS4	3.02±28.0	NH[b]	4.8±28.24	NH	2.01±2.88	NH	NH
66K3	1.52±16.0	0.09±6.65	1.34±2.97	NH	1.03±13.54	2.61±54.0	0.07±0.08
DS18	0.76±71.64	NH	0.64±5.44	NH	ND[c]	0.4±37.48	NH
BS1	0.5±6.59	NH	1.4±11.34	NH	0.76±5.65	NH	NH
20266	1.83±0.85	NH	4.01±28.72	0.006±23.62	0.33±27.39	0.43±2.69	NH
S. saprophyticus							
51K3	3.76±12.83	0.85±7.32	11.5±19.84	0.13±14.58	4.83±96.85	2.86±118.23	0.12±43.44
ES1	NH	NH	NH	NH	NH	NH	NH
5SE7K	0.029±29.86	NH	NH	NH	NH	NH	1.35±4.48
S. equorum							
SI3	0.07±0.16	0.86±24.52	0.66±3.61	NH	NH	NH	NH
SI4	NH	NH	NH	NH	NH	NH	NH
SE4	0.85±14.40	NH	0.61±20.64	NH	NH	NH	NH
SS9	NH	NH	0.82±4.31	NH	NH	NH	0.80±7.15
S. carnosus							
SC28	0.72±69.65	NH	4.85±21.76	NH	1.94±20.10	NH	NH
SC31	0.58±4.94	NH	130.3±34.50	NH	0.71±9.44	NH	NH
SC54	0.56±2.12	NH	7.42±27.44	NH	3.58±15.77	NH	NH
SC55	0.65±2.29	NH	4.71±23.62	NH	2.04±11.78	NH	NH
S. simulans							
Ssm12	2.63±16.81	NH	1.41±17.31	NH	0.3±4.53	NH	NH
Ssm21	1.4±14.80	NH	1.13±37.35	NH	NH	NH	NH

[a] Aminopeptidase activity was measured against amino acid–AMC substrate and the values are the means of results of three different assays±S.D.*10^{-2}.
[b] NH: non-hydrolysed.
[c] ND: non-determined.

and WCs of the strains of *S. carnosus* SC55, SC28 and for CFEs of the strains of *S. xylosus* 66K3 and *S. carnosus* SC54 that were unable to hydrolyse ρNP-caprylate.

The hydrolysis rate of activities found in WCs of all the assayed strains against ρNP-butyrate and caprylate varied from 380 to 12,200 U/mg and from 133 to 3010 U/mg, respectively. Instead, the intracellular esterase activities varied from 360 to 2810 U/mg against ρNP-butyrate and from 54 to 2780 U/mg against ρNP-caprylate. The results obtained suggested that the esterase activity of WCs of most of strains studied was higher than that found in CFEs, indicating that the responsible enzyme (s) is cell-envelope associated.

The only exceptions were the strains of *S. simulans*, which showed comparable activity levels in the three fractions.

On the other hand, WC suspensions of most *S. xylosus* strains against ρNP-butyrate showed higher levels of esterase activity with *S. xylosus* BS1 exhibiting the maximum level of activity (12,200 U/mg of protein). In general, the esterase activity of WCs of most of the assayed strains against this substrate was higher than that found in CFEs (Table 4). In fact, only two strains of *S. equorum* (SI3, SS9), one of *S. carnosus* (SC54) and one of *S. simulans* (Ssm21) showed higher intracellular esterase activities against ρNP-butyrate than that of WCs with values of 2810, 1090, 460 and 790 U/mg, respectively.

Also against ρNP-caprylate the esterase activity of WCs of most of strains studied was higher than that found in CFEs. WCs suspension of most *S. xylosus* strains against this substrate exerted higher activity than that of other species, which showed an esterase activity strain dependent. The highest activity against this substrate was found in WCs of *S. saprophyticus* 51K3 while the strains of *S. carnosus* showed the lowest ρNP-caprylate hydrolysing activities in both WCs and CFEs.

Against ρNP-caprylate, the CFEs of all *S. equorum* strains, of one strain of *S. simulans* (Ssm21) and of one strain of *S. saprophyticus* (5SE7K) exhibited higher activity than that of WCs and *S. equorum* SI3 was the strain that showed the maximum level of activity (2780 U/mg) against this substrate (Table 4).

The substrate ρNP-palmitate was the one hydrolysed at the lowest rates by esterase activities of the 18 staphylococci (Table 4). The extracellular enzymes released by the studied strains were unable to hydrolyse this substrate. The only exceptions were two strains of *S. equorum* (SI3, SI4) and two strains of *S. simulans* (Ssm12, Ssm21), which also showed higher extracellular hydrolytic activity against ρNP-butyrate. The CFEs of 7 strains and the WCs of 11 strains showed ability to hydrolyse ρNP-palmitate. All strains of *S. equorum* showed activities against this substrate varying from 50 to

Table 4
Hydrolysis of ρ-nitrophenyl esters with different chain length of acids by extracellular extracts, whole cells and cell-free extracts of staphylococci

Strains	ρ-Nitrophenyl esters								
	Butyric	Caprylic	Palmitic	Butyric	Caprylic	Palmitic	Butyric	Caprylic	Palmitic
	Extracellular extracts (U/O.D.)			Whole cells (U/mg of protein)			Cell-free extracts (U/mg of protein)		
S. xylosus									
SS4	54.7±5.8	NH[a]	NH	5580±116.0	810±17.5	NH	770±27.5	210±23.4	4.2±0.78
66K3	132±22.0	NH	NH	4770±41.6	1770±60.4	NH	1260±59.9	NH	170±10.5
DS18	89.3±17.0	26.7±4.7	NH	2210±20.21	580±39.6	110±19.15	1110±39.5	270±27.2	NH
BS1	177.3±72.0	NH	NH	12200±700.0	1040±40.4	140±15.54	1760±73.35	190±11.1	NH
20266	76±19.0	NH	NH	4760±140.5	133±11.5	NH	900±21.44	80±12.5	NH
S. saprophyticus									
51K3	200±20.0	NH	NH	3400±149.4	3010±110.5	NH	920±21.44	54±6.27	NH
ES1	196±19.2	NH	NH	710±109.9	210±23.4	NH	360±40.8	160±15.54	NH
5SE7K	81.3±11.3	35.4±5.24	NH	2000±199.7	180±18.5	120±21.31	1060±60.44	380±23.6	30±2.16
S. equorum									
SI3	537.3±33.1	181.3±9.2	21.2±31.0	1890±70.2	1080±80.8	90±10.41	2810±100.5	2780±160.5	68±6.57
SI4	554.6±49.1	589±39.2	13.1±30.0	3460±238.9	650±51.6	120±21.3	1900±98.6	1320±55.3	NH
SE4	90.7±12.5	NH	NH	2100±115.5	142±18.4	50±9.57	500±31.5	170±10.54	NH
SS9	201±11.0	88±10.4	NH	410±23.6	160±14.40	120±25.3	1090±30.8	250±27.2	NH
S. carnosus									
SC28	124±14.0	NH	NH	580±18.0	NH	71±4.5	460±39.8	NH	NH
SC31	66.7±11.3	NH	NH	3900±114.2	460±39.8	140±15.0	1200±75.0	80±10.5	NH
SC54	144±24.0	29.2±50.0	NH	380±30.6	260±20.2	NH	460±39.8	NH	27±3.53
SC55	145±25.0	NH	NH	600±80.3	NH	NH	510±31.5	NH	35±5.28
S. simulans									
Ssm12	678.7±119.4	800±200.0	130.7±54.0	740±70.23	970±29.7	50±4.6	610±40.6	350±28.8	NH
Ssm21	622.7±109.3	189.3±87.5	186.7±79.0	690±32.1	530±31.5	150±15.54	790±32.79	690±39.6	110±11.41

The values are the means of results of three different assays±S.D.
[a] NH: non-hydrolysed.

120 U/mg, but not in the case of the strains belonging to the remaining species.

4. Discussion

Although coagulase negative staphylococci (CNS) play an important role during the ripening of fermented meat products, preventing the rancidity and contributing to the development of sensory properties by producing flavour and aroma compounds (Berdegué et al., 1993; Hammes and Hertel, 1998; Søndergaard and Stahnke, 2002), few data are available on the proteinase, aminopeptidase and esterase enzymes of these microorganisms. So far, the characterization of lipases of the main staphylococcal species has received the most attention (Talon et al., 1995; Rosenstein and Gotz, 2000; Sayari et al., 2001).

In an effort to understand the distribution, specificity and activity levels of enzymes possibly involved in protein and lipid metabolism, 18 strains belonging to five different species of the genus *Staphylococcus* were screened for proteinase, aminopeptidase and esterase activities.

In this study, to obtain a rapid and simple quantification of proteinase activity of the staphylococcal strains a chromogenic substrate, FITC-casein, has been used according to Fadda et al. (1999a,b). These authors found that lactobacilli strains with caseinase activity were also able to hydrolyse sarcoplasmic and myofibrillar proteins. The levels of caseinase activities of the studied staphylococcal strains were rather low, despite most of the same strains were capable to hydrolyse sarcoplasmic and myofibrillar proteins (Mauriello et al., 2002, 2004; Casaburi et al., 2005) when assayed by the agar plate method according to Fransen et al. (1997). So these results suggest the importance of finding a suitable method for a correct detection of positivity and intensity of properties under examination.

The aminopeptidase activity of the studied staphylococci was higher than the proteinase activity but considerably lower than that previously detected in meat lactobacilli (Fadda et al., 1999a,b; Sanz et al., 1999). However, all staphylococci strains preferentially hydrolysed the substrates containing L-methionine, L-phenylalanine and L-leucine as N-terminal residues, which are known to be involved in flavour. Thus, the release of small peptides and these free amino acids by the action of staphylococcal peptidases could partly contribute to taste as well as to the generation of aroma precursors. Recently, the amino acid L-leucine was proposed to be the possible precursor of the microbial formation of 3-methyl butanol with fermented fruit odours and 3-methyl butanal with malt and cacao odours in sausages (Stahnke, 1995; Montel et al., 1996). In fact, 3-methyl butanol and 3-methyl butanal, two compounds derived from the microbial degradation of L-leucine, have been found in dried sausages (Stahnke, 1994), in Parma ham (Hinrichsen and

Pedersen, 1995) and in Spanish Serrano ham (Flores et al., 1997). Likewise, L-phenylalanine has been proposed as another potential precursor of aroma compounds by microbial conversions, especially in cheese (Lee et al., 1985), whereas L-methionine seems to be the precursor of dimethyl-disulfide, a sulphured compound with garlic and cauliflower odour (Maynier et al., 1999).

It is therefore likely that the application of studied staphylococcal strains as starter cultures for dry fermented sausages could imply improvements in the organoleptic properties of these products by means of their exoproteolytic activity although their contribution will be comparatively less significant than that of lactobacilli.

Most of the assayed staphylococcal strains had considerable esterase activity at least against ρNP-butyrate and ρNP-caprylate. In every case, the increase in the chain length of the fatty acid appeared to exert a negative effect on the hydrolysis rates, although this factor could also modify the accessibility of the substrate to the responsible enzymes when using WCs. Our results were in agreement with those reported by Talon and Montel (1997), who found that the specificity of staphylococcal strains varied according to the length of acid carbon. They showed that the cell-free extracts of *S. xylosus*, *S. saprophyticus* and *S. warneri* strains preferentially hydrolysed ρNP-butyrate whereas esters of C6 and C10 were weakly hydrolysed. In contrast, Curtin et al. (2002) reported that staphylococci, brevibacteria, corynebacteria and brachybacteria from the surface of smear cheese displayed similar activities on all the same three esterase substrates included in this study.

The results obtained in this study indicated that the esterase activity of the strains belonging to different species of staphylococci is probably due to intracellular or cell-enveloped associated enzymes since the maximum activity levels were normally found in WCs and, secondly, in CFEs. However, two *S. equorum* strains showed higher level of activity against ρNP-butyrate in CFEs than in WCs despite the fact that the cell-disruption process would have negatively affected the activity recovery in CFEs. Also, the levels of esterase activities found in the extracellular medium of *S. simulans* strains were similar to those found in WC and CFEs.

Branger and Goulet (1987) and Talon and Montel (1997) studying esterase activities of different staphylococcal species found that this activity against short-chain esters (acetate and butyrate) was prevalently intracellular. Moreover, Talon and Montel (1997) found that the staphylococcal strains hydrolysed esters at high rate between 15 and 25 °C while the decrease of water activity and the acidic conditions showed a negative effect on the hydrolysis, inhibiting it. In particular, at pH 5.0 this activity was strongly inhibited. Instead, Sorensen (1997) studied the lipolysis of pork fat by meat starter culture of *S. xylosus* using pork fat emulsion as a model system, and showed that temperature (10–30 °C), pH (5.1–6.0), NaCl concentration (1–8% w/v), and incubation time (6–21 days) were all significant factors controlling lipolysis of whole cell and extracellular extracts.

The capability of staphylococci to hydrolyse esters (C4 to C16) and release short and medium chain free fatty acids at the environmental conditions of dry fermented sausages could have an influence on taste. Stahnke (2002) reported that acetic, propionic, butanoic, pentanoic and hexanoic acids primarily derived from microbial degradation of pyruvate could also be derived from lipid β-oxidation. Thus, the short free fatty acids may be the precursor of short-straight-chain volatile acids, in particular butanoic and propionic. The high levels of these acids may influence the sour taste of dry fermented sausages (Bruna et al., 2000; Bruna et al., 2001).

In conclusion, the studied staphylococci strains possess aminopeptidase and, significant, esterase activities, which constitute relevant technological traits. The diversity of staphylococci esterases in relation to activity levels, substrate specificity and sub-cellular localization seems to be important among species and even among strains of the same species, emphasizing the relevance of these in vitro characterization studies for a rational selection of new starter cultures.

Acknowledgements

This work was supported by a grant from MiPAF (Ministero delle Politiche Agricole e Forestali) project SIQUALTECA and by grant AGL2001-1141 from CICYT (Spain).

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