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Effect of nitrate and incubation conditions on the production of catalase and nitrate reductase by staphylococci

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Abstract

The objective of this work was to study the production of catalase and nitrate reductase by staphylococci in order to understand their role in lipid oxidation during sausage manufacturing. Catalase and nitrate reductase were measured in resting cells and supernatants of staphylococci grown in different conditions. All staphylococci (except *S. warneri*) synthetized nitrate reductase. In static condition, the synthesis was maximal during exponential growth phase, whereas in shaking condition, the synthesis was maximal at the beginning of stationary phase. The production of nitrate reductase was increased in presence of nitrate, this effect was particularly important for the two *S. carnosus* strains which exhibited the highest activity. For all staphylococci, the production of catalase was maximal at the end of the exponential growth phase. The lowest amount of catalase was produced by *S. warneri* and the highest by *S. carnosus*. Only *S. xylosus* 873 and *S. saprophyticus* 852 released high amounts of catalase in the supernatant growth. Staphylococci produced higher amounts of catalase in shaking conditions. Addition of nitrate in the growth media favoured the synthesis of catalase, with a pronounced effect for *S. carnosus*. Nitrate also favoured the release of catalase.

Keywords: Starter cultures; Staphylococci; Catalase; Nitrate reductase

1. Introduction

Strains of *Staphylococcus*, either added as starter cultures or spoiling the raw material, contribute to the sensory properties of fermented sausages. They are well known for their participation in the reddening of sausage through their nitrate reductase activity (Weber, 1994). They also influence the level of many fragrant compounds from different metabolic origins (Berdagué et al., 1993; Stahnke, 1994; Stahnke, 1995; Montel et al., 1996; Talon and Montel, 1997; Talon et al., 1998). Lipid oxidation generates numerous volatile compounds and staphylococci modify the proportion of the different volatiles. So, sausages inoculated with *Staphylococcus carnosus* or *Staphylococcus xylosus* were characterized by dry cured aroma in relation to a high content of ketones together with other volatiles originating from amino acids, and carbohydrates (Montel et al., 1996). Sausages manufactured with *Staphylococcus warneri* had rancid odours correlated with high content of

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aldehydes (Berdagué et al., 1993). So the balance between all these compounds has to be controlled.

To understand the role of staphylococci in lipid oxidation it is necessary to characterize their antioxidant properties. Catalase degrading hydrogen peroxide is an important antioxidant enzyme. Nitrate reductase produces nitrite that can limit lipid oxidation by three indirect mechanisms that may operate simultaneously (Igene et al., 1985). Nitrite may act by: binding heme and preventing release of catalytic iron, binding non heme iron thus inhibiting catalysis, and/or stabilizing olefinic lipids against oxidation. Few studies are available in the literature on the catalase and nitrate reductase of staphylococci. In fact, catalase has been used to establish immunological relationships between different species of staphylococci (Rupprecht and Schleifer, 1979; Schleifer et al., 1979). The catalase of *Staphylococcus aureus* has been studied to establish its role in virulence (Mandell, 1975), and in resistance to hydrogen peroxide stress (Martin and Chaven, 1987). Concerning nitrate reductase, most papers deal with that of *S. aureus* (Burke and Lascelles, 1975; Burke and Lascelles, 1979), although one is about *S. carnosus* nitrate reductase (Neubauer and Götz, 1996).

The aim of this work was to study the production of nitrate reductase and catalase by different *Staphylococcus* species in order to understand their role in lipid oxidation during sausage ripening.

2. Materials and methods

2.1. Growth conditions of the strains

The following staphylococci were studied: *Staphylococcus carnosus* (833, 836), *Staphylococcus xylosus* (831, 873, 16), *Staphylococcus warneri* (863), *Staphylococcus saprophyticus* (852). All the strains were isolated from dry sausage except *S. xylosus* 16 isolated from cheese (Montel et al., 1996).

Strains were grown in MC media: meat extract 10 g/l (Difco, USA), yeast extract 5 g/l (Difco, USA), NaCl 5 g/l, Na_2HPO_4 1 g/l at pH 7.0, or in MC supplemented with 0.03% (w/v) or 0.2% (w/v) of KNO_3 (E. Merck, Germany).

Two conditions of incubation were realized. In the first one, the strains were inoculated in 250 ml Erlenmeyer flask with 50 ml of media, and incubated in rotary shaker at 150 rpm at 25°C. In the second one, strains were inoculated in 50 ml Erlenmeyer flask fitted to the neck and incubated in static condition at 25°C.

Samples were taken after 2, 4, 6, 8, 10, 12 and 24 h of growth. The cultures were centrifuged at 10 000 g for 15 min at 4°C. Pellets were washed with phosphate buffer (20 mM, pH 7.0) and resuspended in this buffer to have an optical density (OD) of 1.0 at $\lambda = 600$ nm. Pellets and culture supernatants were kept frozen at -20°C before analysis.

2.2. Growth measurement

Growth was measured spectrophotometrically at $\lambda = 600$ nm. The growth of the strains was represented by two values: μ growth rate (h^{-1}) and A max = $\log_{10}$(OD final/OD initial) representing the maximal of population reached.

2.3. Catalase assays

Catalase activity was measured on resting cells and on supernatants according to Aebi's method (Aebi, 1974). 0.2 ml of the sample (resting cells with a OD = 1.0 or supernatant) was mixed with 2.8 ml of a solution of 30 mM H_2O_2 (E. Merck, Germany) prepared in phosphate buffer 0.1 M pH 7.0. The activity was measured during 3 min at $\lambda = 240$ nm. Results are expressed in arbitrary units: μmol/min/ml of cells with OD = 1.0 or μmol/min/ml of supernatant corresponding to a cell density of OD = 1.0.

2.4. Nitrate reductase assays

Nitrate reductase was measured on resting cells according to the method of Burke and Lascelles (1975). Resting cells (0.1 or 0.2 ml) were mixed with 0.1 ml of KNO_3 (0.2 M; E. Merck, Germany), 0.3 ml of benzyl viologen (0.49 mM; Sigma Chemical Co., USA), 0.3 ml of dithionite (0.14 M; Sigma Chemical Co., USA) and phosphate buffer 0.1 M pH 7.0 (1.3 ml). The incubation lasted 30 min at 30°C. Then nitrites formed were assayed as described by Burke and Lascelles (1975). Results are expressed in arbitrary units: μmol/30 min/ml of cells with OD = 1.0.

2.5. Nitrite assays

Nitrite was assayed on supernatants according to the colorimetric method of Daniels et al. (1994). Quantity of nitrite was calculated from standard curves establish from a solution of 0.3 mM $NaNO_2$ (Sigma Chemical Co., USA). Results were expressed as μmol/ml.

2.6. Data analysis

The effects of bacterial strains (7 strains), nitrate (3 levels: 0, 0.03, 0.2%) and incubation conditions (2 levels: static, shaking) on the growth of the strains (μ, A max), on the production of nitrite, of nitrate reductase and of catalase were studied by analysis of variance (ANOVA) on STAT-ITCF (Gouet and Philippeau, 1992). Comparisons between means were made according to Newman-Keuls test ($\alpha = 5\%$).

Table 1
Effect of some factors on the growth of Staphylococci

	μ[a]		A max[b]	
Factors	1[c]	2[d]	1	2
Strains	10.3	**	5.5	***
Incubation	65.5	****	90.5	****
Nitrate	0.0	NS	0.4	NS
Interactions				
Strains × Incubation	13.8	**	0.9	NS
Strains × Nitrate	3.4	NS	1.6	NS
Incubation × Nitrate	3.4	*	0.06	NS
Residual factor	3.4		1.0	

[a] μ: growth rate.
[b] A max: logarithmic increase of population = log (OD final/OD initial) maximal.
[c] 1: % of variance explained by the factors: (SSD of factor/SSD of total variation) × 100, where SSD is the sum of the square of the difference.
[d] 2: Significant levels: NS not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3. Results

3.1. Effect of different factors on the growth of staphylococci

The growth rate (μ) of staphylococci was highly influenced by the conditional incubation (Table 1). It varied also with the strains and a significant interaction was noticed between these two factors. This interaction is shown in Table 2. It was obvious that growth rate was higher in shaking than in static incubations for all the strains (Table 2). Under shaking, the strains *S. xylosus* 873, 16 and *S. saprophyticus* 852 had the highest growth rates. The growth rate depended on the interaction between the factors incubation and nitrate (Table 1). Whatever the strains, the growth rate was improved by the presence of nitrate in the culture medium for the staphylococci incubated in static condition (0% of KNO_3 $\mu = 0.07$; 0.03% or 0.2% of KNO_3 $\mu = 0.11$). In contrast, the presence of nitrate in shaking condition did not influence the growth rate of the strains ($\mu = 0.22$ for 0, 0.03 or 0.2% of KNO_3). There were no differences between the two concentrations of nitrate.

Incubation was also the dominant factor influencing the level of population A max (Table 1). Whatever the other conditions and the strains, the

Table 2
Growth rate (μ) and increase of population (A max) of *S. carnosus* 833, 836, *S. xylosus* 831, 873, 16, *S. saprophyticus* 852 and *S. warneri* 863[a]

Strains	833	836	831	873	16	852	863
Interaction: Strains × Incubation (14 means)							
μ[b] static	0.12 d,e	0.11 d,e	0.10 d,e	0.06 e	0.12 d,e	0.10 d,e	0.07 e
μ shaking	0.18 c,d	0.20 b,c	0.21 b,c	0.30 a	0.30 a	0.26 a,b	0.18 c,d
Factor: Strains (7 means)							
A[c] max	0.98 c	1.17 b	1.25 a,b	1.39 a	1.25 a,b	1.41 a	1.10 b,c

[a] Values are source means from ANOVA. For the 2 ANOVA's independently (growth rate μ and population A max), values with different subscripts differ significantly (Newman-Keuls test, $\alpha = 5\%$).
[b] μ (h^{-1}).
[c] A max = log (OD final/OD initial) maximal.

maximal of population was 3 fold higher in shaking than in static conditions (A max = 1.80 in shaking; A max = 0.65 in static). Independently of the other conditions, the strains *S. xylosus* 831, 873, 16 and *S. saprophyticus* 852 reached the highest level of population (Table 2). The maximal level of population was not influenced by the presence of nitrate in the culture media (Table 1).

3.2. Effect of different factors on the production of nitrite and nitrate reductase by staphylococci

The strain *S. warneri* 863 had no nitrate reductase activity, so it was not included in the statistical analysis.

For all the other strains, the reduction of nitrate to nitrite was almost similar and followed the kinetic of reduction shown in Fig. 1 for *S. carnosus* 833. There were major differences in the time of production of nitrite during incubation in static and in shaking conditions. So at mid-exponential growth phase (4 h, Fig. 1A), the reduction of nitrate (0.03%) was finished in static condition whereas it had not begun in shaking condition (Fig. 1B). Similar differences were noticed for the high concentration of nitrate. This was correlated to different time of synthesis of nitrate reductase in the two conditions (Fig. 2). In static condition, all the strains synthesised nitrate reductase during their exponential growth phase as shown in Fig. 2 for *S. carnosus* 833. Whereas, when they are incubated in shaking condition, the synthesis was maximal at the beginning of stationary phase (Fig. 2). To illustrate the effects of the factor incubation, we have selected two values of nitrite (NI) and nitrate reductase (NAR): one at mid-exponential growth (NIE, NARE) and one at beginning of stationary growth phase (NIS, NARS) (Table 3). It was clear that in shaking incubation, no nitrate reductase activity (NARE) was recorded during the exponential growth phase of the strains and so no nitrite (NIE) was assayed in the supernatant during this step. In static conditions, the NARE was maximal and nitrite (NIE) was already found in the supernatant during exponential growth. To sum up, the factor incubation only modified the time course of synthesis of the nitrate reductase and of course the appearance of nitrite. But if we consider only the maximal value of nitrite and nitrate reductase activity, the factor incubation did not influence significantly the maximal activity of nitrate reductase and the maximal level of nitrite recorded (Table 4).

The maximal level of nitrite depended mainly on the factor nitrate (83.6% of the variance), also on the strains and on the interaction of these two factors (Table 4). It was obvious that whatever the other conditions, the highest levels of nitrite were produced in presence of 0.2% of nitrate (Table 5). Regarding the strains, *S. carnosus* 833, 836 and *S. xylosus* 831, 16 produced the highest quantity, whereas the strains *S. saprophyticus* 852 and *S. xylosus* 873 produced the lowest quantity (Table 5).

The synthesis of nitrate reductase depended on the strains, the presence of nitrate and on the interaction between these two factors (Table 4). For the two strains of *S. carnosus* (833, 836), the synthesis of nitrate reductase was enhanced by the presence of nitrate in the culture media; but there were no differences between the two concentrations of nitrate (Table 5). These two strains had also the highest nitrate reductase activity (Table 5). For *S. xylosus* 831 and 16, there was only a small positive effect of nitrate on the synthesis of nitrate reductase. For *S. xylosus* 873 and *S. saprophyticus* 852 there was no significant effect of nitrate, and these two strains produced in all cases the lowest amount of enzyme, thus they produced the lowest amount of nitrite (Table 5).

3.3. Effect of different factors on the production and on the release of catalase by staphylococci

For all the strains, the production of catalase was maximal at the end of exponential growth phase as shown in Fig. 3 for *S. xylosus* 873. During their growth, staphylococci can release catalase in the culture supernatant (Fig. 3). The release of catalase started at the end of exponential growth and increased during the stationary phase (Fig. 3).

To compare the strains and to characterise the effect of the different factors on the production and on the release of catalase, the maximal value of activity assayed was selected for statistical analysis; the results are summarised in Table 6.

The production of catalase by the cells (CC) depended on the strains, on the factors incubation and nitrate and these two factors interacted with the strains (Table 6). Whatever the conditions, *S. warneri* 863 produced the lowest amount of catalase, *S.*

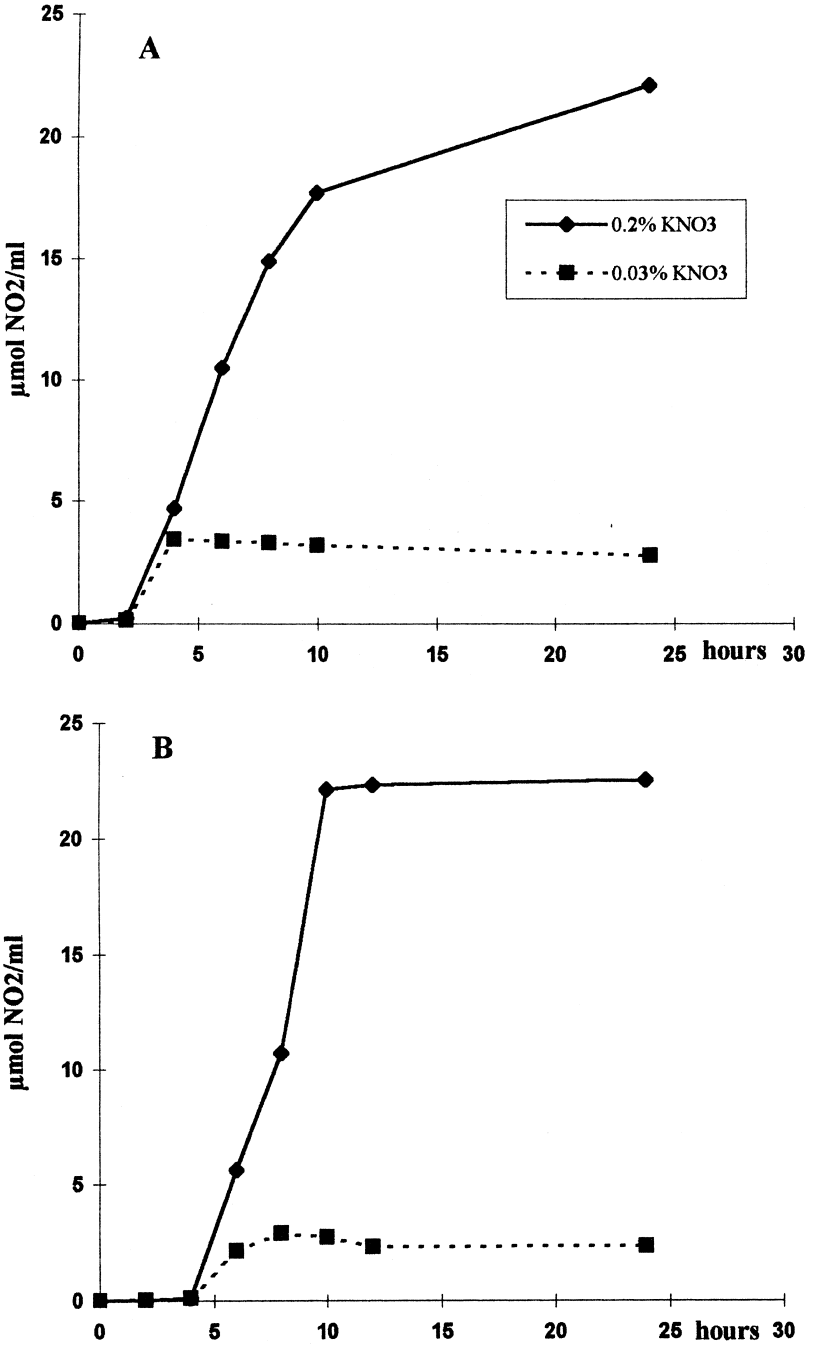

Fig. 1. Production of nitrite by *S. carnosus* 833 during the growth in static condition (A) and in shaking condition (B).

carnosus 833, 836 produced the highest amount (Table 7). As a general rule, staphylococci produced higher amount of catalase when they are grown in shaking conditions (Table 7). The addition of nitrate in the growth media favoured the production of catalase by the two strains of *S. carnosus*; this was also true for *S. xylosus* 16 but only at high concentration of nitrate (0.2%) (Table 7). Nitrate had no

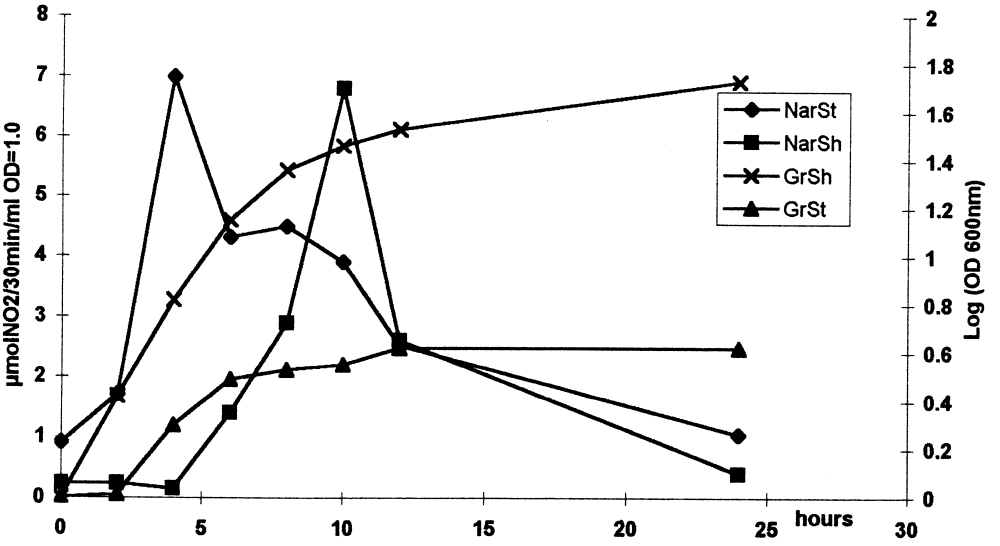

Fig. 2. Growth and nitrate reductase activity of *S. carnosus* 833 incubated in static or in shaking conditions with 0.03% of nitrate. GrSt: growth in static condition; GrSh: growth in shaking condition; NarSt: nitrate reductase in static condition; NarSh: nitrate reductase in shaking condition.

Table 3
Effect of incubation conditions on the quantity of nitrite at the mid-exponential growth phase (NIE) and at the stationary growth phase (NIS) and on the nitrate reductase activity at the mid-exponential growth phase (NARE) and at the stationary growth phase (NARS)[a]

	NIE[b]	NIS[b]	NARE[b]	NARS[b]
Factor incubation[c,d]	32.3%, ****	0.2%, NS	41.4%, ****	17.3%, **
Static	2.96 a	8.79	2.35 a	0.94 b
Shaking	0.25 b	9.68	0.27 b	2.10 a

[a] Values are source means from ANOVA; values with different subscripts differ significantly (Newman-Keuls test, $\alpha = 5\%$).

[b] NIE, NIS: quantity of nitrite expressed in µmol/ml of supernatant, NARE, NARS: activity of nitrate reductase expressed in µmol/30 min/ml of cells with OD = 1.0.

[c] % of variance explained by the factors: (SSD of factor/SSD of total variation) × 100 where SSD is the sum of the square of the difference.

[d] NS not significant; ** $p < 0.01$; **** $p < 0.0001$.

effect on the catalase production by *S. xylosus* 831, 873, *S. saprophyticus* 852 and *S. warneri* 863 (Table 7).

The release of catalase in the supernatant (CS) depended mainly on the factor strains (59% of the variance) and on its interaction with the factors incubation and nitrate (Table 6). Regarding the strains, only two released high amount of catalase: *S. xylosus* 873 and *S. saprophyticus* 852, with *S. xylosus* 873 releasing the highest amount (Table 8). Whatever the conditions, the strains *S. xylosus* 831, 16 and *S. warneri* 863 released small amounts of catalase (Table 8). For *S. xylosus* 873, the release was higher in shaking than in static conditions whereas for *S. saprophyticus* 852 it was the opposite (Table 8). For *S. carnosus* 833, 836, *S. xylosus* 873 and *S. saprophyticus* 852, the release was increased by the presence of nitrate (Table 8). The highest release of catalase was recorded with 0.2% of nitrate for *S. carnosus* 836 and for *S. saprophyticus* 852.

Table 4
Effect of some factors on the production of nitrite and nitrate reductase by Staphylococci

	NI[a]		NAR[a]	
Factors	1[b]	2[c]	1	2
Strains	5.3	****	50.8	****
Incubation	0.2	NS	0.6	NS
Nitrate	83.6	****	16.5	**
Interactions				
Strains × Incubation	0.3	NS	5.5	NS
Strains × Nitrate	0.5	****	21.0	*
Incubation × Nitrate	0.6	*	0.3	NS
Residual factor	0.4		5.4	

[a] NI, NAR: maximal quantity of nitrite, maximal activity of nitrate reductase independently of the growth phase.

[b] 1: % of variance explained by the factors: (SSD of factor/SSD of total variation) × 100, where SSD is the sum of the square of the difference.

[c] 2: Significant levels: NS not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

For *S. carnosus* 833 and *S. xylosus* 873, the positive effect of nitrate was noticed at a concentration of 0.03%.

4. Discussion

As facultative anaerobes, growth of staphylococci is better under aerobic conditions. In anaerobic conditions, the presence of nitrate resulted in a higher growth. This result agreed with those of Neubauer and Götz (1996) on *S. carnosus* and those of Burke and Lascelles (1975) on *S. aureus*.

The synthesis of nitrate reductase by staphylococci was influenced by the incubation conditions. In static incubation, the synthesis of the nitrate reductase was maximal during exponential growth phase. In shaking incubation, the synthesis was maximal at the beginning of the stationary phase. This could be explained by the fact that in shaking incubation, staphylococci may consume oxygen during the growth; so when the maximum of population was reached, i.e. beginning of stationary phase, oxygen was limiting and the nitrate reductase was induced. Borch and Molin (1989) had already noticed that the amount of dissolved oxygen decreased, in spite of agitation, during the growth of lactic acid bacteria. Our results on the induction of staphylococcal nitrate reductase synthesis by anaerobiosis are in agreement with the data of the literature, more particularly with those on *S. aureus* (Burke and Lascelles, 1979), on *S. carnosus* TM300 (Neubauer and Götz, 1996) and on *S. xylosus*, *Staphylococcus epidermidis* (Miralles et al., 1996).

Addition of nitrate in the culture media increased around 5 fold the production of nitrate reductase for *S. carnosus*. This result was in agreement with those of Neubauer and Götz (1996) for *S. carnosus* and Burke and Lascelles (1975) for *S. aureus*. However,

Table 5
Effect of nitrate on the production of nitrite (NI) and nitrate reductase (NAR) by *S. carnosus* 833, 836, *S. xylosus* 831, 873, 16 and *S. saprophyticus* 852[a]

	Strains	833	836	831	873	16	852
Interaction: Strains × Nitrate (18 means)							
NI[b]	0% KNO_3	0.00 e	0.10 e	0.00 e	0.05 e	0.00 e	0.03 e
	0.03% KNO_3	2.97 d	2.74 d	3.28 d	2.38 d	2.00 d	2.03 d
	0.2% KNO_3	21.90 a	17.25 a	16.20 a	7.85 c	17.30 a	10.25 b
Interaction: Strains × Nitrate (18 means)							
NAR[c]	0% KNO_3	1.41 c	1.14 c	1.32 c	0.53 c	1.44 c	0.75 c
	0.03% KNO_3	6.78 a	4.92 a	2.38 b,c	0.80 c	2.28 b,c	1.01 c
	0.2% KNO_3	7.09 a	6.11 a	2.49 b,c	0.68 c	2.18 b,c	1.43 c

[a] Values are source means from ANOVA. For the 2 ANOVA's independently (NI and NAR), values with different subscripts differ significantly (Newman-Keuls test, $\alpha = 5\%$).

[b] NI: quantity of nitrite expressed in μmol/ml of supernatant.

[c] NAR: activity of nitrate reductase expressed in μmol/30 min/ml of cells with OD = 1.0.

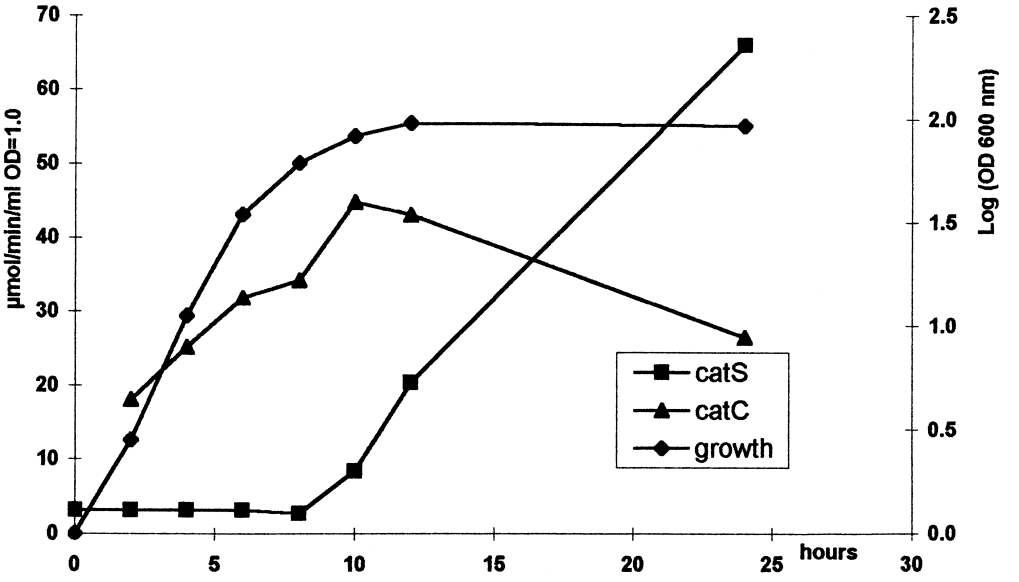

Fig. 3. Growth and catalase activity of *S. xylosus* 873 incubated in shaking conditions with 0.03% of nitrate. CatS: activity of catalase assayed in the growth supernatant; CatC: activity of catalase assayed in the cells.

Table 6
Effect of some factors on the production and on the release of catalase by Staphylococci

	CC[a]		CS[a]	
Factors	1[b]	2[c]	1	2
Strains	31.4	****	58.9	****
Incubation	18.6	****	0.8	NS
Nitrate	8.7	**	2.6	NS
Interactions				
Strains × Incubation	14.0	**	20.3	**
Strains × Nitrate	21.9	**	12.2	*
Incubation × Nitrate	1.0	NS	0.1	NS
Residual factor	4.4		4.9	

[a] CC: catalase activity of the cells, CS: catalase activity in the supernatant.

[b] 1: % of variance explained by the factors: (SSD of factor/SSD of total variation) × 100, where SSD is the sum of the square of the difference.

[c] 2: Significant levels: NS not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

in our study, the effect of nitrate on the synthesis of nitrate reductase by *S. xylosus* was weak and for *S. saprophyticus*, no effect was noticed.

Catalase is mainly cell associated (Loewen, 1996) but some strains excrete catalase in the supernatant (Naclerio et al., 1995). In our study, the seven staphylococci had a cell-bound catalase activity, and only two strains (*S. xylosus* 873 and *S. saprophyticus* 852) released high amount of catalase in the culture supernatant. The release of catalase does not seem to be a common feature for the staphylococci, as Miralles et al. (1996) did not find any catalase activity in the culture supernatant of four strains of *S. xylosus* and one of *S. epidermidis*.

As shown for other species of micro-organisms, the specific activity of catalase of staphylococci increases steadily with cell growth to a maximal level at the onset of, or during stationary phase (Finn and Condon, 1975; Hassan and Fridovich, 1978; Martin and Chaven, 1987).

It has been shown for *E. coli* that synthesis of catalase is co-induced with the respiratory chain components (Hassan and Fridovich, 1978). In aerobic incubation, oxygen is the preferred electron acceptor for the electron transport chain, but in anaerobic incubation, nitrate can induce the synthesis of electron chain components. This was also true for *S. carnosus*, so high level of catalase was synthetised either in aerobiosis, or in anaerobiosis with nitrate.

In conclusion, the staphylococci investigated synthetised catalase and except for *S. warneri*, they

Table 7
Effect of incubation and nitrate on the production of catalase by the cells (CC[a]) of *S. carnosus* 833, 836, *S. xylosus* 831, 873, 16, *S. saprophyticus* 852 and *S. warneri* 863[b]

Strains	833	836	831	873	16	852	863
Factor: Strains (7 means)							
	46.36 a	47.77 a	33.35 b	32.84 b	36.79 a,b	41.49 a,b	16.30 c
Interaction: Strains × Incubation (14 means)							
Static	39.54 a,b	51.85 a	16.65 c	25.79 b,c,	30.07 b,c	25.59 b,c,	12.31 c
Shaking	53.18 a	43.68 a,b	50.05 a	39.90 a,b	43.51 a,b	57.40 a	20.28 c
Interaction: Strains × Nitrate (21 means)							
0%	31.69 c,d	25.18 d	33.66 c,d	34.78 c,d	24.60 d	40.71 a,b,c	15.82 e
0.03%	45.79 a,b,c	64.44 a	36.78 b,c	32.64 c,d	28.51 d	37.24 b,c	18.56 e
0.2%	61.60 a	53.67 a,b	29.61 c,d	31.11 c,d	57.17 a,b	46.53 a,b,c	14.51 e

[a] CC: activity expressed in μmol/min/ml of cells with OD = 1.0.
[b] Values are source means from ANOVA. For the factor strains and the 2 interactions independently (strains × incubation and strains × nitrate), values with different subscripts differ significantly (Newman-Keuls test, $\alpha = 5\%$).

Table 8
Effect of incubation and nitrate on the release of catalase by the cells (CS[a]) of *S. carnosus* 833, 836, *S. xylosus* 831, 873, 16, *S. saprophyticus* 852 and *S. warneri* 863[b]

Strains	833	836	831	873	16	852	863
Factor: Strains (7 means)							
	3.75 c	9.63 c	2.43 c	31.16 a	0.53 c	18.76 b	0.46 c
Interaction: Strains × Incubation (14 means)							
Static	5.64 c	9.30 b,c	1.93 c	15.27 b, c	1.05 c	24.25 b	0.45 c
Shaking	1.86 c	9.96 b,c	2.94 c	47.05 a	0.00 c	13.26 c	0.48 c
Interaction: Strains × Nitrate (21 means)							
0%	0.75 d	2.89 d	2.69 d	25.30 b	0.00 d	12.88 b,c	0.89 d
0.03%	6.72 c	7.06 c	2.41 d	42.62 a	0.99 d	11.78 b,c	0.30 d
0.2%	3.77 d	18.95 b, c	2.20 d	25.26 b	0.60 d	31.61 a,b	0.20 d

[a] CS: activity expressed in μmol/min/ml of supernatant corresponding to a cells density of OD = 1.0.
[b] Values are source means from ANOVA. For the factor strains and the 2 interactions independently (strains × incubation and strains × nitrate), values with different subscripts differ significantly (Newman-Keuls test, $\alpha = 5\%$).

also synthetised nitrate reductase. *S. warneri*, having no nitrate reductase activity and a low catalase activity, inoculated in sausages generated rancid products (Berdagué et al., 1993). On the contrary, *S. carnosus* had high nitrate reductase and catalase activities in anaerobic condition in media with nitrate, conditions encountered in sausage manufacturing. This could be correlated to the low amount of volatiles from lipid oxidation in sausages inoculated with *S. carnosus* (Berdagué et al., 1993). For the three strains of *S. xylosus*, there was a great variability in the level of catalase and nitrate reductase produced, but the level was always above that of *S. warneri*. These *S. xylosus* inoculated in sausage model were able to limit lipid oxidation (Montel et al., 1996). However, other antioxidant enzymes, such as superoxide dismutase could also participate in the inhibition of lipid oxidation. Work is in progress to characterize the synthesis of these enzymes by *S. carnosus* and *S. xylosus*.

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References

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

Berdagué, J.L., Monteil, P., Montel, M.C., Talon, R., 1993. Effects of starter cultures on the formation of flavour compounds in dry sausage. Meat Sci. 35, 275–287.

Borch, E., Molin, G., 1989. The aerobic growth and product formation of *Lactobacillus*, *Leuconostoc*, *Brochothrix* and *Carnobacterium* in batch cultures. Appl. Microbiol. Biotechnol. 30, 81–88.

Burke, K., Lascelles, J., 1975. Nitrate reductase system in *Staphylococcus aureus* wild type and mutants. J. Bacteriol. 123, 308–316.

Burke, K., Lascelles, J., 1979. Partial purification and some properties of the *Staphylococcusaureus* cytoplasmic nitrate reductase. J. Bacteriol. 139, 120–125.

Daniels, L., Hanson, R.S., Phillips, J.A., 1994. Chemical analysis. In: Gerhardt, P. (Ed.), Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, pp. 512–554.

Finn, G.J., Condon, S., 1975. Regulation of catalase synthesis in *Salmonella typhimurium*. J. Bacteriol. 123, 570–579.

Gouet, J.P., Philippeau, G., 1992. Comment interpréter les résultats d'une régression linéaire. In: STAT-ITCF, Institut Techniques des Céréales et des Fourrrages (Eds.), Paris, pp. 1–55.

Igene, J.O., Yamauchi, K., Pearson, A.M., Gray, J.I., 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavor in cured meat. Food Chem. 18, 1–18.

Hassan, M.H., Fridovich, I., 1978. Regulation of synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. 253, 6445–6450.

Loewen, P., 1996. Probing the structure of catalase HPII of *Escherichia coli*, a review. Gene 179, 39–44.

Mandell, G.L., 1975. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. J. Clin. Invest. 55, 561–566.

Martin, S.E., Chaven, S., 1987. Synthesis of catalase in *Staphylococcus aureus* MF-31. Appl. Environ. Microbiol. 53, 1207–1209.

Miralles, M.C., Flores, J., Perez-Martinez, G., 1996. Biochemical tests for the selection of *Staphylococcus* strains as potential meat starter cultures. Food Microbiol. 13, 1–10.

Montel, M.C., Reitz, J., Talon, R., Berdagué, J.L., Rousset-Akrim, S., 1996. Biochemical activities of *Micrococcaceae* and their effects on the aromatic profiles and odours of a dry sausage model. Food Microbiol. 13, 489–499.

Naclerio, G., Baccigalupi, L., Caruso, C., De Felice, M., Ricca, E., 1995. *Bacillus subtilis* vegetative catalase is an extracellular enzyme. Appl. Environ. Microbiol. 61, 4471–4473.

Neubauer, H., Götz, F., 1996. Physiology and interaction of nitrate and nitrite reduction in *Staphylococcus carnosus*. J. Bacteriol. 178, 2005–2009.

Rupprecht, M., Schleifer, K.H., 1979. A comparative immunological study of catalases from coagulase-positive staphylococci. Arch. Microbiol. 120, 53–56.

Schleifer, K.H., Meyer, S.A., Rupprecht, M., 1979. Relatedness among coagulase-negative staphylococci: deoxyribonucleic acid reassociation and comparative immunological studies. Arch. Microbiol. 122, 93–101.

Stahnke, L.H., 1994. Aroma components from dried sausages fermented with *Staphylococcus xylosus*. Meat Sci. 38, 39–53.

Stahnke, L.H., 1995. Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels. Meat Sci. 41, 179–223.

Talon, R., Montel, M.C., 1997. Hydrolysis of esters by staphylococci. Int. J. Food Microbiol. 36, 207–214.

Talon, R., Chastagnac, C., Vergnais, L., Montel, M.C., Berdagué, J.L., 1998. Production of esters by staphylococci. Int. J. Food Microbiol. 45, 143–150.

Weber, H., 1994. Dry sausage manufacture, the importance of protective cultures and their metabolic products. Fleischwirtsch. 74, 278–282.