

# Growth and effect of staphylococci and lactic acid bacteria on unsaturated free fatty acids

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## Abstract

The growth and the effects of four species of staphylococci and six lactic acid bacteria (LAB) of the genus *Carnobacterium*, *Lactobacillus* and *Pediococcus* on unsaturated free fatty acids were studied. The strains were grown in complex medium supplemented either with oleic, linoleic or linolenic acids. Growth was followed and oxidation of the substrates measured by TBARS. The strains of *Staphylococcus xylosus* 873, 16, *Staphylococcus warneri* 863 and *Staphylococcus saprophyticus* grew well on all the substrates. Whereas, the growth of the two strains of *Staphylococcus carnosus* and *Staphylococcus xylosus* 831 was inhibited in the media with linolenic acid. The addition of manganese to this media allowed good growth of these strains. All the LAB did not grow well in the media with linoleic acid, but their growth was favoured by addition of manganese to the media. Under our conditions, only linoleic and linolenic acids were oxidised. All the strains had no prooxidant activity. All the staphylococci limited oxidation of linoleic acid and had a small effect on linolenic acid. LAB did not limit oxidation of linoleic acid. With manganese in the media: the oxidation of the sterile controls was delayed for 2 days and then increased; strains of *S. carnosus* and *S. xylosus* inhibited oxidation of linolenic acid; and *Lactobacillus plantarum* and *Pediococcus pentosaceus* limited oxidation of linoleic acid. The two *Carnobacterium*, whatever the conditions, had no antioxidant properties. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Staphylococci; Lactic acid bacteria; Free fatty acids; Oxidation

## 1. Introduction

Lipid oxidation is one of the primary mechanisms of quality deterioration in foods, especially meat products. The changes in quality are manifested by adverse changes in flavour, colour, texture and nutritive value, and the possible production of toxic compounds (Gray, Gomaa & Buckley, 1996; Morrissey, Sheehy, Galvin, Kerry & Buckley, 1998). On the other hand, oxidation is necessary to develop the desirable flavour of dry cured ham or fermented sausages (Chizzolini, Novelli & Zanardi, 1998; Ladikos & Lougovois, 1990).

During sausage processing, several factors influence the rate of lipid oxidation such as composition of raw meats, grinding and adding exogenous components: salt, nitrite, spices, antioxidants (Kanner, 1994). The manufacturing of sausages often includes starter cultures. Bacterial components of starters consist of staphylococci

and lactic acid bacteria (LAB). LAB (*Lactobacillus*–*Pediococcus*) are well known for their acidification of the product (Hammes, Bantleon & Min, 1990). Staphylococci ensure colour development by nitrate reductase activity (Lücke & Hechelmann, 1987), they also contribute to the development of dry sausage aroma by influencing the composition of volatile compounds in the products. In particular, they modulate the level and the nature of volatiles originating from lipid oxidation (Berdagué, Monteil, Montel & Talon, 1993; Montel, Reitz, Talon, Berdagué & Rousset-Akrim, 1996; Stahnke 1994, 1995). However, the antioxidant potential of starter cultures are not well known. Few studies have been conducted on catalase activity of LAB (Engesser & Hammes, 1994) and *Staphylococcus aureus* (Martin & Chaven, 1987). Data are available on the effect of some micro-organisms on the peroxides of lard (Smith & Alford, 1969; Alford, Smith & Lilly, 1971) and on oxidised methyl oleate (Lilly, Smith & Alford, 1970). These authors have shown that *S. aureus* reduced the peroxide values of these substrates. Smith and Alford (1969) also showed that *Lactobacillus casei*

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and *Pediococcus acidilactici* had no effect on the peroxides of fresh lard.

As the role of bacteria on lipid oxidation in meat fermentation is not well documented, we have studied the growth of different staphylococci and lactic acid bacteria on unsaturated free fatty acids and their effect on the oxidation of these acids, to further understand their role in the development of the flavour of cured meat products.

## 2. Materials and methods

### 2.1. Bacterial strains

#### 2.1.1. Staphylococci

*Staphylococcus carnosus* (833, 836), *Staphylococcus xylosus* (831, 873, 16), *Staphylococcus warneri* (863) and *Staphylococcus saprophyticus* (852) were studied.

#### 2.1.2. Lactic acid bacteria

*Carnobacterium divergens* (210), *Carnobacterium piscicola* (545), *Lactobacillus sakei* (206), *Lactobacillus plantarum* (lpl), *Lactobacillus curvatus* (411) and *Pediococcus pentosaceus* (716) were studied.

### 2.2. Growth media

Growth and oxidation were studied in MC media (meat extract 10 g/l; yeast extract 5 g/l; Na<sub>2</sub>HPO<sub>4</sub> 2 g/l; NaCl 5 g/l; glucose 1 g/l; agar 3 g/l) at pH 6 for all strains. After sterilisation, either melted pork fat, oleic, linoleic, or linolenic acid was added to a final concentration of 0.5 g/l.

For the staphylococci, all these substrates were studied in media MC. Linolenic acid was also studied in MC supplemented with manganese MCMn (0.05 g/l).

For the lactic acid bacteria, only linoleic acid was studied in the two media: MC and MCMn (0.05 g/l).

All media were emulsified with an Ultra Turrax T25 homogenizer at 100,000 rpm for 1 min. The media (4 ml) were distributed in small flasks and then inoculated.

The strains were inoculated at approximately 10<sup>6</sup> cells/ml. All samples, inoculated and controls, were incubated at 25°C and samples were analysed after 0, 2, 5, 8, 15 and 20 days.

### 2.3. Analysis

Viable counts of staphylococci were enumerated with Chapman medium, those of lactic acid bacteria with APT medium incubated at 25°C for 48 h. Results are expressed in log of CFU/ml (log N).

Oxidation was evaluated by thiobarbituric acid reactive substances (TBARS) (Lynch & Frei, 1993). Results are expressed in µmol of malonaldehyde/g of lipids.

### 2.4. Data analysis

For staphylococci the experimental design was as follows. The effects of substrates (5 levels: melted pork fat, oleic, linoleic, linolenic and linolenic + manganese), bacterial strains (7 levels) and incubation times (6 levels) on the growth of the bacteria (log N) and on the oxidation of the substrates (TBARS) were studied by variance analysis using STAT-ITCF (Gouet & Philippeau, 1992).

For LAB, the experimental design was as followed. The effects of substrates (2 levels: linoleic and linoleic + manganese), bacterial strains (6 levels) and incubation times (5 levels) on the growth of the bacteria (log N) and on the oxidation of the substrates (TBARS) were studied by variance analysis as described above.

The percentage of variance explained by experimental factors was calculated: % of variance explained = SSD of factor/SSD of the model × 100 (SSD is the sum of the squares of the differences). Comparisons between means were made according to Newman–Keuls test ( $\alpha = 5\%$ ).

## 3. Results

### 3.1. Growth of the staphylococci on the different substrates

The effects of different factors on the growth of staphylococci are summarised in Table 1. Incubation times significantly influenced the growth of the strains. As a general rule, the strains, inoculated at a range from 6 to 6.5 log, grew during the first 2 days of incubation, then their number decreased to the initial number and stayed constant during incubation (Table 2). However, the substrates significantly modified the growth of the strains and a strong interaction between substrates and strains was observed, it explained 31.4% of the variance (Table 1). The growths of *S. xylosus* 873, 16, *S. warneri* 863 and *S. saprophyticus* 852 were similar in all the substrates (Table 2). The growths of the two *S. carnosus* (833, 836) were strongly inhibited in presence of linolenic acid. A similar tendency was seen for *S. xylosus* 831. When manganese was added to the media with linolenic acid, the growth of both *S. carnosus* and *S. xylosus* 831 were improved (Table 2).

### 3.2. Growth of the lactic acid bacteria (LAB) in linoleic media

The effects of different factors on the growth of LAB are summarised in Table 1. The growth was dependent on the strains (26.2% of the variance) and varied during the incubation period (22% of the variance). Also the interaction between these two factors was important (Table 3). Whatever the conditions, only *C. piscicola* and

Table 1  
Effect of different factors on the growth of staphylococci and lactic acid bacteria

Factors	Staphylococci		Lactic acid bacteria	
	% of variance <sup>b</sup>	Significance of levels (2)	% of variance (1)	Significance of levels (2)
Substrates <sup>a</sup>	10.5	****	4.0	*
Strains	3.8	*	26.2	***
Incubation times	7.9	****	22.0	***
Main interactions				
Substrates–strains	31.4	****	13.4	**
Substrates–incubation times	9.1	*	1.2	NS
Strains–incubation times	11.2	NS	22.8	**
Residual factor	23.3		10.3	

<sup>a</sup> Melted pork fat, oleic acid, linoleic acid, linolenic acid and linolenic + manganese for the staphylococci, linoleic acid and linoleic + manganese for the lactic acid bacteria.

<sup>b</sup> The results of the variance analyses are indicated by: (1) the % of variance explained by the factors (SSD of factor/SSD of total variation × 100) and (2) the significance levels \*,  $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ; NS, non significant.

Table 2  
Effect of substrates on the growth (log N) of the staphylococci<sup>a</sup>

Strains	833	836	831	873	16	863	852
Pork fat	7.38a,b	7.60a	6.45 a,b	6.84 a,b	6.80 a,b	6.99 a,b	7.17 a,b
Oleic acid	7.43 a,b	7.31 a,b	6.61 a,b	6.88 a,b	6.66 a,b	6.56 a,b	6.84 a,b
Linoleic acid	7.67 a	7.45 a,b	6.65 a,b	6.71 a,b	5.97 b,c	6.90 a,b	6.65 a,b
Linolenic acid	3.92 a	7.45 a,b	6.65 a,b	6.71 a,b	6.23 a,b	7.41 a,b	7.16 a,b
Linolenic + Mn	6.81 a,b	6.86 a,b	8.82 a	6.59 a,b	6.59 a,b	ND	ND
Initial value	6.44	6.53	6.10/7.20*	5.87	6.00	6.10	6.21

<sup>a</sup> Values are source means from AVOVA; a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ); strains: *S. carnosus* 833, 836; *S. xylophilus* 831, 873, 16; *S. warneri* 863; *S. saprophyticus* 852; ND: not done; Mn: manganese; Initial value: means of inoculation for all the substrates, \*7.20 initial inoculation of the media linolenic + Mn for the strain 831.

Table 3  
Kinetic growth (log N) of the lactic acid bacteria<sup>a</sup>

Strains	<i>C. divergens</i>	<i>C. piscicola</i>	<i>L. sakei</i>	<i>L. curvatus</i>	<i>L. plantarum</i>	<i>P. pentosaceus</i>
0 days	6.28 a,b,c	5.00 b,c,d	6.38 a,b,c	7.81 a,b	7.38 a,b,c	7.54 a,b,c
2 days	6.53 a,b,c	7.95 a	5.77 a,b,c	6.88 a,b,c	7.60 a,b	7.52 a,b,c
5 days	6.55 a,b,c	6.89 a,b,c	5.16 b,c,d	5.64 a,b,c	7.02 a,b,c	7.09 a,b,c
8 days	5.90 a,b,c	6.86 a,b,c	4.32 c,d	4.65 b,c,d	6.07 a,b,c	6.69 a,b,c
15 days	5.36 a,b,c,d	6.68 a,b,c	2.85 d	4.98 b,c,d	4.95 b,c,d	6.77 a,b,c

<sup>a</sup> Values are source means from ANOVA; a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ).

*L. plantarum* grew during the first 2 days of incubation. The population of *C. divergens* and *P. pentosaceus* did not change during incubation. *L. sakei* and *L. curvatus* decreased during incubation, this decrease was more marked for *L. sakei*.

Growth was significantly influenced by the substrate (presence or not of manganese) and this factor interacted with the strains (Table 1). Except for *L. sakei*, all strains grew better in the media supplemented with manganese (Table 4).

### 3.3. Oxidation of the substrates in the presence of different staphylococci

Results on the main factors involved in the oxidation of substrates are presented in Table 5. It is clear that oxidation mainly depended on the substrate. This factor explained 80% of the variance. Also the oxidation of the substrates varied significantly during incubation.

Pork fat and mono-unsaturated fatty acid were poorly oxidised during the incubation periods (Table 6).

Table 4  
Growth (log N) of lactic acid bacteria in the presence of linoleic acid<sup>a</sup>

Strains	<i>C. divergens</i>	<i>C. piscicola</i>	<i>L. sakei</i>	<i>L. curvatus</i>	<i>L. plantarum</i>	<i>P. pentosaceus</i>
Linoleic	5.31 c	6.21 a,b,c	5.64 a,b,c	5.48 b,c	6.08 b,c	7.04 a,b
Linoleic + Mn	6.94 a,b	7.14 a,b	4.15 d	6.51 a,b,c	7.12 a,b	7.20 a

<sup>a</sup> Values are source means from ANOVA; a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ); Mn: manganese.

Table 5  
Effect of different factors on the oxidation of the substrates

Factors	Staphylococci		Lactic acid bacteria	
	% of variance <sup>b</sup> (1)	Significance of levels (2)	% of variance (1)	Significance of levels (2)
Substrates <sup>a</sup>	80.0	****	5.7	**
Strains	1.2	***	14.5	**
Incubation times	3.1	****	45.0	****
Main interactions				
Substrates–strains	1.6	*	8.1	*
Substrates–incubation times	8.0	***	4.3	NS
Strains–incubation times	1.8	NS	13.0	NS
Residual factor	4.0		9.2	

<sup>a</sup> Melted pork fat, oleic acid, linoleic acid, linolenic acid and linolenic + manganese for the staphylococci; linoleic acid and linoleic + manganese for the lactic acid bacteria.

<sup>b</sup> The results of the variance analysis are indicated by: (1) the % of variance explained by the factors (SSD of factor/SSD of total variation  $\times 100$ ) and (2) the significance levels \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; \*\*\*,  $p \leq 0.001$ ; \*\*\*\*,  $p \leq 0.0001$ ; NS, non significant.

Table 6  
Kinetic evolution of the oxidation of the substrates in the sterile control media<sup>a</sup>

Times (days)	0	2	5	8	15	20
Pork fat	3.12 e	2.23 e	3.28 e	3.68 e	3.27 e	3.27 e
C18:1	3.21 e	3.73 e	3.73 e	3.67 e	2.32 e	2.19 e
C18:2	5.12 e	11.92 d	24.85 c	22.40 c	15.61 c,d	13.19 d
C18:2 + Mn	3.63 e	3.66 e	15.71 c,d	30.47 b	34.00 b	ND
C18:3	11.26 d	31.26 b	48.18 a	35.16 a,b	32.77 b	32.01 b
C18:3 + Mn	5.24 e	5.83 e	20.59 c	23.40 c	22.03 c	ND

<sup>a</sup> Oxidation is expressed in  $\mu\text{mol/g}$  of substrate (test TBARS); a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ); ND: not done; Mn: manganese; C18:1: oleic acid; C18:2: linoleic acid; C18:2 and C18:2 + Mn were done with the same lot of substrate; C18:3: linolenic acid; C18:3 and C18:3 + Mn were done with two different lots of substrate.

Tri-unsaturated (C18:3) and di-unsaturated (C18:2) fatty acids were sensitive to oxidation (Table 6). The oxidation of the two poly-unsaturated fatty acids started at the beginning of the incubation and reached a maximal value after 5 days of incubation in the control sterile media without manganese. The decrease in TBARS values after 5 days was certainly due to loss of volatile compounds not measured by this method. However, the presence of manganese in the media influenced the oxidation of linoleic and linolenic acids. Thus in the sterile control with manganese, the oxidation of linoleic and linolenic acids was inhibited during the first 2 days of incubation (Table 6). Afterwards it increased and reached a significant level after 8 days

incubation. The oxidation of linolenic acid in media with or without manganese was different. This was due to manganese but also to the two different lots of substrate, in two trials the oxidation at the beginning of the experiment was already different (Table 6, 11.26 for C18:3 and 5.24 for C18:3 + Mn).

The strain of staphylococci interacted with the oxidation of the substrates (Table 5). All strains had no effect on the oxidation of pork fat or oleic acid (Table 7). However, all species inhibited the oxidation of linoleic acid (C18:2). This inhibition was seen on the second day of incubation and lasted for all the incubation period. With linolenic acid (C18:3), a small inhibition of the oxidation was noticed after 5 days of incubation for the

Table 7  
Effect of the staphylococci on the oxidation of the substrates

Strains	Control	833	836	831	873	16	863	852
Pork fat <sup>a</sup>	3.90 d	2.64 d	2.94 d	2.70 d	2.52 d	2.64 d	2.87c	3.44 d
C18:1 <sup>a</sup>	3.14 d	2.57 d	2.22 d	2.36 d	2.32 d	2.30 d	2.35 d	2.41 d
C18:2 <sup>a</sup>	15.51 c	2.82 d	2.99 d	5.63 d	3.74 d	3.54 d	4.05 d	3.80 d
C18:3								
5 days	48.18 a	30.93 b	31.86 b	28.50 b	36.90 a,b	43.10 a	40.60 a	48.00a
20 days	32.01 b	29.56 b	36.66 b	29.85 b	26.06 b	34.17 b	32.64 b	30.70 b
C18:3 + Mn								
5 days	20.59 c	4.77 d	3.09 c	4.11 d	6.33 d	3.88 d	ND	ND
15 days	22.03 c	2.86 d	3.58 d	2.86 d	4.40 d	3.67 d	ND	ND

<sup>a</sup> Values are sources means from ANOVA; a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ); oxidation is expressed in  $\mu\text{mol/g}$  of substrate (test TBARS); C18:1: oleic acid; C18:2: linolenic acid; C18:3: linolenic acid; C18:3 and C18:3 + Mn were done with two different lots of substrate; Mn: manganese; strains: *S. carnosus* 833, 836; *S. xylosus* 831, 973, 16; *S. warneri* 863; *S. saprophyticus* 852; ND: not done.

two strains of *S. carnosus* 833, 836 and *S. xylosus* 831, 873 (Table 7). However, after 5 days, oxidation was similar in all the samples and followed the kinetics of the control sample (Tables 6 and 7). With manganese in the media, the two *S. carnosus* and the three *S. xylosus* inhibited oxidation of linolenic acid during all the incubation period (Table 7).

### 3.4. Oxidation of linoleic acid in the presence of lactic acid bacteria

Results on the main factors involved in the oxidation of linoleic are presented in Table 5. The oxidation of linoleic varied during incubation (45% of the variance). Oxidation of this acid increased during incubation (Table 6). Lactic acid bacteria and the presence or not of manganese in the media interacted with oxidation of linoleic acid (Table 5).

In the media without manganese, only *L. plantarum* inhibited oxidation of linoleic acid (Table 8). This inhibition lasted during the first 8 days of incubation, but at 15 days the oxidation of linoleic was similar to that of the control (Table 8). For the other lactic acid bacteria, no significant inhibition was recorded during the incubation period. With manganese in the media, after 8 days of incubation not only *L. plantarum* limited the

oxidation, but also *L. sakei*, *L. curvatus* and *P. pentosaceus* (Table 8). But after 15 days, inhibition was significant only for *L. plantarum* and *P. pentosaceus*. The two species of *Carnobacterium* again showed no significant inhibition of oxidation of linoleic acid.

## 4. Discussion

Staphylococci grew on all the substrates except linolenic acid (Table 2). In the presence of this acid, the strains of *S. carnosus* did not grow and lysis occurred. This inhibition of growth was not observed with manganese in the media. The two strains of *S. carnosus* were susceptible to oxidation of fatty acids. This susceptibility was also seen for the strains of *S. xylosus*, but to a lesser extent. On the contrary, the growth of *S. warneri* and *S. saprophyticus* was not affected in oxidised linolenic acid media. Thus, it appears that the different species of staphylococci have different susceptibilities to oxidised media.

Knapp and Melly (1986) found that polyunsaturated fatty acids had bactericidal effects and that the toxicity of these acids increased with increasing unsaturation. Many gram-positive species were susceptible to arachidonic acid. Among the species tested, *S. aureus* and

Table 8  
Effect of the lactic acid bacteria on the oxidation of linoleic acid<sup>a</sup>

Strains	Control	<i>C. divergens</i>	<i>C. piscicola</i>	<i>L. sakei</i>	<i>L. curvatus</i>	<i>L. plantarum</i>	<i>P. pentosaceus</i>
C18:2							
5 days	24.85 a,b	18.17 b	19.84 b	22.29 b	23.00 b	9.62 c	19.31 b
15 days	15.61 b,c	21.44 b	18.20 b	20.36 b	21.00 b	16.04 b,c	20.33 b
C18:2 + Mn							
8 days	30.47 a	20.33 b	20.68 b	5.24 c	7.40 c	5.86 c	3.86 c,d
15 days	34.00 a	31.80 a	35.19 a	14.73 b,c	26.28 a,b	7.25 c	5.96 c

<sup>a</sup> a, b, c, d: statistical groups obtained with the Newman–Keuls test ( $\alpha = 5\%$ ); oxidation is expressed in  $\mu\text{mol/g}$  of substrate (test TBARS); C18:2: linoleic acid; Mn: manganese.

*Lactobacillus acidophilus* were extremely susceptible. These authors concluded that these bactericidal effects of the polyunsaturated fatty acids were mediated by a peroxidative process involving  $H_2O_2$  and iron of bacterial origin.

All the LAB did not grow very well in the media with linoleic acid, they stayed at their original level of inoculation or decreased (Table 3). The growth of the LAB could have been inhibited by this di-unsaturated acid. This result confirms the inhibitory effect of unsaturated fatty acids found by Knapp and Melly (1986).

Manganese favoured the growth of most of the LAB (Table 4). LAB are often fastidious organisms with high nutritional requirements and supplementation of media with manganese is either essential or stimulatory (Kandler & Weiss, 1986). The presence of manganese in spices or starter cultures used to manufacture sausages stimulated the lactic acid fermentation of carbohydrates by LAB (Coventry & Hickey, 1993; Raccach & Marshall, 1985; Zaika & Kissinger, 1984).

Many studies have shown that susceptibility and severity of oxidation depend on the degree and amounts of unsaturated fatty acids (Kanner, 1994; Gray et al., 1996). Our results agree with this observation, as the rate of oxidation decreased in the order linolenic > linoleic > oleic, oleic and pork fat had a degree of oxidation of almost zero under our conditions. In sausage, free fatty acids increased during the ripening process. Linolenic and linoleic acids represent respectively about 1 and 12% of the total free fatty acids (Johansson, Molley, Geenen & Demeyer, 1996) and thus represent a significant percentage that will oxidise during sausage processing.

Staphylococci and LAB interacted with the oxidation of the unsaturated free fatty acids. Neither staphylococci nor LAB had pro-oxidant properties as no increase in oxidation of the free fatty acids was seen. *L. plantarum*, *L. sakei* and *L. curvatus* are able to produce  $H_2O_2$ , which is an oxidation agent (Hammes & Knauf, 1994) and it is often suggested that these LAB could be involved in colour or flavour faults in sausages (Buckenhüskes, 1993; Weber, 1994). However, there is no data clearly indicating that LAB are responsible for these defects.

Staphylococci and some LAB had antioxidant properties. In the linoleic media without manganese, it was clear that staphylococci had a higher capacity to inhibit oxidation than LAB. All the staphylococci strongly inhibited the oxidation during all the incubation period, whereas *L. plantarum* had a small effect (Tables 7 and 8). It is difficult to compare our results to those in the literature because there have been few such studies. However, Lilly et al. (1970) and Alford et al. (1971) have shown that *S. aureus* inhibited the oxidation of fresh lard and decreased the concentration of peroxides in rancid lard but that *Lactobacillus casei* and *P. acidilactici* had no effect.

With manganese (Mn) in the media, oxidation of unsaturated fatty acids was modified. In the sterile control, the oxidation of linoleic and linolenic acids was delayed for the first 2 days of incubation and then increased (Table 6). This can be explained by the fact that simple Mn (II) salts will scavenge superoxide anions ( $O_2^-$ ), and thus, prevent lipid peroxidation (Archibald, 1986; Engesser & Hammes, 1994). However, oxidation of linolenic in the media with Mn was strongly inhibited in the presence of *S. carnosus* and *S. xylosus*, compared to the sterile control (Table 7). These two species had superoxide dismutase (SOD) with Mn as cofactor (Barrière, Montel & Talon, 1998). In the presence of Mn, the synthesis of this enzyme was stimulated, and then the dismutation of superoxide anion increased, thus limiting the oxidation of linolenic acid. Also with Mn, *L. plantarum* and *P. pentosaceus* inhibited the oxidation of linoleic acid during all the incubation period whereas *L. curvatus* and *L. sakei* inhibited the oxidation only during the first 8 days of incubation (Table 8). LAB had no SOD, but crude extracts of *L. plantarum* grown in media with Mn have high levels of  $O_2^-$  scavenging activity. This was due to intracellular Mn in *L. plantarum* scavenging  $O_2^-$  to an extent similar to that of the SOD enzyme (Archibald, 1986). Many other LAB are able to concentrate Mn and so could also scavenge  $O_2^-$ . Moreover, *L. plantarum* and *P. pentosaceus* synthesise pseudocatalase when they are grown with Mn (Hammes & Knauf, 1994). *L. sakei* synthesised a catalase in media with heme, but *L. curvatus* did not exhibit any catalase activity (Hammes & Knauf, 1994). Heme was not added in our experiments, so catalase activity cannot explain the antioxidant property of *L. sakei*.

In conclusion, the different species of staphylococci limited oxidation of linoleic acid whereas LAB were not able to have this effect in media without manganese. But with manganese, the antioxidant properties of *S. carnosus*, *S. xylosus*, *L. sakei*, *L. plantarum*, *L. curvatus* and *P. pentosaceus* were enhanced. More work is necessary to characterise the antioxidant capacities of these 3 genus. At present, work is in progress in our laboratory on the catalase and SOD of *S. carnosus* and *S. xylosus*.

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