# PRODUCTION OF CATALASE AND SUPEROXIDE DISMUTASE BY *STAPHYLOCOCCUS CARNOSUS* AND *STAPHYLOCOCCUS XYLOSUS*

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## BACKGROUND

In dry sausages, lipids, glucids and proteins degradation leads to the formation of volatile compounds. Many of them (up to 60%) come from lipid oxidation (Berdagué *et al.*, 1993). Some of them, such as ketones and secondary alcohols participate to the flavour of the product whereas others, such as alkanes, alkenes and high levels of aldehydes are involved in rancidity. The flavour depends on a subtle balance between all these different compounds. *Staphylococcus carnosus* and *Staphylococcus xylosus* are usually used as starter cultures in sausage manufacturing and they modify the proportion of volatile compounds. In order to improve flavour of sausage, it is important to understand the role of *Staphylococci* in lipid oxidation and then to characterise their antioxidant enzymes. Two antioxidant enzymes are essential by preventing reactive oxygen species accumulation : the catalase and the superoxide dismutase (Sod). These enzymes are still unknown in *S. carnosus* and *S. xylosus*.

## OBJECTIVES

The aim of this work was to characterise the production of catalase and Sod by strains of *S. carnosus* and *S. xylosus*.

## METHODS

**Strains and growth conditions :** *S. carnosus* (833) and *S. xylosus* (C2a) (R. Brückner, Germany) were used. All the cultures were realised in a MC media (meat extract 10 g/l, yeast extract 5 g/l, NaCl 5 g/l and $Na_2HPO_4$ 2 g/l) prepared in phosphate buffer 0.067 M pH 6.

In order to characterise the effect of oxygenation on catalase and Sod synthesis, the strains were grown in different conditions of aeration: high aerobic conditions (cultures were realised in a flask oxygenated by air pressure), aerobic conditions (cultures were grown with shaking at 150 rpm) and facultative anaerobic conditions (cultures were realised in tubes fitted to the neck) with or without $NO_3$ 0.03% or $NO_2$ 0.02%.

The growth of strains was followed by spectrophotometric method at λ 600 nm and expressed as log (final $OD_{600}$/initial $OD_{600}$).

**Preparation of enzymatic extracts :** Cultures of *S. carnosus* (833) and *S. xylosus* (C2a) were harvested by centrifugation at 620 g for 10 min. They were washed in 0.02 M potassium phosphate buffer pH 7, pelleted by centrifugation as before and resuspended in potassium phosphate buffer. Then, they were disrupted by sonication. Unbroken cells were removed by centrifugation at 620 g for 10 min. Cell extracts were ultracentrifugated at 100 000 g for 1 hour. Supernatants constituted the cytosolic fraction (CF) and were stored frozen. Pellets were washed in potassium phosphate buffer and ultracentrifugated at 100 000 g for 30 min. Finally, they were resuspended in potassium phosphate buffer and constituted the membrane fractions (MF).

The protein concentration was determined by the method of Bradford (1976) with bovine serum albumin as the standard.

**Measure of catalase and Sod activities :** Catalase catalyses the conversion of hydrogen peroxide into water and oxygen. Catalase activity was measured on CF and MF of *S. carnosus* (833) and *S. xylosus* (C2a), according to the Aebi's protocol Aebi, 1974). The method consists to measure hydrogen peroxide disappearance with a spectrophotometer at λ 240 nm.

Sod accelerates the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Sod activity was assayed on the CF of *S. carnosus* (833) and *S. xylosus* (C2a) with the SD125 Randox kit, according to the manufacturer's instructions. This method employs xanthine and xanthine oxidase to generate superoxide radicals and then the Sod activity is measured by the degree of inhibition of this reaction with a spectrophotometer at λ 505 nm.

**Visualisation of catalase and Sod activity on polyacrylamide gels :** Potential catalase isoenzymes were separated on a 5% nondenaturing polyacrylamide gel and stained for catalase activity with ferric chloride-potassium ferricyanide (Woodbury *et al.*, 1971). Potential Sod isoenzymes were separated on 10% nondenaturing polyacrylamide gels and stained for Sod activity with nitroblue tetrazolium (Beauchamp & Fridovich, 1971). To identify the type of Sod a duplicate gel was incubated with 15 mM $H_2O_2$ inactivating FeSod.

## RESULTS AND DISCUSSION

### I Growth and kinetic of catalase and Sod production by *S. carnosus* and *S. xylosus* cultivated in high aerobic and facultative anaerobic conditions

Growth of *S. carnosus* and *S. xylosus* cultivated in high aerobic and in facultative anaerobic conditions was followed every two hours. Prelevements were made to prepare enzymatic extracts. On these extracts, catalase and Sod activities were measured.

For the two strains, a catalase activity was detected in the cytosol and in the membranes. *S. carnosus* excreted catalase in the supernatant whereas *S. xylosus* didn't excrete catalase or a few amount (data not shown). By nondenaturing electrophoresis, a major band was detected in cytosolic fractions, respectively at Rf 0.51 and Rf 0.52 for *S. carnosus* and *S. xylosus*. In membrane fractions, a single band was dectected for. *S carnosus* and *S. xylosus* at the same Rf than the bands detected in the cytosolic fraction (data not shown). Catalases are indeed mainly cytosolic (Loewen, 1996) but some are localised in the membranes (Katsuwon & Anderson, 1992) and some strains excrete catalase in supernatant (Naclerio *et al.*, 1995). Catalase seems to be expressed in a growth dependant manner reaching a maximum of production in early growth phase (Figure 1). This is not surprising because catalases are generally synthesised in stationary phase (Finn & Condon, 1975).

For *S. carnosus* and *S. xylosus*, a Sod activity was detected only in the cytosolic fraction. For *S. carnosus*, Sod seemed to be synthesise in early stationary phase but for *S. xylosus*, Sod didn't seem to be expressed in a growth phase dependant manner. By nondenaturing gels, a single band was visualised at Rf 0.58 for *S. carnosus* and at Rf 0.43 for *S. xylosus*. No inhibition of Sod activity was observed for the two strains with $H_2O_2$, indicating that *S. carnosus* and *S. xylosus* doesn't have FeSod (data not shown). As the prokaryotes generally possess a FeSod and/or a MnSod, *S. carnosus* and *S xylosus* probably have a MnSod.

**II Effect of oxygen on catalase and superoxide dismutase production by *S. carnosus* and *S. xylosus***

The effect of oxygen was tested on cells in early stationary phase. The population maximum decreased with anaerobic conditions (Table 1). *S. carnosus* excreted catalase in supernatant in anaerobic conditions. For the *S. carnosus* and *S. xylosus*, there was no important variation of Sod activity in the different conditions of oxygenation. On the contrary, catalase activity in the cytosol and in the membranes decreased with the diminution of oxygen. This induction of synthesis by oxygen has been demonstrated for many other bacteria (Finn & Condon, 1975). For *S. carnosus*, the addition of $NO_3$ or $NO_2$ led to an increase of catalase activity in the cytosol and in the membranes. For *S. xylosus*, it had no effect on catalase activity in the cytosol but increased catalase activity in the membranes.

## CONCLUSION

*S. carnosus* and *S. xylosus* used as starter cultures possess the antioxidants enzymes catalase and superoxide dismutase. These enzymes will contribute to the inhibition of oxidation of unsaturated fatty acids (Talon *et al.*, 1998). To understand their role in flavour, it's important to characterise their conditions of production.


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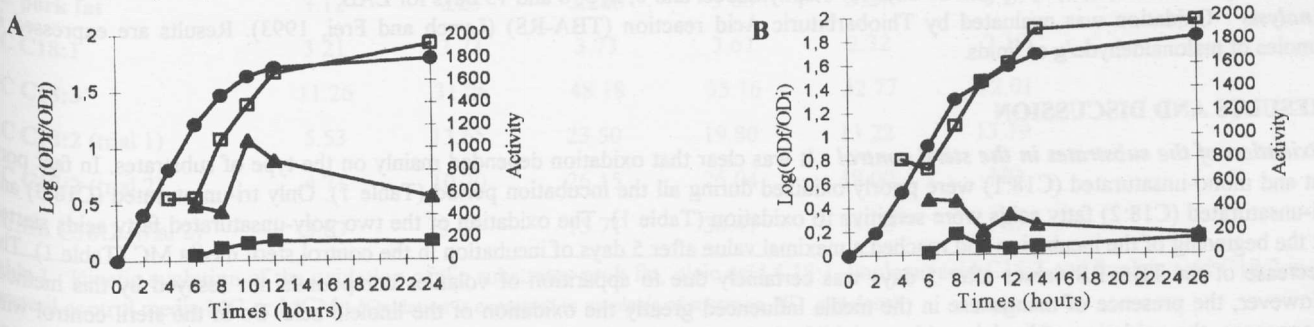
**Figure 1**: Evolution of growth (●), of catalase activity in the cytosolic fractions (□) and in the membrane fractions (■), of superoxide dismutase activity in cytosolic fractions (▲) for *Staphylococcus carnosus* (A) and *Staphylococcus xylosus* (B).




Catalase activity is expressed in µmol/min/mg and Sod activity is expressed in nmol/min/mg.

**Table 1** : Catalase and Sod activities of *S. carnosus* and *S. xylosus* cultivated in different conditions of oxygenation.

| | | High aerobic conditions | Aerobic conditions | Facultative anaerobic conditions | Facultative anaerobic conditions with $NO_3$ | Facultative anaerobic conditions with $NO_2$ |
|---|---|---|---|---|---|---|
| *S. carnosus* | A max | 1.8 | 1.4 | 0.8 | 0.8 | 0.7 |
| | Catalase activity in CF | 1671.0 | 988.8 | 509.4 | 631.3 | 852.3 |
| | Catalase activity in MF | 179.8 | 85.6 | 18.0 | 66.6 | 51.4 |
| | Catalase activity in supernatant | 0.0 | 0.0 | 40.4 | 174.5 | 51.9 |
| | Sod activity in CF | 8.9 | 5.0 | 5.1 | 5.8 | 7.0 |
| *S. xylosus* | A max | 1.7 | 1.6 | 0.5 | 0.6 | 0.4 |
| | Catalase activity in CF | 1902.6 | 593.8 | 276.9 | 333.3 | 274.5 |
| | Catalase activity in MF | 69.1 | 29.2 | 42.8 | 100.0 | 66.7 |
| | Catalase activity in supernatant | 9.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Sod activity in CF | 2.5 | 4.6 | 4.8 | 3.8 | 4.9 |

All the activities are expressed in µmol/min/mg. A max = log (final OD/initial OD). CF and MF mean respectively cytosolic fraction and membrane fraction.