CHARACTERIZATION OF A SUPEROXIDE DISMUTASE-DEFICIENT MUTANT AND A CATALASE-DEFICIENT MUTANT IN *STAPHYLOCOCCUS XYLOSUS*

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Background

Staphylococcus xylosus is an anaerobic facultative bacterium used as starter culture in meat fermented products. It ensures color development by its nitrate reductase activity and protects the cured color by its catalase *activity (Lücke et al., 1986; Talon et al.,1999)*. It also contributes to the typical aroma, mainly by modulating the level and the nature of volatiles coming from lipid oxidation (*Berdagué et al., 1993; Montel et al., 1996; Stahnke 1995a, b, c*). Its antioxidant activities (i.e. catalase and superoxide dismutase) are thought to be involved in these sensorial qualities (*Rozier et al., 1971*). *S. xylosus* contains a single superoxide dismutase, the corresponding gene *sodA* was cloned and sequenced (*Barrière et al., 2000*). It also contains two catalases genes, one gene *katA* was cloned and sequenced (*Barrière et al., 2000*). Two mutants were constructed by inactivation respectively, of the *sodA* gene and the *katA* gene in the chromosome of *S. xylosus*.

Objective

The objective of this work was to characterize the *sodA* and the *katA* mutants obtained and in particular their resistance to oxidative stress.

Methods

The *sodA* mutant and the *katA* mutant were obtained by allelic exchange as described by Brückner (1997). Cells were grown in MC medium (*Talon et al., 2000*) or in chemically defined medium (CDM) (*Hussain et al., 1991*). Growth was monitored by OD_{600} with a spectrophotometer. To test the effect of hydrogen peroxide on cells survival, hydrogen peroxide was added in exponential growth phase. After one hour of challenge, hydrogen peroxide was removed by the addition of catalase (2000 U/ml) and serial dilutions of cultures with physiological water were plated on MC agar.

Results

All the mutants exhibited a normal aerobic growth in MC medium (data not shown). However, the *katA* mutant was more sensitive to hydrogen peroxide than wild type strain (Figure 1). The *sodA* mutant was more sensitive to hyperbaric oxygen (Figure 2) and to paraquat (Figure 3) (an aromatic compound that generates superoxide radicals within cells) because it is unable to detoxify superoxide radicals. In *E. coli*, double mutant *sodA sodB* is unable to grow aerobically if branched-chain amino acids are missing (*Carlioz et al., 1986*). This conditional auxotrophy was explained by the inactivation of an enzyme involved in the biosynthesis of branched-chain amino acids. In *S. xylosus*, the *sodA* mutant exhibited also an impaired growth if branched-chain amino acids are missing (Figure 4).

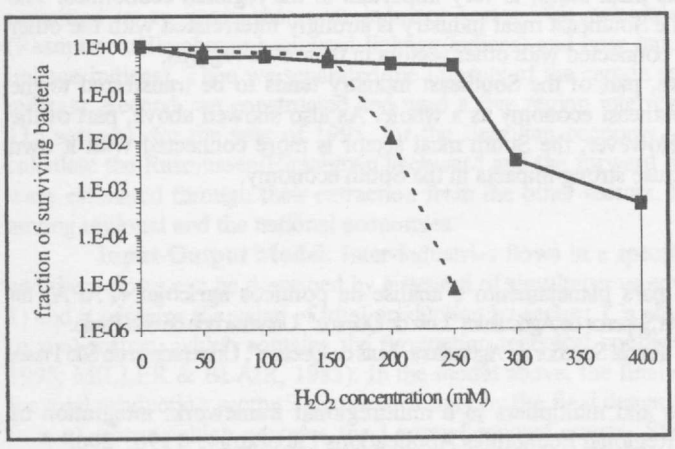

Figure 1: Effect of hydrogen peroxide on survival of the *katA* mutant and wild type strain. Dotted lane: survival of the *katA* mutant; continue lane: survival of wild type strain.

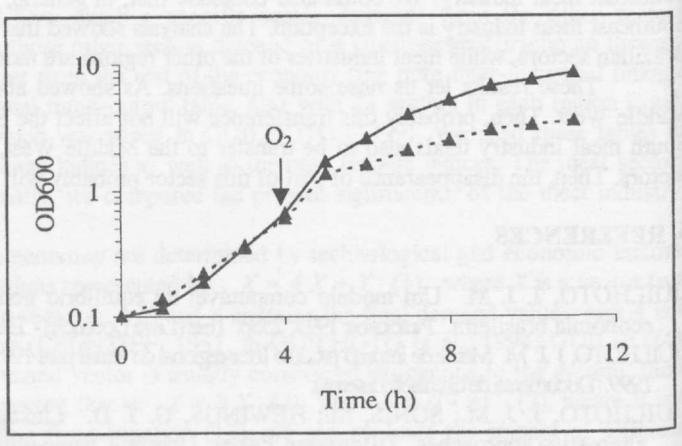

Figure 2: Effect of hyperbaric oxygen on the growth of *sodA* mutant and wild type strain. Dotted lane: growth of the *sodA* mutant; continue lane: growth of wild type strain. Addition of hyperbaric oxygen is indicated by an arrow.

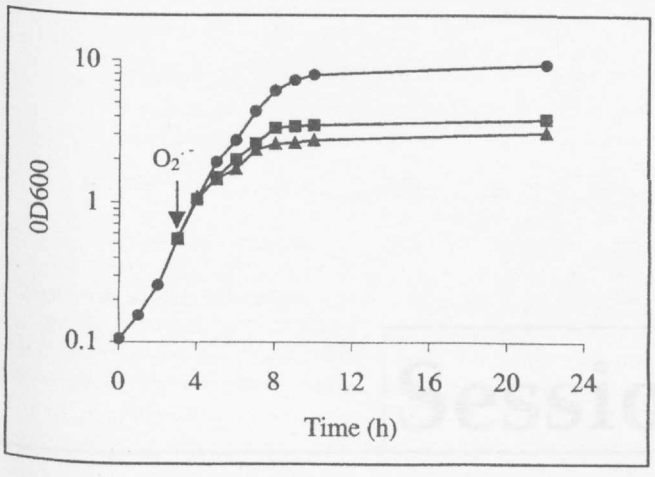

Figure 3: Effect of paraquat on the growth of the *sodA* mutant. Circles: growth without paraquat; squares: growth with 50 µM paraquat and triangles growth with 500 µM paraquat. Addition of paraquat is indicated by an arrow.

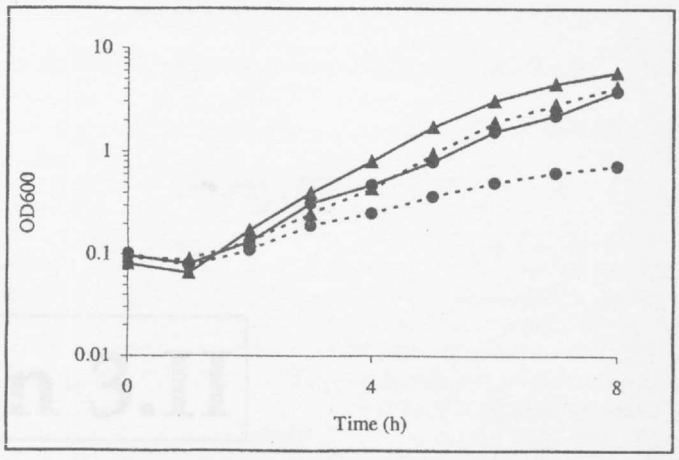

Figure 4: Growth of *sodA* mutant and wild type strain in CDM medium. Dotted lanes: *sodA* mutant; continue lanes: wild type strain. Triangles: growth with 20 amino acids, circles with all amino acids except Leu, Ile and Val.

Conclusion

In conclusion, the *sodA* and the *katA* genes are not essential for aerobic growth but seems to protect cells against oxidative stress.

Acknowledgements

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