INDUCTION OF STRESS PROTEINS IN *LEUCONOSTOC OENOS* TO PERFORM DIRECT INOCULATION OF WINE

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SUMMARY

The enhancement or induction of the protein synthesis was clearly observed in cells of *L. oenos* labeled with $^{35}$S for five proteins during heat shock at 42°C and acid shock at pH 3. Furthermore, no stress protein was induced after exposure of *L. oenos* to ethanol shock 10% (v/v). Moreover, survival of *L. oenos* in wine and ability to perform malolactic fermentation was improved after direct inoculation when cells were pretreated at 42°C.

INTRODUCTION

*Leuconostoc oenos* is a lactic acid bacteria used as starter culture for malolactic fermentation (MLF) in wine and cider. The conversion of L-malic acid into L-lactic acid and CO$_2$ deacidifies wine, which leads to a significant influence on its quality and stability (Davis *et al.*, 1985). However, many factors appear to affect this reaction which presently cannot be controlled. This may lead to a number of processing problems, time consuming and risk of alteration of wine. When the MLF is desired, the addition of bacteria is a general practice (Kunkee, 1991) but cells undergo a rapid death due to the harsh environments (pH between 3.0 and 3.5, presence of ethanol and SO$_2$).

It is also becoming clear that exposure to heat, ethanol or acid stress can afford protection against another often unrelated, hostile environment. This adaptative response requires
stress protein synthesis. *Salmonella typhimurium* (Foster *et al*, 1990) and *Escherichia coli* (Goodson and Rowbury, 1989) can become more acid tolerant following exposure to mild acid conditions.

In this paper, the patterns of proteins which are synthesized in *L. oenos* labeled with $^{35}$S methionine and $^{35}$S cysteine during acid, heat and ethanol stress were examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Our results suggest that *L. oenos* could be adapted to survive and to grow in wine after direct inoculation if cells were preincubated at 42°C.

### MATERIALS AND METHODS

#### Growth conditions.

The parental stain *L. oenos* Lo 84.13 was used for all experiments (Institut d'Oenologie de Bordeaux, France). *L. oenos* was grown at 30°C in FT 80 medium pH 5.3 (Cavin *et al*, 1989). FT 80 was modified by addition of meat extract instead of casamino acids in order to improve the growth. Methionine assay medium (20g/l) (Difco) supplemented with glucose (5 g/l), fructose (5 g/l), tween 80 (1 g/l) and D-L malate (10 g/l) was used for pulse-labeling experiments (MAM20) and preincubation of bacteria before direct inoculation in wine.

#### In vivo protein labeling with $^{35}$S.

The protein labeling method was carried out according to Guzzo *et al* (1991) with important modifications due to the physiological properties of *L. oenos* (well-known for its low growth rate (0.2 h$^{-1}$) and its complex nutritional requirement (Fourcassie *et al*, 1992)). Bacteria were grown in 10 ml of FT 80 to an optical density of 0.4 at 600 nm. The cells were collected by centrifugation at 6,000 x g for 10 min, washed in MAM20 and resuspended in 1 ml of different mediums for labeling experiments: MAM20, pH 5.3, 30°C; MAM20, pH 3.0, 30°C; MAM20, pH 5.3, 30°C, ethanol 10% (v/v); MAM20, pH 5.3, 42°C. After 10 minutes, labeling was carried out with 10 μCi/ml of Trans $^{35}$S label (ICN Biomedicals, Inc) for one hour. All aliquots were centrifuged at 12,000 x g for 10 minutes, and the cell pellets were washed with TE (10 mM Tris HCl, 1 mM EDTA, pH 8).

#### Sample preparation and gel electrophoresis

The cell pellets were lysed in 60 μl of loading buffer and 25 mg of micro-glass beads. This mixture was vortexed for 30 minutes at room temperature and then boiled for 10 minutes. Samples were analyzed by SDS-PAGE according to Laemmli (1970). The separating gel 12% acrylamide was stained with coomassie blue, dried and autoradiographed.

#### Preparation of wines in order to test bacterial survival.

Four different wines were prepared using commercial pasteurized red grape juice (L-malic acid (3.5 g/l); fermentescible sugars (145 g/l); pH 3.4). After addition of 1.5 g/l of L-malic acid, four samples of 2.5 l were dispensed into 4 l flasks. Addition of sucrose and adjustment of pH was made (with 2 M tartaric

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