EFFECT OF PHENOLIC ACIDS ON GLUCOSE AND MALIC ACID METABOLISM OF *LACTOBACILLUS HILGARDII*

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INTRODUCTION

Lactic acid bacteria can interact with wine altering its composition in a way which can, under certain circunstances, be considered beneficial to the quality of the final product. This type of "positive" interaction includes the malolactic fermentation in which, among other reactions, bacteria decarboxylate L-malic acid to L-lactic acid reducing wine acidity. Some strains of wine lactic acid bacteria can also cause wine spoilage, producing unacceptable acetic acid and ethyl acetate levels and other "off-flavours". Of the lactic acid bacteria implicated in "spoilage" conditions, strains of *Lactobacillus* are among the most cited. *Lact. hilgardii* has been identified as a major cause of spoilage in Port wines (Couto & Hogg, 1994)

Phenolic compounds are abundant in wine being extracted from the initial grape material and from wood used for storage. The phenolic composition of wines is very complex and includes phenolic acids in concentrations ranging from 100 to 200 mg l^{-1}, depending on the grape variety and vinification process. Several published studies indicate that some wine phenolic acids have antimicrobial activity against lactic acid bacteria while others can stimulate their growth. (Vivas *et al*., 1997)

In this work, four phenolic acids that occur naturally in wine (*p*-coumaric, caffeic, ferulic and protocatechuic acids), which were previously found to affect growth of *Lact. hilgardii* (Campos *et al.*, 2003), were tested for their effects on glucose and L-malic acid metabolism.

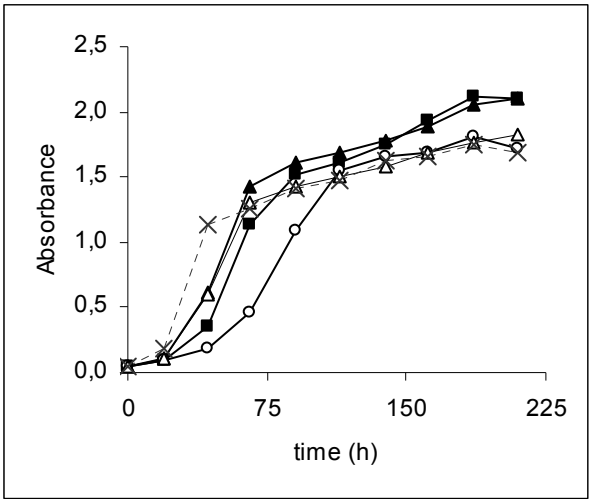
MATERIAL AND METHODS

Lact. hilgardii strain 5, isolated from Port wine was used for being taxonomically representative of the predominant ethanol-tolerant species found in Port wine (Couto & Hogg, 1994). Growth experiments were performed in modified liquid MRS/TJ medium (pH 4.5, 5% v/v ethanol) supplemented with L-malic acid (4.0 g l^{-1}) and with different phenolic acids at 500 mg l^{-1}. Cell growth was monitored spectrophotometrically and glucose and organic acids (malic, lactic and acetic) concentrations were determined by HPLC-UV-IR.

Cultures were grown to late exponential phase, transferred to modified MRS/TJ medium and incubated aerobically at 25ºC for 10 days. During the experiment, samples were collected to monitor growth and concentration of glucose and organic acids.

RESULTS

(a)



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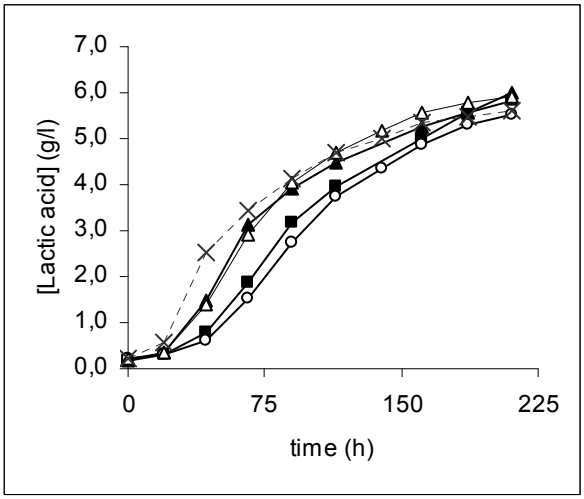
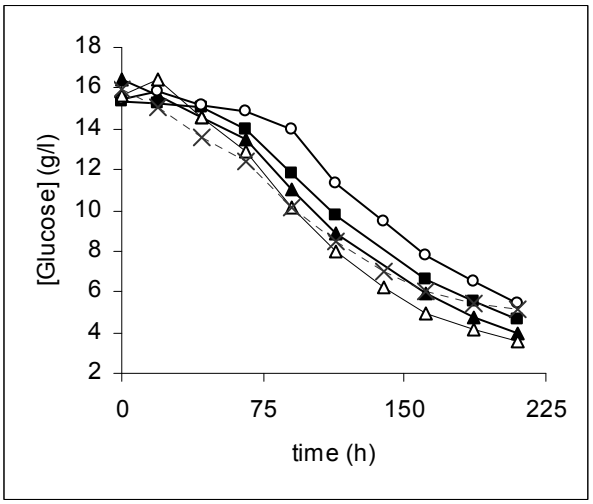
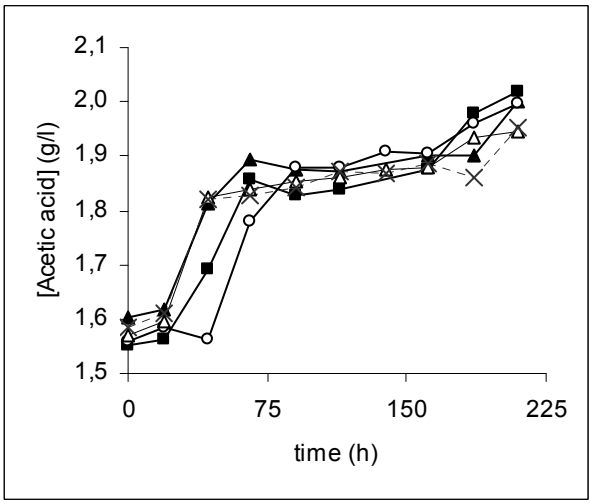


(b)



(e)



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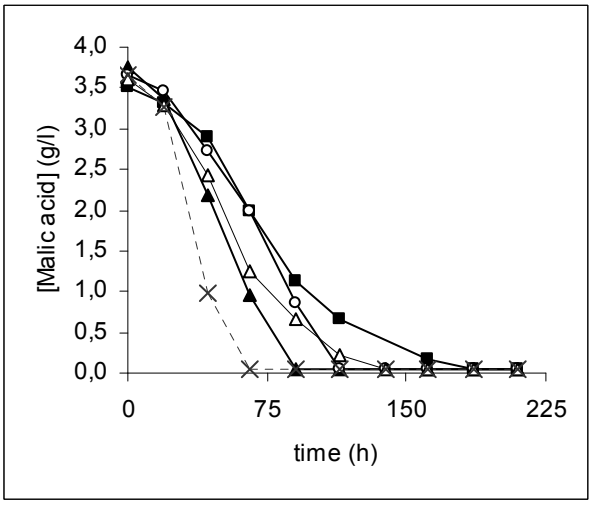

Figure 1 – Growth (a) and metabolism of glucose (b) and organic acids (c-e) by *Lact. hilgardii*5 in MRS/TJ medium (pH 4.5, 5% ethanol) supplemented with 4.0 g l^{-1} L-malic acid and 500 mg l^{-1} phenolic acids; (▲) caffeic acid, (■) ferulic acid (○) *p*-coumaric acid, (▽) protocatechuic acid, (X) control

DISCUSSION

Experimental results obtained indicated that all tested phenolic acids had a negative effect on the progress of the malolactic fermentation , best shown as an extension of the total time to completion, particularly in the cases of ferulic and *p*-coumaric acids (Fig. 1c-1d). *p*-Coumaric demonstrated an inhibitory effect on growth of *Lact. hilgardii* (Fig. 1a). The presence of ferulic and caffeic acids in the growth medium apparently increased the final cell density (Fig. 1a) while extending the time for completion of the malolactic fermentation (Fig. 1c-1d). Glucose consumption was not significatively affected by the presence of phenolic acids in the growth medium, except in the case of *p*-coumaric acid (Fig. 1b). Acetic acid production was delayed by the presence of *p*-coumaric and ferulic acids in the growth medium (Fig. 1e).

These results indicate that the phenolic acids tested influence the malolactic fermentation to a greater extent than the glucose consumption.

REFERENCES

- Campos, F.M., Couto, J. A., Hogg, T.A. (2003) Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*, *Journal of Applied Microbiology,* 94, 167-174
- Couto, J. A. and Hogg, T. A. (1994) Diversity of ethanol-tolerant *lactobacilli* isolated from Douro fortified wine: clustering and identification by numerical analysis of electrophoretic protein profiles. *Journal of Applied Bacteriology* 76, 487-491.
- Vivas, N., Lonvaud-Funel, A. and Glories, Y. (1997) Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. *Food Microbiology*, 14, 291-300