Use of Chemical Probes to Detect Cellular Targets for Phenolic Acids in Wine Lactic Acid Bacteria

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ABSTRACT

Several chemical probes were used to evaluate the effect of *p*-coumaric acid on different cell structures of *Lact. hilgardii 5* and *O. oeni* VF. Minimum Inhibitory Concentrations (MIC) were determined in liquid MRS/TJ medium for each probe. Subsequently, experiments were performed to assess cell recovery after exposure to *p*-coumaric acid in liquid medium supplemented with chemical probes at sub-inhibitory concentrations. The results indicate that sodium chloride, chloramfenicol and rifampicin considerably delayed cellular growth (in comparison with the control assay). These results suggest that besides affecting the cytoplasmic membrane, *p*-coumaric acid could also interfere at the level of protein synthesis.

INTRODUCTION

Lactic acid bacteria (LAB) have a major impact on wine quality, being being responsible for the malolactic fermentation, a natural deacidification process which positively modifies the flavour profiles of certain wine styles. *Oenococcus oeni* is the main species responsible for this fermentation (Dicks *et al*., 1995). On the other hand, some LAB are implicated in wine spoilage. In the case of sweet fortified wines (such as Port), *Lactobacillus hilgardii* is considered to be a major cause of spoilage (Couto and Hogg, 1994).

As a class of compounds, phenolics have particular importance in wine due to their impact on sensory attributes such as colour, bitterness, astringency and aroma, as well as and their antioxidant and antimicrobial properties. Wine phenolic acids (particularly *p*-coumaric acid) are known to exhibit inhibitory activity towards lactic acid bacteria (Campos *et al*., 2003).

Although phenol-derived compounds are known to act at the cytoplasmic membrane level, the mechanism of toxicity of phenolic acids on wine lactic bacteria is not yet fully understood. Chemical probes have been used as indicators of cellular lesions at particular structures (Teixeira, 1995). Despite not being absolutely conclusive, this approach offers some insights on the primary target structures or functions which may be affected by physical or chemical stresses.

In this work, several chemical probes (see Table 1) were used (at sub-lethal concentration levels) in an attempt to identify the main cellular target structures for *p*-coumaric acid in *O. oeni* and *Lact. hilgardii*.

MATERIAL AND METHODS

Bacterial strains:
- *Lact. hilgardii* strain 5 - isolated from contaminated Port
- *O. oeni* Viniflora Oenos – commercial starter culture (Christian Hansen, Hrevidre, Denmark)

Growth conditions
- MRS (de Man, Rogosa and Sharpe) + TJ (Tomato Juice), pH = 4.5, 5% (v/v) ethanol
- Lactic acid bacteria were grown to late exponential phase (4 days, aerobic at 25ºC)

Minimum Inhibitory Concentration (MIC)
- MIC for each probe/strain combination were determined in MRS/TJ using binary dilutions and visual detection of bacterial growth after incubation for 7 days at 25ºC. Sub-inhibitory concentrations were used in the following experiments (Table 1)

LAB recovery following exposure to chemical stress (with *p*-coumaric acid)
- An inactivation experiment (see Fig. 1) was devised using chemical stress with *p*-coumaric acid followed by recovery in MRS/TJ medium without and with chemical probes at the sub-inhibitory levels presented in Table 1.

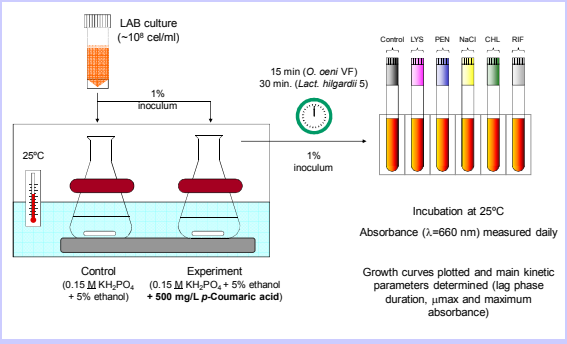

Figure 1 – Inactivation experiment of lactic acid bacteria with p-coumaric acid followed by recovery in MRS/TJ supplemented with different chemical probes (at sub-inhibitory levels).

RESULTS

Table 1 – Minimum Inhibitory Concentrations (MIC) and tested concentrations of chemical probes used in this experiment

			O. oeni VF		*L. hilgardii 5*	
Chemical Probe	**Target structure / function**	**Mechanism of action**	**MIC (mg/L)**	**Tested Conc. (mg/L)**	**MIC (mg/L)**	**Tested Conc. (mg/L)**
Penicillin G	Cell Wall	Inhibits peptoglycan synthesis	3.0	2.2	10	5.0
Lysozyme	Cell Wall	Hydrolyses peptoglycan (NAG-NAM linkages)	113	93	12	9.3
Sodium Chloride	Cell Membrane	Increases osmotic pressure (induces plasmolysis)	32 x 10^3	28 x 10^3	28 x 10^3	24 x 10^3
Rifampicin	RNA Synthesis	Inhibits bacterial RNA polymerase	3.2	2.3	5.5	5.0
Cloramphenicol	Protein Synthesis	Inhibits peptydil-transferases (peptide extension)	3.3	1.3	7.5	2.9

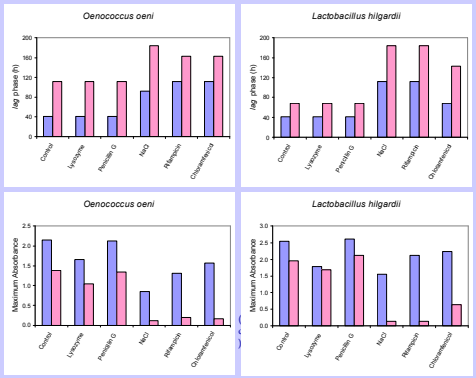

Figure 2 – Influence of different chemical probes on LAB recovery following *p*-coumaric acid exposure; blue bars represent unexposed cells (control) and pink bars represent exposed cells (experiment)

DISCUSSION

- Exposure to *p*-coumaric acid caused an increase in *lag* phase duration and a decrease in maximum absorbance during recovery of both tested bacteria (comparatively to non-exposed cells); no differences were found in μmax of exposed and unexposed cells (results not shown).

- In cells exposed to *p*-coumaric acid (pink bars), the presence of sodium chloride, rifampicin and chloramphenicol in the growth medium delayed the recovery of injured cells of both *O.oeni* and *Lact. hilgardii*; lysozyme and penicillin G, apparently did not have an effect on cell recovery.

- These results suggest that *p*-coumaric acid exposure affects cell membrane integrity and protein synthesis is required to repair or overcome this damage; apparently, the cell wall was not a primary target for this phenolic acid.

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