

## FLUORIMETRIC DETECTION OF CELL MEMBRANE LESIONS IN WINE LACTIC ACID BACTERIA EXPOSED TO PHENOLIC COMPOUNDS

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Some phenolic compounds are known to have membrane-active properties against microorganisms causing extensive leakage of cell constituents such as proteins, nucleic acids, ATP, and inorganic ions (1). The phenolic composition of wines is very complex and includes phenolics acids, which can have antimicrobial properties (2), and aldehydes which can contribute positively to the overall wine aroma (particularly vanillin). In this work, phenolic acids and aldehydes were tested for their effects on the cell membrane of lactic acid bacteria from wine (*Lact. hilgardii* 5 and *O. oeni* VF). A fluorescence bacterial viability kit (LIVE/DEAD BacLight), which is based on cell membrane integrity, was used to differentiate viable and non-viable cells. Cultures of lactic bacteria were centrifuged, washed and exposed to chemical stress with phenolic compounds (in ethanol solutions). Subsequently, cells were dyed with a propidium iodide/SYTO 9 mixture after which the fluorescence of viable cells was measured. The results indicate that changes in cytoplasmic membrane permeability occur in LAB after exposure to phenolic acids and aldehydes. All phenolic acids affected membrane integrity of both bacteria, p-Coumaric acid having the strongest effect. Phenolic aldehydes (especially cinnamaldehyde) caused an increase in membrane permeability of *O. oeni* VF. In *Lact. hilgardii* 5, these compounds apparently had a protective effect against membrane integrity loss caused by ethanol.

### REFERENCES

- [1] Denyer, S. & Hugo, W. B. (1990), Mechanisms of action of chemical biocides, Society of Applied Bacteriology Technical Series n°27, 1st Edition, Blackwell Publishing, London, UK  
[2] 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

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