

Antimicrobial peptides from *Saccharomyces cerevisiae* induce physiological changes in *Hanseniaspora guilliermondii*

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Saccharomyces cerevisiae secretes antimicrobial peptides (AMPs) during alcoholic fermentation that are active against other wine-related yeasts (e.g. *Hanseniaspora guilliermondii*) (Albergaria *et al.*, 2010) and bacteria (e.g. *Oenococcus oeni*) (Osborne and Edwards, 2007). In the present study we assessed the physiological changes induced by those AMPs on sensitive yeast cells of *Hanseniaspora guilliermondii*, namely membrane permeability and intracellular pH (pHi) alterations. Membrane permeability was evaluated by staining cells with propidium iodide (PI) and pHi by the fluorescence ratio imaging microscopy (FRIM) technique (Guldfeldt and Arneborg, 1998). Results showed that after 20 min of incubation with inhibitory concentrations of AMPs, the average pHi of cells dropped from 6.5 to 5.4. After 8 h of incubation, 32% of the cells had lost their Δ pH (=pHi-pHext) and after 24 h that percentage rose to 77%. The culturability (plating) and viability (PI staining) of the sensitive yeast cells also decreased in the presence of the AMPs. After 24 h of exposure to AMPs, 61% of the cells were dead (PI-stained) and the number of viable cells fell from 1×10^5 to 1.5 CFU/ml, which means that virtually all cells (99.999%) became unculturable but a sub-population of 39% of cells remained in a viable but non-culturable (VBNC) state. However, those VBNC cells were able to recover their culturability after incubation at optimal growth conditions. Our study revealed that the mode of action of these AMPs seems to be primarily targeted to the cell membranes, reducing their permeability and preventing cells to maintain pH homeostasis.

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References:

- Albergaria, H., Francisco, D., Gori, K., Arneborg, N., Gírio, F. (2010) *Appl Microbiol Biotechnol* **86**: 965-972.
- Osborne, J.P., and Edwards, C.G. (2007) *Int J Food Microbiol* **118**: 27-34.
- Guldfeldt, L.U., and Arneborg, N. (1998) *Appl Env Microbiol* **64**: 530-534