Poster

Development of a Semicontinuous System to Evaluate the Effects of K2 Toxins on Wine Yeasts

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ABSTRACT

Some yeast species can be infected by viral sequences from the Totiviridae family, which encode toxins (1). These toxins provide a competitive advantage to the yeast carriers by inducing the death of sensitive cells (2). Additionally, "killer" yeasts must possess another auxiliary virus, called LA, responsible for providing the capsid and the replication functions for the killer virus. This study focuses on wine yeasts infected with the M2 killer virus, which encodes the K2 toxin, maintained by the auxiliary virus, L2.

The main objective of this work is to develop and assay a semicontinuous culture system that allows maintaining the culture in its exponential growth phase for as long as possible. This will enable the evaluation of competition between infected and non-carrier yeasts, as well as the effects of these toxins on wild wine yeasts. Since toxins are only active in a narrow range of pH the variation in the pH of the culture, caused by yeast metabolism, must be monitored and controlled using a pH -coupled peristaltic bomb that adds fresh medium to keep cells in proliferative state while keeping pH in the toxin active range.

To assess competitiveness between two yeast strains and observe the behavior and evolution in co-culture, strains must be genetically labeled to distinguish between them, to differentiate between yeasts that produce K2 and those that do not. Our last goal is to select yeast strains suitable for their use in wineries, so we have chosen to label the strains with spontaneous resistance mutations. This strategy allows the marked strain to be used in the food industry without restrictions. The spontaneous generation of mutants is carried out using different protocols, in which various toxins are employed, such as sulfometuron (3), copper sulfate (4), or 2-deoxyglucose (5).

In this poster we present preliminary results on the setup of the pH controlled bioreactor and the analysis of different strategies to isolate yeast spontaneous mutants.

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