Interactions between *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii*: cell-cell contact mechanism

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Several studies have shown that the early death of non-*Saccharomyces* during wine fermentations are due to yeast-yeast interactions induced by *Saccharomyces cerevisiae* (Sc) through different mechanisms such as growth arrest mediated by a cell-cell contact mechanism (Nissen et al. 2003) and death mediated by killer-like toxins (Pérez-Nevado et al 2006; Albergaria et al. 2010). Besides, previous work also showed that death of non-*Saccharomyces* in co-cultivation with Sc is always triggered at the end of exponential growth phase (Pérez-Nevado et al 2006). In order to investigate the role of cell-cell contact in the early death of non-*Saccharomyces*, we performed assays in which Sc cells pre-grown at enological growth conditions for 12 and 48 h, respectively, were in direct contact with *Hanseniaspora guilliermondii* (Hg) cells at high cellular density (10⁷-10⁸ cells/ml) in a carbon-free medium. As a negative control we performed similar assays in which Sc and Hg cells were separated by a dialysis tube (pore cut-off of 1000 kDa) and as a positive control a single Hg culture. Results showed that Hg cell density decreased by 2 orders of magnitude (i.e. from 10⁸-10⁶ cells/ml) in contact with 48 h-grown Sc cells, while its viability remained unchanged (10⁸ cfu/ml) in the presence of 12 h-grown Sc cells. Moreover, Hg viability was not affected both in the dialysis tube experiments and single culture, which confirmed the death-induced cell-cell contact phenomenon.

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