

## RESEARCH ARTICLE

# Proteomics insights into the responses of *Saccharomyces cerevisiae* during mixed-culture alcoholic fermentation with *Lachancea thermotolerans*

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**One sentence summary:** The response of *S. cerevisiae* to cocultivation with *L. thermotolerans* during alcoholic fermentations has been investigated using tandem mass tag-based proteomics combined with flow cytometry.

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## ABSTRACT

The response of *Saccharomyces cerevisiae* to cocultivation with *Lachancea thermotolerans* during alcoholic fermentations has been investigated using tandem mass tag (TMT)-based proteomics. At two key time-points, *S. cerevisiae* was sorted from single *S. cerevisiae* fermentations and from mixed fermentations using flow cytometry sorting. Results showed that the purity of sorted *S. cerevisiae* was above 96% throughout the whole mixed-culture fermentation, thereby validating our sorting methodology. By comparing protein expression of *S. cerevisiae* with and without *L. thermotolerans*, 26 proteins were identified as significantly regulated proteins at the early death phase (T1), and 32 significantly regulated proteins were identified at the late death phase (T2) of *L. thermotolerans* in mixed cultures. At T1, proteins involved in endocytosis, increasing nutrient availability, cell rescue and resistance to stresses were upregulated, and proteins involved in proline synthesis and apoptosis were downregulated. At T2, proteins involved in protein synthesis and stress responses were up- and downregulated, respectively. These data indicate that *S. cerevisiae* was stressed by the presence of *L. thermotolerans* at T1, using both defensive and fighting strategies to keep itself in a dominant position, and that it at T2 was relieved from stress, perhaps increasing its enzymatic machinery to ensure better survival.

**Keywords:** yeast-yeast interactions; flow cytometry; cell sorting; TMT-based proteomics; alcoholic fermentations; proteomic response