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Identification of novel GAPDH-derived antimicrobial peptides secreted by *Saccharomyces cerevisiae* and involved in wine microbial interactions

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Abstract *Saccharomyces cerevisiae* plays a primordial role in alcoholic fermentation and has a vast worldwide application in the production of fuel-ethanol, food and beverages. The dominance of *S. cerevisiae* over other microbial species during alcoholic fermentations has been traditionally ascribed to its higher ethanol tolerance. However, recent studies suggested that other phenomena, such as microbial interactions mediated by killer-like toxins, might play an important role. Here we show that *S. cerevisiae* secretes antimicrobial peptides (AMPs) during alcoholic fermentation that are active against a wide variety of wine-related yeasts (e.g. *Dekkera bruxellensis*) and bacteria (e.g. *Oenococcus oeni*). Mass spectrometry analyses revealed that these AMPs correspond to fragments of the *S. cerevisiae* glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein. The involvement of GAPDH-derived peptides in wine microbial interactions was further sustained by results obtained in mixed cultures performed with *S. cerevisiae* single mutants deleted in each of the GAPDH codifying genes (*TDH1-3*) and also with a *S. cerevisiae* mutant deleted in the *YCA1* gene, which codifies the apoptosis-involved enzyme metacaspase. These findings are discussed in the context of wine microbial interactions, biopreservation potential and the role of GAPDH in the defence system of *S. cerevisiae*.

Keywords Antimicrobial peptides · Wine microbial interactions · Alcoholic fermentation · Biopreservation · Metacaspases · Glyceraldehyde-3-phosphate dehydrogenase

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Introduction

Alcoholic fermentation is the main biotransformation that occurs during winemaking, brewery and fuel-ethanol production. Since these industrial processes are conducted under non-sterile growth conditions, a huge variety of microorganisms is present and can participate in the fermentative process. Although several yeast and bacteria are able to perform alcoholic fermentation, *Saccharomyces cerevisiae* is the dominant microorganism in all those processes, being usually called the "wine yeast". During spontaneous wine fermentations, there is a consistent growth pattern in which the non-*Saccharomyces* species belonging to the natural microflora of grape musts (e.g. *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *Candida stellata*, *Kluyveromyces thermotolerans*, *Kluyveromyces marxianus* and *Torulaspora delbrueckii*) grow during the early stages of fermentation (up to 4–5 % *v*/