**Exploring the Biotechnological Potential of *Starmerella bacillaris* beyond winemaking**

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In the wine industry, where *Saccharomyces cerevisiae* yeast derivatives are commonly used to enhance wine quality attributes, there is still a lack of knowledge regarding the utilization of non-*Saccharomyces* strains derived products. In the first part of this PhD program, different strains of *Starmerella bacillaris,* a non-*Saccharomyces* yeast naturally present on the grape skin surface,were grown in grape must-like conditions. After cell harvesting, cell wall and cytoplasm were separated by means of mechanical lysis. Carbohydrates and protein content of these two fractions were then investigated in comparison with those obtained from *Saccharomyces cerevisiae,* grown in same conditions, spotting key differences between these species, in a framework of evaluating the capabilities of *Starmerella bacillaris* lees recycling for derivatives manufacturing.

***Starmerella bacillaris* come risorsa biotecnologica oltre la vinificazione**

Nell’industria enologica moderna, i derivati di lievito ottenuti da *Saccharomyces cerivisae* sono utilizzati per migliorare gli attributi qualitativi del vino. Tuttavia, poco conosciuto risulta ancora l’impiego di derivati ottenuti da specie non-*Saccharomyces*. Nella prima parte di questo programma di dottorato sono stati propagati in condizioni fermentative diversi ceppi di *Starmerella bacillaris*. Dopo aver collezionato la biomassa, le pareti cellulari e il contenuto citoplasmatico sono stati separati a mezzo di lisi meccanica. La composizione in carboidrati e proteine di queste due frazioni sono state determinate e messe a confronto con quelle ottenute da *Saccharomyces cerevisiae,* propagato nelle stesse condizioni. È stato quindi possibile individuare le principali caratteristiche di *Starmerella bacillaris* per un prossimo sviluppo di derivati di lievito applicabili in enologia

**Key words:** *Starmerella bacillaris*, non-saccharomyces yeast, yeast cell wall, yeast protein extracts

# 1. Introduction

This PhD project funded by PON resources and carried on with the close partnership and economic support of Groupe SOFRALAB (Magenta – France) is focused on yeast derivatives applications in winemaking. Products obtained by yeasts are applied to improve qualitative attributes of wine mimicking in a controlled manner the spontaneousageing on lees process, which has been carried on from decades by wine producers simply maintaining the wine in contact with the leftover of the alcoholic fermentation (Ángeles Pozo-Bayón *et al.,* 2009). Although the use of yeast derivatives in winemaking is currently limited to those derived from *Saccharomyces* *cerevisiae* strains (OIV OENO 576A-2017), there is a growing interest in non-*Saccharomyces* species with the aim of discovering new biotechnological tools for the winemaking process. Additionally, the demand for circular economy practices encourages the reuse of by-products, such as wine fermentation lees in winemaking. For this reason, the work conducted so far focused on the characterisation of lees of *Starmerella bacillaris,* a wine yeast that has shown high physiological variability and desirable technological properties (Raymond Eder and Rosa 2021), in two cellular fractions that finds application in yeast derivatives sector: cell walls and yeast extracts.

# 2. Materials and methods

**Biomass growth and cell harvesting**

A set of 6 strains of *Starmerella bacillaris* (***SB***) and 6 strains of *Saccharomyces cerevisiae* (***SC***) were inoculated at 106 cells/mL in MS300 synthetic must (Bely, Sablayrolles, and Barre 1990) at 200 g/L of glucose and fructose in 1:1 ratio and pH 3,2. Fermentations were performed at 25 °C under agitation for 40 hours. Cell concentration during the growth was monitored with a Cyflow cytofluorimeter (Sysmex Partec, Goerlitz, Germany).

**Cell lysis**

Cell breakage protocol was defined based on works of Avramia & Amariei (2022), with glass beads (425 – 600 µm of diameter from Sigma, St. Louis, MO, USA). After recovery of the liquid fraction, the lysed cells suspension was centrifuged at 5200 g for 30 minutes and supernatant, accounting for soluble cytoplasmic matters, was collected and lyophilized as whole extract (WE); the pellet containing the insoluble yeast cell walls (YCW) was washed for three times and lyophilized.

**Acid hydrolysis and carbohydrates analysis**

***Table 2.*** *Sugar composition of whole extract (WE). Results are expressed as grams per 100 grams of whole extract dry weight.* *Protein content is measured in the same way as BSA equivalent. Different letters stand for significative differences at p<0,001*

To hydrolyse carbohydrates composing the two fractions, freeze-dried material (WE and YCW) was treated according to Dallies et al. (1998) with H2SO4 in vacuum sealed tubes. Analysis of monomers liberated from acid hydrolysis was performed upon derivatization according to Wang et al. (2020). Protein concentration of WE were determined with Bradford assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as calibration standard. Protein visualisation was obtained through SDS-PAGE adapted from Laemmli (1970).

***Table 1.*** *Sugar composition of yeast cell wall (YCW). Results are expressed as grams per 100 grams of cell wall dry weight. Different letters stand for significative differences at p<0,001*

# 3. Results and discussion

**Yeast cell wall (YCW)**

As depicted in Table 1, *SB* resulted to have a cell wall richer in glucosamine and poorer in mannose and glucose, resulting in a global lower sugar concentration. As these three sugars are obtained during the acid hydrolysis starting respectively from chitin, mannoproteins and glucan, it is possible to infer some characteristic of *SB* composition*.* Firstly, the lower relative carbohydrates content comparing to *SC*, mainly driven by glucan content, suggests a cell wall for SB characterized by the higher presence of other components like proteins or lipids*.* Secondly, the chitin and its metabolism seem to play a much important role in the cell wall of this specie. It is possible to hypothesize a relevant role in must chitinase binding capabilities for cell walls of *SB* as this kind of activity has been reported in Chardonnay and model wine as dependent on chitin cellular concentration (Ndlovu, Divol, and Bauer 2018).

**Whole extract (WE)**

The cytoplasmic material of *SB* (Table 2) follows the same trend, except for mannose content. The most noticeable difference in WE composition between species regards however the protein content, where *SB* marks an average +52% in respect to *SC*. Proteins are not only quantitatively different, but on SDS-PAGE those coming from *SB* exhibit a wider molecular size distribution in the range 200 – 31 KDa, this might be of interest to obtain protein-based derivatives with oenological applications. The lower amount of WE glucose in *SB* might be impacted by a lower capability of storing reserve carbohydrates, like glucose and trehalose, in comparison to *SC*.

# 4. References

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