**Use of chitosan from sustainable sources to reduce the sulphur dioxide use in wine production**

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The first three activities of the PhD thesis project are described. Firstly, a screening for SO2 and chitosan resistance was performed among 65 non-*Saccharomyces* strains, belonging to species present in vinification, such as *Metschnikowia pulcherrima*, *Zygosaccharomyces bailii*, *Torulaspora delbruekii*, *Hanseniaspora* spp., *Lachancea thermotolerans*, *Candida zemplinina*, *Pichia* spp. In the second step, selected strains were tested for chitosan resistance in laboratory scale fermentation, whereas in the third step the same strains were evaluated for resistance to commercial and insect-based chitosan on agarized grape must medium.

Utilizzo del chitosano da fonti sostenibili per ridurre l’impiego dell’anidride solforosa nella produzione del vino

In questo lavoro vengono descritte le prime tre attività del progetto di tesi di dottorato. In primo luogo, è stato effettuato uno screening di resistenza alla SO2 e al chitosano tra 65 lieviti non-*Saccharomyces*, appartenenti alle specie presenti in vinificazione, come *Metschnikowia pulcherrima*, *Zygosaccharomyces bailii*, *Torulaspora delbruekii*, *Hanseniaspora* spp, *Lachancea thermotolerans*, *Candida zemplinina*, *Meyerozyma caribbica*, *Pichia* spp. Nella seconda fase, i ceppi selezionati sono stati testati per la resistenza al chitosano in fermentazione su scala di laboratorio, mentre nella terza fase gli stessi ceppi sono stati valutati per la resistenza al chitosano commerciale e da insetto su terreno a base di mosto d'uva agarizzato.

**Key words**: Chitosan, resistance screening, non-*Saccharomyces* yeasts, antimicrobial activity, sustainable sources.

# **1. Introduction**

In accordance with the PhD thesis project previously described (Tedesco, 2022), this poster reports the main results of the first three activities concerning:

(A1) the resistance screening to 150 mg/L of SO2 and 100 mg/L of commercial chitosan among non-*Saccharomyces* yeast strains, belonging to UNIBAS yeast collection (University of Basilicata – Italy) and including the species most frequent in winemaking;

(A2) the laboratory scale fermentations in pasteurised grape must, inoculated with selected strains, and added with 50 mg/L of SO2; 100 mg/L of chitosan and 20 mg/L of SO2 + 100 mg/L of chitosan;

(A3) the resistance tests on agarized grape must, added with 100, 200, 300 and 400 mg/L of both commercial and insect-based chitosan.

# **2. Materials and Methods**

# In the first screening, sixty-five non-*Saccharomyces* yeast strains were tested. The resistance test was carried out in 96-well microtiter plates, containing the Yeast Nitrogen Base medium (Capece et al., 2020), added with 150 ppm of SO2 and 100 ppm of chitosan. The inoculum level for each strain was set at 1x106 cells/mL and the strain growth level was followed by OD600 evaluation.

# On the basis of the first screening, twenty-two yeast strains were selected and tested in laboratory scale fermentations (inoculum level of 1x104 cells/mL) using pasteurized grape must, added with the antimicrobial compounds. The tested conditions were the following: (a) 50 mg/L of SO2, the amount frequently used during cellar fermentations; (b) 100 mg/L of chitosan, the amount authorized by the OIV (oiv-eno-338a-2009); (c) 20 mg/L of SO2 and 100 mg/L of chitosan, in order to try to reduce the use of sulphur dioxide; (d) without antimicrobial compounds (positive control); (e) without inoculum and antimicrobials (negative control). In order to study the influence of antimicrobials on the inoculated non-*Saccharomyces* strains, the kinetic of fermentation and the yeast viability were monitored after 48 h of incubation.

The same selected yeasts strains were tested for plate resistance, using agarized grape must added with the following doses of commercial and insect-based chitosan 100; 200; 300 and 400 mg/L. The growth level was monitored after 48 h of incubation.

**3. Results and Discussion**

## **3.1 Preliminary screening**

The resistance level to SO2 and chitosan was determined by the ratio between strain growth in broth with and without the antimicrobial compounds. The test showed that the assayed non-*Saccharomyces* strains exhibited a high variability to sulphur dioxide and chitosan tolerance. Strains like *M. pulcherrima*, *Mey. caribbica*, *L. thermotolerans* showed high susceptibility to chitosan, while the others are more resistant to both antimicrobial compounds, although a strain variability was observed (Fig. 1).

Based on the obtained results, twenty-two strains were selected, by choosing those chitosan-sensitive and SO2-resistant and SO2-sensitive and chitosan-resistant.

**Figure 1** *Resistance to SO2 and commercial chitosan of 65 non-Saccharomyces strains*



**3.2 Chitosan resistance of selected strains**

The resistance level of selected strains during lab-scale fermentations were calculated monitoring the cells growth level after 48h of incubation. Resistance to tested antimicrobials was calculated as the ratio between the number of generations in grape must with and without the antimicrobials. The results showed that, in some cases, the chitosan has a higher antimicrobial activity than SO2 (i.e. *Metschnikowia*)and vice versa. In few cases, the better action is given by the combined use of both, as reported in Table 1.

As the evaluation of chitosan resistance on agarized medium, the tolerance level was reported as the maximum doses allowing the strain growth. The results showed that strains exhibit different levels of chitosan resistance, and, in some cases, the use of a lower dose of insect chitosan showed better antimicrobial activity (Tab. 1).

**Table 1** *Strain resistance to antimicrobials evaluated in fermentation (reported as percentage of survival to antimicrobials) and as growth on agarized grape must (expressed as the highest tolerated dose)*

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Grape must fermentation** | **Agarized grape must** |
| **Strain** | **Species** | **SO2**(%) | **Chitosan** **(%)** | **SO2 + chitosan (%)** | **Commercial chitosan (mg/L)** | **Insect chitosan (mg/L)** |
| AII-136 | *M. pulcherrima* | 17,9 | 18,7 | 16,2 | 200 | 200 |
| 4-11 | *M. pulcherrima* | 74,9 | 19,4 | 44,8 | 200 | 200 |
| 4R1 | *M. pulcherrima* | 75,4 | 37,1 | 29,3 | 200 | 200 |
| CR-1 | *Z. bailii* | 92,4 | 83,2 | 80,8 | 300 | 200 |
| CR-2 | *Z. bailii* | 100 | 100 | 89,5 | 400 | 300 |
| 425 | *T. delbruekii* | 72,5 | 48,2 | 48,5 | 400 | 400 |
| LC2-1 | *T. delbruekii* | 93,8 | 74,5 | 96,5 | 400 | 400 |
| AP1 | *H. uvarum* | 0 | 81,1 | 16,7 | 200 | 300 |
| 1P3 | *H. uvarum* | 76,9 | 100 | 100 | 300 | 300 |
| 2R9 | *H. guilliermondii* | 23,3 | 100 | 86,2 | 200 | 200 |
| TM5-2 | *H. guilliermondii* | 6,4 | 59,1 | 60,4 | 200 | 300 |
| ND1 | *H. osmophila* | 56,3 | 82,2 | 73,3 | 400 | 400 |
| AII-134 | *L. thermotolerans* | 22,3 | 100 | 59,4 | 300 | 300 |
| 4-14 | *L. thermotolerans* | 41,9 | 80,2 | 58,4 | 300 | 300 |
| TSE | *C. zemplinina* | 90,5 | 100 | 100 | 300 | 300 |
| FCB6 | *C. zemplinina* | 52,2 | 100 | 78,9 | 400 | 400 |
| AII-171 | *M. caribbica* | 59,1 | 29,4 | 29,9 | 100 | 100 |
| AII-82 | *M. caribbica* | 91,5 | 59,2 | 33,1 | 200 | 100 |
| AII-177 | *P. kudriavzevii* | 74,5 | 87,4 | 75,5 | 400 | 400 |
| 4-16 | *P. kudriavsevii* | 61,6 | 85 | 79,1 | 400 | 400 |
| AII-110 | *P. kluyveri* | 66,5 | 68,6 | 83,8 | 400 | 400 |
| AII-186 | *P. anomala* | 76 | 83,2 | 84 | 200 | 200 |

# **4. References**

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