Research on the origin and the side effects of chitosan stabilizing properties in wine

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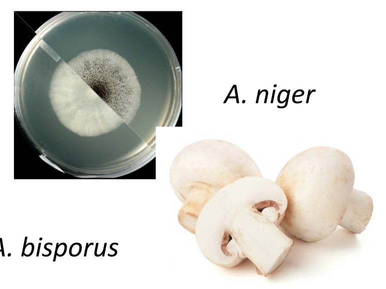
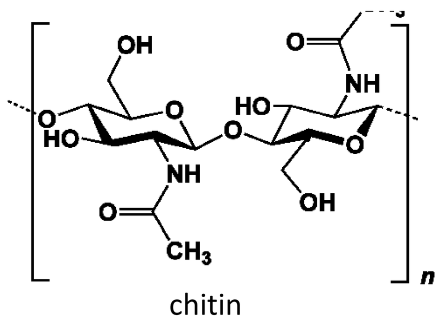
Fungal **chitosan** is a polysaccharide made up of glucosamine and *N*-acetyl-glucosamine and derived from chitin-glucan from ***Aspergillus niger*** or ***Agaricus bisporus***. It has been authorized in 2009 as an antiseptic agent in wine (OIV). At the maximum dose of 10g/hl, it was shown to efficiently eliminate *Brettanomyces bruxellensis*, a spoilage agent in red wines. Although fungal chitosan is highly renewable, biocompatible (ADI equivalent to sucrose) and non-allergenic, winemakers very often prefer to use sulfites (SO_2), though they are classified as priority food allergens. Indeed, fungal chitosan appears as a poorly reliable product because of many conflicting reports and advices on its efficiency and on its side effects on wine quality. These contradictions could be explained by the heterogeneity of the fungal chitosan preparations traded, wines diversity (chemical composition, winemaking itineraries), but also, by the recently highlighted huge genetic diversity prevailing in wine microbial species.

The **CHITOWINE** project (2018-2021), funded by the ANR, aims first of all at identifying the situations in which chitosan is effective and those in which it is not. The first results are summarized in this poster.

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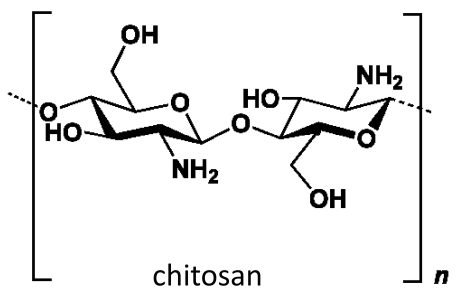
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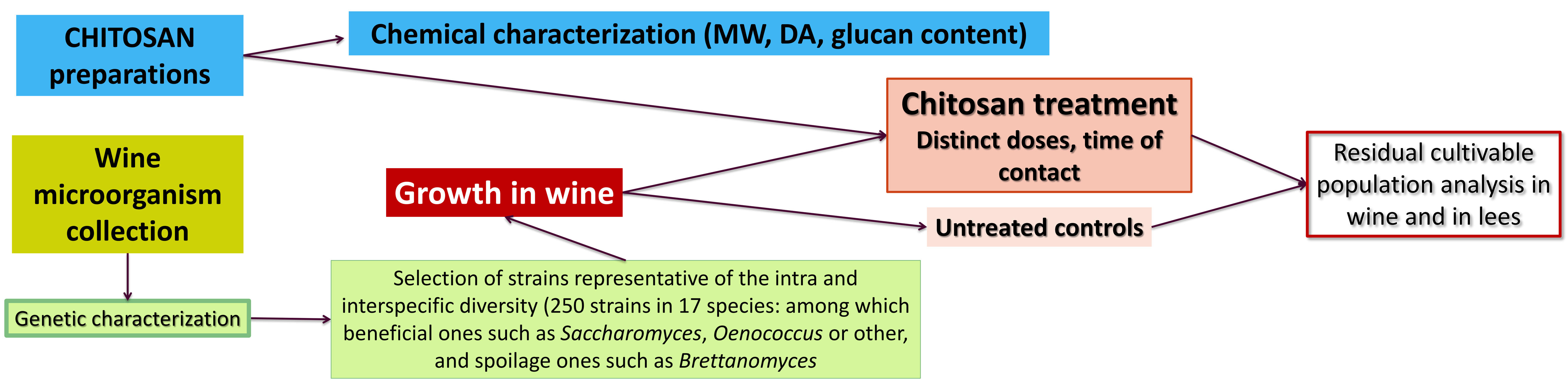
SCREENING METHODOLOGY

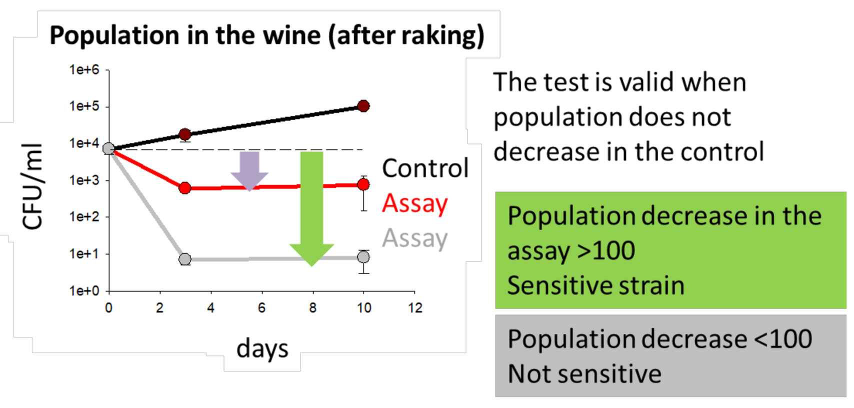


FIRST RESULTS

Chitosan selection and characterization

Many fungal chitosan preparations were analyzed and two were selected for further study: F1 displayed a mean MW (estimated by HPLC-SEC) of 30 kDa and a DA (estimated by ^{1}H-NMR) of 9.6%, while F4 displayed a mean MW of 400 kDa and a DA of 15.8%. Both displayed low glucan content (1,5-2%).

Example of proportion of sensitive strains in three distinct species

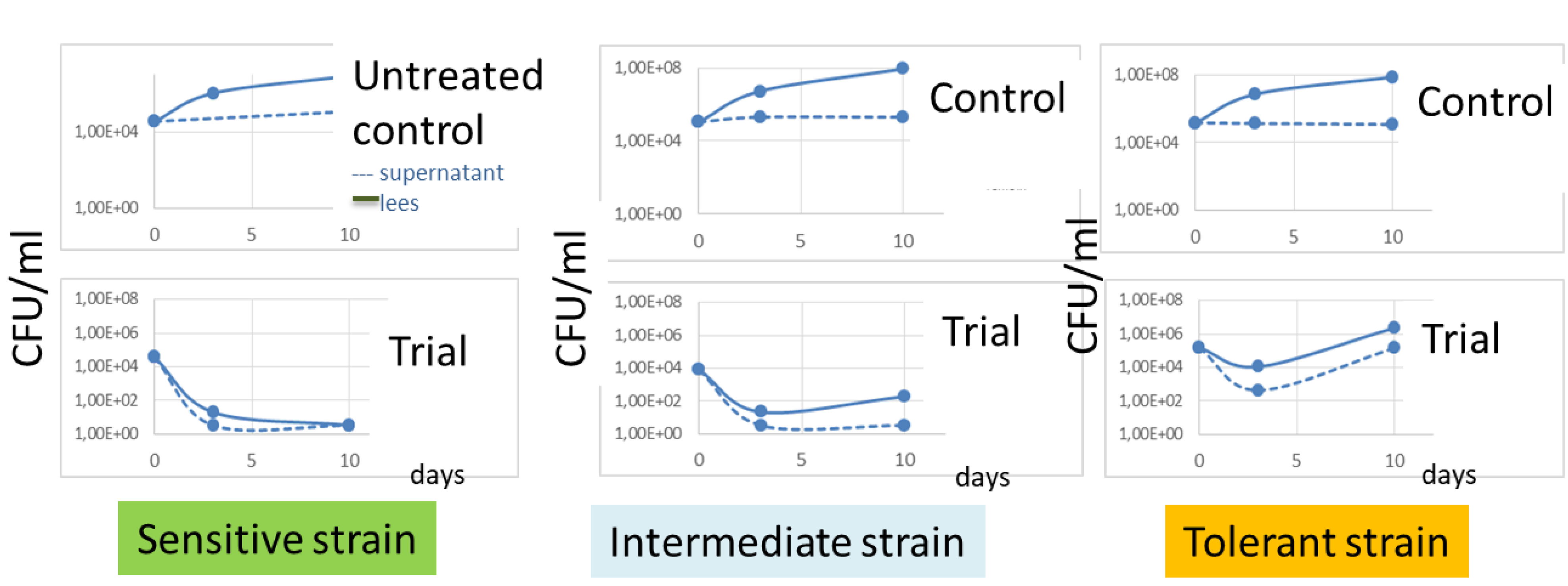


Species	Number of studied strains	% sensitive strains F1	% sensitive strains F4
S. cerevisiae	27	74%	33%
O. oeni	32	94%	73%
B. bruxellensis	53	89%	75%

When the criterion for sensitivity is the reduction of the population >100 after treatment + raking, the most sensitive species is *O. oeni*, followed by *B. bruxellensis* and *S cerevisiae*. Moreover, F1 is significantly more efficient than F4 in the wine used.

In all species, there is a small proportion of resistant strains in which the population remains high in the wine, in the experimental conditions used.

Kinetic analysis of population decrease upon chitosan treatment



A kinetic analysis of the evolution of the populations in the wine and in the lees makes it possible to distinguish different profiles of resistance to the treatment, whatever the species.

In all cases, microorganisms display a slight tendency to sediment in control cultures (lees enrichment compared with supernatant).

This phenomenon is much more important in the presence of chitosan: microorganisms fall at the bottom of the test tube. In half of the cases (all species combined), this is accompanied by a total loss of cultivability in wine and lees (very sensitive strain). In other cases (30%), some of the sedimented cells survive in the lees. And finally, in 20% of the cases, after a loss of initial cultivability, the microorganisms develop again in the lees as in the wine. This last category of tolerant strains is more common in the case of F4.

CONCLUSIONS / PERSPECTIVES

All strains and species tested are affected (at least transiently): Chitosan is a broad-spectrum antiseptic agent. But…All chitosan are not equal

Even the best chitosan preparations are not always effective on all strains to be eliminated. Keeping the lees may be dangerous in certain cases. Chitosan can also affect the fermentative species present at the time of treatment.

The comparison of different chitosan preparations, the further analysis of the physiological consequences of treatment, or the comparison of the biochemical and genetic properties of resistant and sensitive strains will help us better understand chitosan mechanism of action, to better control its activity,

Furthermore, further work will be necessary to state about the consequence of the treatment on the sensorial properties of the wine, before providing new advices for a better use of chitosan treatment.