POLYSACCHARIDES AND GLYCEROL PRODUCTION BY NON-*SACCHAROMYCES* WINE YEASTS IN MIXED FERMENTATION•

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1. INTRODUCTION

In the last few years the attention of wine industry has been addressed to the research of new and improved wine-yeast strains useful for the production of different types and styles of wines. In this context, several studies report on the advantages deriving from the utilization of non-*Saccharomyces* yeasts in mixed starter cultures with *Saccharomyces cerevisiae* (Ciani *et al*., 2010). In fact, although non-*Saccharomyces* are often referred as spoilage yeasts, they are also characterized by the production of high glycerol concentrations (Ciani *et al*., 1996; Romano *et al.*, 1997) and of specific enzymes involved in the release of aromatic compounds (Rosi *et al*., 1994; Esteve-Zarzoso *et al*., 1998). Less is known on their contribution to the production of polysaccharides. Since polysaccharides in wine are important to improve mouthfullness, richness and aromatic persistence, to stabilize colour and avoid protein and tartrate instability (Feuillat, 2003), with this study we evaluated the ability of non-*Saccharomyces* yeasts to produce total polysaccharides during fermentation carried out by pure and mixed cultures with a commercial starter strain of *Saccharomyces cerevisiae.* Moreover, the effect of non-*Saccharomyces* yeasts on glycerol production was evaluated in mixed fermentation trials.

2. MATERIALS AND METHODS

2.1. Yeasts

Eighty-nine non-*Saccharomyces* yeasts, isolated from grape-must of different origins, and three *S. cerevisiae* strains, all identified by means of PCR-RFLP of Internal Transcribed Spacers (ITS) according to the method of Esteve-Zarzoso *et al.* (1999), were utilized to inoculate pure fermentation trials. A commercial selected starter strain of *S. cerevisiae*, Lalvin EC1118 (Lallemand Inc., F), was utilized in mixed fermentation trials with non-*Saccharomyces* yeasts (tab. 1).

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2.2. Fermentation trials

Pure fermentation trials were performed in 140 mL of pasteurized must (sugar 27 %) inoculated with 5 % of pre-culture. Mixed fermentation trials were carried out in 450 mL of sterile grape must (sugar 21 %) inoculated with 48h pre-cultures of *Saccharomyces*/non-*Saccharomyces* to obtain a initial concentration of 10^7cell mL^{-1} of each strain (inoculum ratio 1:1). Pure cultures of non-*Saccharomyces* and *S. cerevisiae* yeasts, inoculated at 10^7 cell mL^{-1}, were utilized as controls. All the fermentations, carried out in double at 25 °C were weighted every day until the end of fermentation (constant weight for two consecutive days).

Tab. 1 - Yeast strains utilized.

Strain	Code n.	Strain	Code n.
Candida diversa	C1	*Zygosaccharomyces florentinus*	Z1-Z4
Candida stellata	C2	*Zygosaccharomyces bailii*	Z5-Z10
Candida beechii	C3	*Zygosaccharomyces bisporus*	Z11-Z16
Candida montana	C4	*Zygosaccharomyces rouxii*	Z17
Candida tropicalis	C5	*Zygosaccharomyces fermentati*	Z18
Candida apicola	C6-C8	*Hanseniaspora uvarum*	H1-H4
Candida bombicola	C9	*Hanseniaspora vineae*	H5
Candida vinaria	C10-C12	*Hanseniaspora osmophila*	H6-H8
Candida cantarellii	C13	*Hanseniaspora valbyensis*	H9-H10
Pichia fermentans	P1-P5	*Torulaspora delbrueckii*	T1-T9
Pichia guilliermondii	P6	*Metschnikowia pulcherrima*	M1-M7
Pichia membranifaciens	P7-P10	*Saccharomycodes ludwigii*	L1-L12
Pichia anomala	P11	*Issatchenkia terricola*	I1
Pichia fluxuum	P12-P13	*Schizosaccharomyces pombe*	Sp1
Pichia kluyveri	P14	*Saccharomyces cerevisiae*	S1-S3
Kluyveromyces thermotolerans	K1-K4	*S. cerevisiae* Lalvin EC1118	S4

2.3. Chemical analysis

Total polysaccharides, determined by HPLC according to Domizio *et al.* (2010), and evaluated as difference among the concentration in fermented juice and that in must, were expressed as mannans. Glycerol was determined enzymatically (kit no. 10148270035, R-Biopharm AG, Darmstadt, D). All results are means of duplicate determinations.

3. RESULTS

Most of the non-*Saccharomyces* yeasts tested in pure culture produced more polysaccharides than the *S. cerevisiae* control strains (fig. 1). Moreover, strains ascribed to the genera *Zygosaccharomyces, Hanseniaspora*, *Candida* and *Pichia*, showed a wide inter- and intrageneric biodiversity for this character, while a lower variability was observed for the other genera. In particular, all *Saccharomycodes ludwigii* strains showed a polysaccharides production ranging from 200 to 280 mg L^{-1} with the exception of strain L10 (320 mg L^{-1}). Similarly, *Metschnikowia pulcherrima* strains ranged from 130 to 180 mg L^{-1} and those ascribed to *Torulaspora delbrueckii* from 160 to 200 mg L^{-1} with the exception of strain T5 that produced 253 mg L^{-1}. The highest production of polysaccharides was shown by the only *Schizosaccharomyces pombe* strain tested, that reached a concentration of 712 mg L^{-1}.

Fifteen non-*Saccharomyces* strains, selected on the basis of their polysaccharide production, were then evaluated for their ability to produce polysaccharides and glycerol in pure and mixed fermentation trials with a starter strain of *S. cerevisiae*. Polysaccharide production was deeply influenced by the presence of *S. cerevisiae* and decreased in mixed fermentations trials (fig. 2). However, in spite of this reduction, 50 % of the fermentation trials carried out by mixed cultures resulted in higher polysaccharide production than that obtained by *S. cerevisiae* in pure culture. In particular, the associations *S. cerevisiae/Schiz. pombe* Sp1, *S. cerevisiae/Saccharomycodes ludwigii* L8 and L10 showed the highest concentrations of polysaccharides (482 mgL^{-1}, 216 mgL^{-1} and 201 mgL^{-1}, respectively). Glycerol production was rather variable in fermentation trials carried out by pure cultures of selected non-*Saccharomyces* yeasts, but become comparable in mixed fermentation trials, with the exception of mixed cultures with *S. ludwigii* L10 and *S. pombe* Sp1, that maintained high glycerol production also in the presence of *S. cerevisiae* (fig. 2). Thus the influence of mixed cultures on this parameter was dependent on the non-*Saccharomyces* strain utilized.

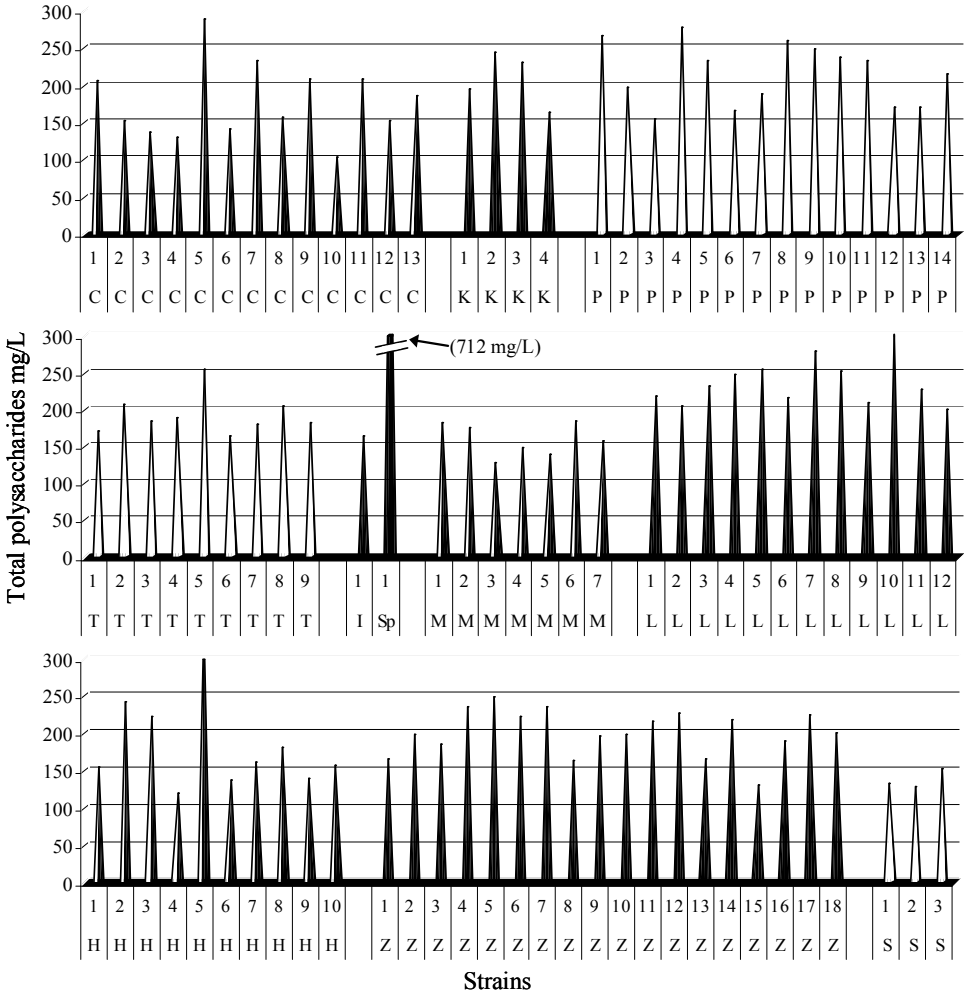

Fig. 1 - Total polysaccharide concentration in fermentations carried out by pure cultures.

4. CONCLUSIONS

In spite of the effect of the *S. cerevisiae* starter strain on the final concentration of total polysaccharides, the utilization of non-*Saccharomyces/S. cerevisiae* mixed cultures generally results in higher concentrations of these metabolites as compared to pure culture of *S. cerevisiae*. Moreover, depending on the non-*Saccharomyces* strain utilized a marked effect on the concentration of glycerol is observed. Thus, even though further studies will be needed to optimize the management of fermentations carried out by mixed cultures, the results here presented indicate that selected non-*Saccharomyces* yeasts may positively interact with *S. cerevisiae*, and represent potential and innovative tools to increase wine complexity.

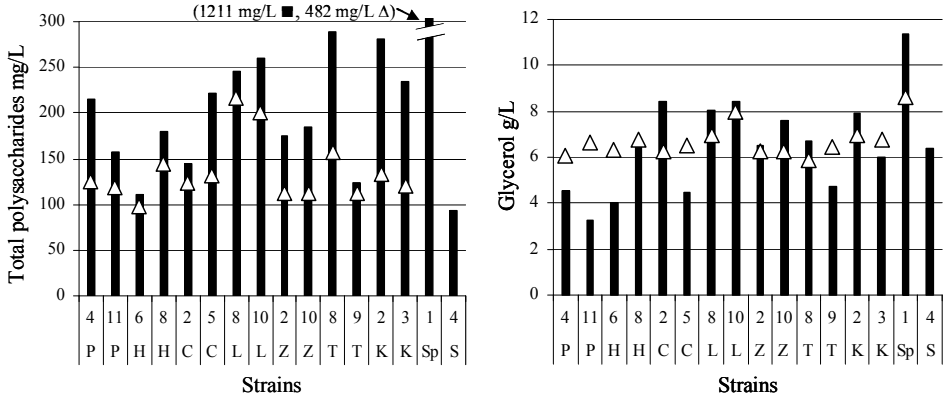

Fig. 2 -. Total polysaccharides (left panel) and glycerol (right panel) content in wines produced by pure (■) and mixed cultures (Δ).

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Abstract

A great variability in the amount of polysaccharides recovered at the end of fermentations carried out by pure cultures of 89 non-*Saccharomyces* yeasts was observed. The utilization of the best polysaccharides producers in mixed cultures with *S. cerevisiae* resulted in considerable increases in the final concentration of polysaccharides and showed a strain dependent effect on glycerol production as compared to pure culture of *S. cerevisiae.* Thus, selected non-*Saccharomyces* yeasts may positively interact with *S. cerevisiae*, and represent potential and innovative tools to increase wine complexity.

Literature cited

Ciani M., Ferraro L. - 1996 - Enhanced glycerol content in wines made with immobilized *Candida stellata* cells. *Appl Environ. Microbiol.*, 62, 128–132.

Ciani M., Comitini F., Mannazzu I., Domizio P. – 2010 - Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Res.,* 10, 123–133.

Domizio P., Romani C., Comitini F., Gobbi M., Lencioni L., Mannazzu I., Ciani M. - 2010 - Potential spoilage non-*Saccharomyces* yeasts in mixed cultures with *S. cerevisiae*. *Ann. Microbiol.,* DOI 10.1007/s13213-010-0125-1.

Esteve-Zarzoso B., Manzanares P., Ramón D., Querol A. - 1998 - The role of non-*Saccharomyces* yeasts in industrial winemaking. *Internat. Microbiol.*, 1, 143–148.

Esteve-Zarzoso B., Belloch C., Uruburu F., Querol A. – 1999 - Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *Int. J. Syst. Bacteriol.*, 49, 329–337.

Feuillat M. - 2003 - Yeast macromolecules: origin, composition, and enological interest. *Am. J. Enol. Vitic.*, 54, 211-213.

Romano P., Suzzi G., Domizio P., Fatichenti F. – 1997 - Glycerol and other fermentation products of apiculate wine yeasts. *J. Appl. Microbiol.,* 82, 615-618.

Rosi I., Vinella M., Domizio P. - 1994 - Characterization of β-glucosidase activity in yeasts of oenological origin. *J. Appl. Bacteriol.*, 77, 519-527.