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

Cristina ROMANI[1,4], Paola DOMIZIO[1], Livio LENCIONI[1], Mirko GOBBI[2], Francesca COMITINI[2], Maurizio CIANI[2], Ilaria MANNAZZU[3]
[1] DiBA, Università degli Studi di Firenze, via Donizetti, 6 - 50144 Firenze, I.
[2] DiSAIFET, Università Politecnica delle Marche, via Brecce Bianche, 232 - 60131 Ancona, I.
[3] DiSAABA, Università degli Studi di Sassari, Viale Italia 39 - 07100 Sassari, I..
[4] Consorzio Tuscania, p.za Strozzi, 1 - 50123 Firenze, I.
E-mail: cristina.romani@unifi.it; cristina.romani@consorziotuscania.it

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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 contribute 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).

2.2. Fermentation trials

Pure fermentation trials were performed in 140 mL of pasteurized must (sugar 27 %)

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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 10^7 cell 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	*Pichia fermentans*	P1-P5
Candida stellata	C2	*Pichia guilliermondii*	P6
Candida beechii	C3	*Pichia membranifaciens*	P7-P10
Candida montana	C4	*Pichia anomala*	P11
Candida tropicalis	C5	*Pichia fluxuum*	P12-P13
Candida apicola	C6-C8	*Pichia kluyveri*	P14
Candida bombicola	C9	*Saccharomycodes ludwigii*	L1-L12
Candida vinaria	C10-C12	*Saccharomyces cerevisiae*	S1-S3
Candida cantarellii	C13	*S. cerevisiae* Lalvin EC1118	S4
Hanseniaspora uvarum	H1-H4	*Schizosaccharomyces pombe*	Sp1
Hanseniaspora vineae	H5	*Torulaspora delbrueckii*	T1-T9
Hanseniaspora osmophila	H6-H8	*Zygosaccharomyces florentinus*	Z1-Z4
Hanseniaspora valbyensis	H9-H10	*Zygosaccharomyces bailii*	Z5-Z10
Kluyveromyces thermotolerans	K1-K4	*Zygosaccharomyces bisporus*	Z11-Z16
Issatchenkia terricola	I1	*Zygosaccharomyces rouxii*	Z17
Metschnikowia pulcherrima	M1-M7	*Zygosaccharomyces fermentati*	Z18

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 *S. 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, *M. pulcherrima* strains ranged from 130 to 180 mg L^{-1} and those ascribed to *T. 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 *S. pombe* strain tested, that reached a concentration of 712 mg L^{-1}.

Fifteen non-*Saccharomyces* strains, selected on the basis of their fermentative performances (data not shown), were then evaluated for their ability to produce polysaccharides and glycerol in pure and mixed fermentation trials with a starter strain of *S.*

cerevisiae. Polysaccharides 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 polysaccharides productions than that obtained by *S. cerevisiae* in pure culture. In particular, the associations *S. cerevisiae/S. pombe* Sp1, *S. cerevisiae/S. ludwigii* L8 and L10 showed the highest concentrations of polysaccharides (482 mg L^{-1}, 216 mg L^{-1} and 201 mg L^{-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 productions 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.

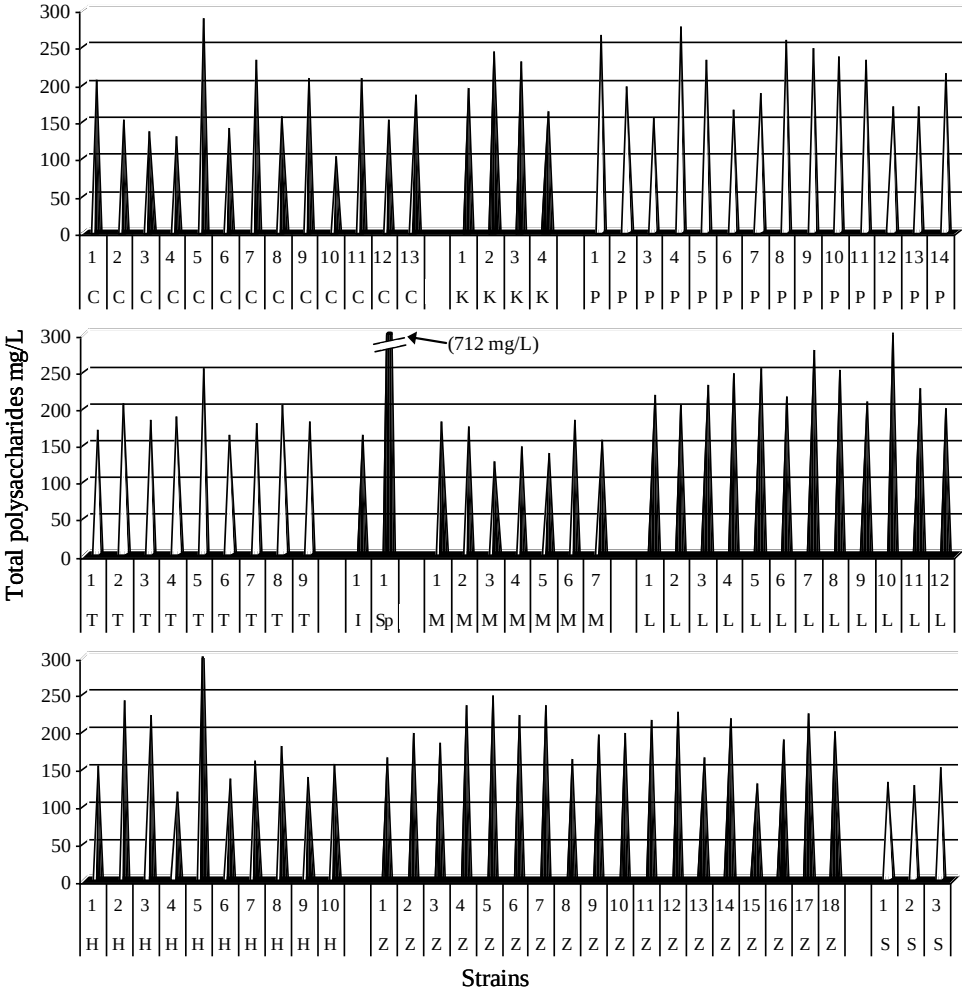

Fig. 1 - Total polysaccharides concentration in fermentations carried out by pure cultures.

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.

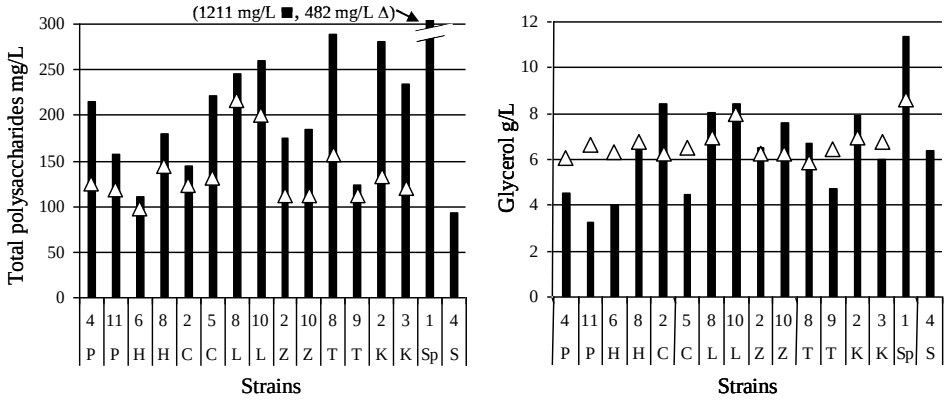

Fig. 2 -. Total polysaccharides (left panel) and glycerol (right panel) content in wines produced by pure (■) and mixed cultures (Δ).

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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 fifteen non-Saccharomyces strains 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.

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