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Cell wall polysaccharides released during the alcoholic fermentation by *Schizosaccharomyces pombe* and *S. japonicus*: quantification and characterization

P. Domizio [a, b, *], Y. Liu [c], L.F. Bisson [a], D. Barile [c, d]

[a] Department of Viticulture & Enology, University of California-Davis, Davis, CA 95616, USA
[b] Dipartimento di Gestione dei Sistemi Agrari, Alimentari e Forestali (GESAAF), Università degli Studi di Firenze, 50144 Firenze, Italy
[c] Department of Foods Science & Technology, University of California-Davis, Davis, CA 95616, USA
[d] Foods for Health Institute, University of California-Davis, Davis, CA 95616, USA

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ABSTRACT

The present work demonstrates that yeasts belonging to the *Schizosaccharomyces* genus release a high quantity of polysaccharides of cell wall origin starting from the onset of the alcoholic fermentation. By the end of the alcoholic fermentation, all of the *Schizosaccharomyces* yeast strains released a quantity of polysaccharides approximately 3–7 times higher than that released by a commercial *Saccharomyces cerevisiae* yeast strain under the same fermentative conditions of synthetic juice. A higher content of polysaccharide was found in media fermented by *Schizosaccharomyces japonicus* with respect to that of *Schizosaccharomyces pombe*. Some of the strains evaluated were also able to produce high levels of pyruvic acid, which has been shown to be an important compound for color stability of wine. The presence of strains with different malic acid consumption patterns along with high polysaccharide release would enable production of naturally modified wines with enhanced mouth feel and reduced acidity. The chemical analysis of the released polysaccharides demonstrated divergence between the two yeast species *S. pombe* and *S. japonicus*. A different mannose/galactose ratio and a different percentage of proteins was observed on the polysaccharides released by *S. pombe* as compared to *S. japonicus*. Analysis of the proteins released in the media revealed the presence of a glycoprotein with a molecular size around 32–33 kDa only for the species *S. japonicus*. Mass spectrometry analysis of carbohydrate moieties showed similar proportions among the *N*-glycan chains released in the media by both yeast species but differences between the two species were also observed. These observations suggest a possible role of rapid MALDI-TOF screening of *N*-glycans compositional fingerprint as a taxonomic tool for this genus. Polysaccharides release in the media, in particular galactomannoproteins in significant amounts, could make these yeasts particularly interesting also for the industrial production of exogenous polysaccharide preparations.

1. Introduction

The addition of commercial products containing polysaccharides derived from yeast cells wall to wine (in particular mannoproteins) is becoming a common practice during the wine-making process (Pozo-Bayón et al., 2009). Positive enological properties associated with the polysaccharides and mannoprotein content of wine have been reported: reduction in protein and tartrate instability (Brown et al., 2007; Dupin et al., 2000; Gerbaud et al., 1997; Gonzalez-Ramos et al., 2008; Moine-Ledoux and Dubourdieu, 1999; Lubbers et al., 1994; Waters et al., 1994), improvement of mouth-feel (Vidal et al., 2004), increase of sweetness and roundness (Guadalupe and Ayestarán, 2007; Rosi et al., 1998), decrease in astringency (Escot et al., 2001; Quijada-Morín et al., 2014), prevention of tannin aggregation and precipitation (Poncet-Legrand et al., 2007), addition of complexity and aromatic persistence (Chalier et al., 2007; Lubbers et al.,1994), stabilization of the color of red wines (Fuster and Escot, 2002; Riou

* Corresponding author. Dipartimento di Gestione dei Sistemi Agrari, Alimentari e Forestali (GESAAF), Università degli Studi di Firenze, Via Donizetti, 50144 Firenze, Italy.
E-mail address: domizio@unifi.it (P. Domizio).

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et al., 2002), and stability of the foam of sparkling wine (Vanrell et al., 2007). In contrast, Guadalupe and Ayestarán (2008), using commercial mannoprotein-rich preparations in Tempranillo must, observed a reduction of the content of wine proanthocyanidins and wine stable pigments. Thus the nature of the polysaccharide is an important factor in defining the potential impact on the wine.

Guadalupe et al. (2010) suggested that using yeasts during the alcoholic fermentation production that are able to release mannoproteins would be less costly than use of exogenous mannoproteins. However yeast release variable portions of mannoproteins and the typical yeast present during fermentation, *S. cerevisiae*, releases low amounts of polysaccharides, normally ranging from 50 to 150 mg/L (Rosi et al., 2000). Therefore, an interesting alternative to the addition of these commercial exogenous polysaccharides-based products would be the use of yeasts able to release high quantity of polysaccharides during the alcoholic fermentation. Several studies have shown that non-*Saccharomyces* yeasts are generally characterized by the capacity to release a high quantity of polysaccharides (Comitini et al., 2011; Domizio et al., 2011a, 2011b, 2014; Giovani et al., 2012; Gobbi et al., 2013). In most of these analyses, the concentration of polysaccharides released by the non-*Saccharomyces* yeasts was much higher when compared with *S. cerevisiae* yeasts used as controls under the same conditions.

Schizosaccharomyces yeast strains have found application in winemaking because of their ability to reduce malic acid in grape juice and/or wine (Ciani, 1995; Dharmadhikari and Wilker, 1998; Gao and Fleet, 1995; Magyar and Panyik, 1989; Munyon and Nagel, 1977; Rankine, 1966; Silva et al., 2003; Snow and Gallander, 1979; Thornton and Rodriguez, 1996; Yokotsuka et al., 1993). Recently, Benito et al. (2012, 2014) reported benefits in addition to the demalication activity deriving from using *S. pombe* yeast in wine fermentation, such as the production of pyruvic acid and the possibility to reduce ethyl carbamate in wine, through the removal of its urea precursor, as a consequence of urease activity. Pyruvic acid seems to be of particular interest for the color stability of the wine. Indeed, a strong correlation between the amount of pyruvic acid released and the formation of vitisin A (a pyranoanthocyanin, a natural polyphenol found in grapes) has been observed (Morata et al., 2003). Moreover, malic acid consumption by *Schizosaccharomyces* yeasts permit non-bacterial biological deacidification and averting production of amines. In this context, in order to avoid the risk of biogenic amines formation by lactic bacteria during malolactic fermentation, Benito et al. (2015) proposed mixed fermentation by using two different yeasts: *S. pombe*, to consume malic acid, and *Lachancea thermotolerans* to produce lactic acid to balance the acidity of wines produced from low acidity musts. *S. pombe* is also able to utilize D-gluconate as an alternative carbon and energy source for growth during glucose starvation (Tsai et al., 1995). Therefore, the possibility to reduce gluconic acid in wines produced from rotten grapes by using *S. pombe* strains could represent an interesting approach. However, glucose addition rapidly inhibits gluconate degradation. For this reason, *S. pombe* use has been proposed as a way to remove gluconic acid from wine to be subsequently subjected to biological aging (Peinado et al., 2004). However, in following studies, Peinado et al. (2007, 2009), successfully used glucose-transport-deficient mutants of *S. pombe* mutant strains to reduce the content in gluconic acid also of grape juice obtained from rotten grapes. Thus this yeast has already been proposed for use in wine production. The ability to release polysaccharides with beneficial effects would also be of interest.

A study comparing the quantity of polysaccharides released at the end of alcoholic fermentation by eighty-nine non-*Saccharomyces* yeasts strains, found that the only *Schizosaccharomyces* strain tested released the highest level of polysaccharides (712 mg/L), about 5 times higher than the average of those released by three *S. cerevisiae* strains, used as controls (Romani et al., 2010). In contrast, Giovani et al. (2012) found that the only *S. pombe* strain tested released a quantity of polysaccharides (203 mg/L) lower than that released by three *S. cerevisiae* strains (ranging from 225 to 264 mg/L) when tested under the same conditions. Both of these studies used a single strain of *Schizosaccharomyces* and the differences could be due to strain effects or to the growth conditions of the studies.

The presence of a high quantity of polysaccharides from the beginning of the alcoholic fermentation process could promote the formation of polysaccharide-tannin complexes that stabilize the reactive tannins and in turn enhance the mouthfeel of red wine as well as enable other types of interactions of benefit to wine stability. Therefore, considering the elevated quantity of polysaccharides released by the strain of *Schizosaccharomyces* (712 mg/L), as previously reported by Romani et al. (2010) and the variability in release reported in the literature (Giovani et al., 2012), in the present work we evaluated the ability of different strains belonging to the genus *Schizosaccharomyces* to release polysaccharides during the alcoholic fermentation.

2. Materials and methods

2.1. Yeast strains

Nine yeast strains belonging to the genus *Schizosaccharomyces* from the yeast culture collection of the Department of Agricultural, Food and Forestry Systems (GESAAF, University of Florence, Italy) and the Department of Viticulture & Enology University of California-Davis, (Davis) were used (Table 1). Six strains were ascribed to the species *Schizosaccharomyces pombe* and three to the species *Schizosaccharomyces japonicus* by D1-D2 domain analysis.

A commercial strain, Lalvin EC1118 (Lallemand Inc., Montreal, Canada), was used as reference strain for *S. cerevisiae* and for comparison determinations.

2.2. Fermentation trials

The fermentations were carried out in duplicate at 27 °C in 200 mL Erlenmeyer flasks containing 150 mL of a synthetic grape juice medium "Minimal Must Medium" (MMM) (Spiropoulos et al., 2000). The medium was sterilized by filtration. The flasks were inoculated at optical density of 0.1 ($OD_{600\ nm}$), with 48-h precultures grown in 10 mL of YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) (Oxoid Unipath Ltd, Hampshire, UK), at 25 °C in a roller drum. The levels of sugar and total assimilable nitrogen were 220 g/L and 208 mg/L, respectively. The assimilable nitrogen concentration was obtained by using 0.2 g/L of

Table 1
Origin and source of the *Schizosaccharomyces* strains used in the present study.

Code	Species	Strain	Origin	Source
# 1	*Schizosaccharomyces japonicus*	13	GESAAF[a]	Wine
# 2	*Schizosaccharomyces pombe*	227	UCD[b]	Unkown
# 3	*Schizosaccharomyces pombe*	582	UCD[b]	Sherry wine
# 4	*Schizosaccharomyces pombe*	583	UCD[b]	Sherry wine
# 5	*Schizosaccharomyces pombe*	584	UCD[b]	Wine
# 6	*Schizosaccharomyces pombe*	687	UCD[b]	Wine
# 7	*Schizosaccharomyces pombe*	807	UCD[b]	Unkown
# 8	*Schizosaccharomyces japonicus*	2096	UCD[b]	Wine
# 9	*Schizosaccharomyces japonicus*	2489	UCD[b]	Wine

[a] Dipartimento di Gestione dei Sistemi Agrari, Alimentari e Forestali, Università degli Studi di Firenze, Italy.
[b] Department of Viticulture & Enology, University of California-Davis, Davis.