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Behaviour of *Saccharomyces cerevisiae* wine strains during adaptation to unfavourable conditions of fermentation on synthetic medium: Cell lipid composition, membrane integrity, viability and fermentative activity

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Abstract

During must fermentation wine strains are exposed to a variety of biotic and abiotic stresses which, when prevailing over the cellular defence systems, can affect cell viability with negative consequences on the progression of the fermentative process. To investigate the ability of wine strains to survive and adapt to unfavourable conditions of fermentation, the lipid composition, membrane integrity, cell viability and fermentative activity of three strains of *Saccharomyces cerevisiae* were analysed during hypoxic growth in a sugar-rich medium lacking lipid nutrients. These are stressful conditions, not unusual during must fermentation, which, by affecting lipid biosynthesis may exert a negative effect on yeast viability. The results obtained showed that the three strains were able to modulate cell lipid composition during fermentation. However, only two of them, which showed highest viability and membrane integrity at the end of the fermentation process, reached a fatty acid composition which seemed to be optimal for a successful adaptation. In particular, C16/TFA and UFA/TFA ratios, more than total lipid and ergosterol contents, seem to be involved in yeast adaptation.

Keywords: Wine yeast; *Saccharomyces cerevisiae*; Viability; Lipid composition; Membrane integrity; Fatty acids

1. Introduction

During wine production yeast strains are subjected to a variety of biotic and abiotic stresses which, when prevailing over the cellular defence systems, can affect cell viability, with negative consequences on the progression of the fermentative process (Attfield, 1997; Zuzuarregui and del Olmo 2004a; Zuzuarregui et al., 2005). Thus, to avoid stuck or sluggish fermentations, wine strains should be able to counteract the effects exerted by environmental stressors through the activation of an adequate stress response (Ivorra et al., 1999; Trabalzini et al., 2003; Zuzuarregui and del Olmo 2004a; Zuzuarregui and del Olmo 2004b). Accordingly, the existence of a correlation between fermentative behaviour and stress resistance has been shown in *Saccharomyces cerevisiae* wine strains (Ivorra et al., 1999; Querol et al., 2003; Zuzuarregui and del Olmo 2004b).

Among the environmental factors that influence the progression of must fermentation, oxygen availability and ethanol accumulation are of primary importance due to their effect on composition and functional properties of cell membranes.

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Oxygen deprivation negatively affects lipid biosynthesis either directly, by blocking oxygen-dependent enzymes (e.g. Δ9-desaturase, squalene epoxydase, the lanosterol demethylation complex), or indirectly, by causing the accumulation of saturated fatty acids (SFAs) and precursors of ergosterol, which regulate the expression of acetyl-CoA carboxylase and hydroxymethylglutaryl-CoA reductase, respectively (Bloomfield and Bloch, 1960; Parks, 1978; Henry, 1982; Wakil et al., 1983; Ratledge and Evans, 1989; Hammond, 1993). Thus, under anaerobiosis, yeast cells are unable to complete the biosynthesis of unsaturated fatty acids (UFAs) and ergosterol, and accumulate intermediates of lipid metabolism (Bardi et al., 1998; Bardi et al., 1999; Belviso et al., 2004). In these conditions, if lipid nutrients are not available, *S. cerevisiae* cells progressively change the composition of their lipid fractions, reducing the surface area of organelle membranes and diluting their lipid content until the limit of viability (Henry, 1982). It thus follows that *S. cerevisiae* viability is low during growth in the absence of oxygen and lipid nutrients (Fornairon-Bonnefond et al., 2002) and that the number of generations produced by wine strains may depend on the initial sterol content (Deytieux et al., 2005).

Lipid composition of yeast cell membranes and ethanol tolerance are strictly related (Thomas et al., 1978; Piper, 1995). In particular, the ability to operate acyl chain unsaturation (Thomas et al., 1978, Chi and Arneborg, 1999; You et al., 2003) and ergosterol biosynthesis (Shobayashi et al., 2005) seems to be essential for ethanol tolerance, particularly during grape must fermentation, a process that yeasts carry out under hypoxic conditions and increasing ethanol concentrations. These conditions compromise the biosynthesis of sterols and fatty acids thus causing variations in the amount and composition of the lipid fraction of cell membranes. The adaptive response to produced ethanol was evaluated by Arneborg et al., (1995) in chemostat grown cells of *S. cerevisiae.* A part from that work, most of the data regarding the correlation between ethanol tolerance and cell lipid composition derived from the analysis of cells subjected to ethanol shock (Thomas et al., 1978; You et al., 2003; Aguilera et al., 2006) while, to our knowledge, no attempt has been made to assess changes in the lipid composition of several *S. cerevisiae* strains, with different ethanol tolerances, during the adaptation to self-produced ethanol in batch fermentations.

In the present study we investigated the ability of three strains of *S. cerevisiae* to adapt to unfavourable conditions of fermentation, in terms of cell lipid composition, membrane integrity, viability and fermentative activity. The rationale was that, during hypoxic growth in a sugar-rich medium lacking lipid nutrients, the ability to modulate cell lipid composition may be one of the factors involved in yeast survival and adaptation to stressful conditions of fermentation.

2. Materials and methods

2.1. Strains and culture conditions

The following *S. cerevisiae* strains were used: BY4743, a laboratory strain (*S. cerevisiae* MATa/MATα Δ *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0* purchased from Euroscarf, Frankfurt, Germany); L2056, a commercial enological strain (Lallemand, Montreal, Canada); M25, a flor wine strain commonly utilized for must fermentation at the industrial level, deposited with the Culture Collection of DiSAABA (Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agroalimentari, University of Sassari, Sardinia, Italy). Bench-scale fermentations were carried out in triplicate in SJ medium (2 g l^{-1} YNB without amino acids, 7 g l^{-1} ammonium sulphate, 120 g l^{-1} glucose, 120 g l^{-1} fructose, 30 mg l^{-1} leucin, 20 mg l^{-1} histidine, 20 mg l^{-1} uracil, pH 4.4). Briefly, yeast strains were pre-cultured aerobically in YEPD (20 g l^{-1} glucose, 10 g l^{-1} yeast extract, 20 g l^{-1} peptone) (liquid:air ratio, 1:10), and 5×10^5 cells ml^{-1} were inoculated in 100 ml flasks containing 75 ml SJ medium (liquid:air ratio, 7.5:10) equipped with glass capillary stoppers and incubated statically at 20 °C for 25 days. A flask for each sampling time was inoculated, and both the culture broth and cells underwent the analyses described below. Yeast growth was determined by viable plate counting. All experiments were carried out at least in triplicate from independent pre-cultures.

2.2. Analytical determinations of fermented SJ medium

The 10139106035 and 10176290035 enzymatic kits (R-Biopharm Boehringer–Mannheim, Germany) were used for the determination of the residual glucose and fructose contents and for the production of ethanol, respectively, at the time points indicated during fermentation.

2.3. Cell lipid extraction

Cells were collected at days 1, 3, 7 and 20 by centrifugation (5 min at 625 × g), washed in sterile water and freeze dried. The pellets were powdered in a mortar, weighted and subjected to lipid extraction. This was performed according to Taylor and Parks (1978) modified as described by Belviso et al., (2004). Pentadecanoic acid in chloroform was added as internal standard. The lipid extract was dried in a rotary evaporator (Rotovapor, Laborota 4000, Heidolph Instruments GMBH & Co KG, Schwabach, Deutschland) and dissolved in 5 ml chloroform for storage at −25 °C for no longer than 2 weeks. Fatty acids and sterols contents were referred to the dry weight of freeze dried cells.

2.4. Determination of cellular fatty acid content

The methyl esters of the fatty acids contained in lipid extract prepared as described above, were obtained according to Christie (1982), and then analyzed by gas chromatography with a DANI GC 1000 DPC, equipped with an FID detector (DANI Instruments s.p.a., Milan, Italy) on a DB-5 capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific Inc., Folson, CA, USA). The operating conditions were: temperature from 80 °C to 120 °C at 4 °C min^{-1}, from 120 °C to 220 °C at 5 °C min^{-1}, from 220 °C to