Food Microbiology 29 (2012) 121–131

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

The impact of co-inoculation with *Oenococcus oeni* on the trancriptome of *Saccharomyces cerevisiae* and on the flavour-active metabolite profiles during fermentation in synthetic must

Debra Rossouw, Maret Du Toit, Florian F Bauer*

Institute for Wine Biotechnology, University of Stellenbosch, Stellenbosch, South Africa

ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form
12 August 2011
Accepted 10 September 2011
Available online 16 September 2011

Keywords:
Transcriptome
Saccharomyces cerevisiae
Oenococcus oeni
Alcoholic fermentation
Malolactic fermentation

ABSTRACT

Co-inoculation of commercial yeast strains with a bacterial starter culture at the beginning of fermentation of certain varietal grape juices is rapidly becoming a preferred option in the global wine industry, and frequently replaces the previously dominant sequential inoculation strategy where bacterial strains, responsible for malolactic fermentation, are inoculated after alcoholic fermentation has been completed. However, while several studies have highlighted potential advantages of co-inoculation, such studies have mainly focused on broad fermentation properties of the mixed cultures, and no data exist regarding the impact of this strategy on many oenologically relevant attributes of specific wine yeast strains such as aroma production. Here we investigate the impact of co-inoculation on a commercial yeast strain during alcoholic fermentation by comparing the transcriptome of this strain in yeast-only and in co-inoculated fermentations of synthetic must. The data show that a significant number of genes are differentially expressed in this strain in these two conditions. Some of the differentially expressed genes appear to respond to chemical changes in the fermenting must that are linked to bacterial metabolic activities, whereas others might represent a direct response of the yeast to the presence of a competing organism.

1. Introduction

Alcoholic fermentation of grape juice to produce wine is a highly complex process resulting from the combined metabolic activities of various species of yeast and bacteria. The metabolic activities of these microorganisms collectively transform the starting juice into the final fermented product, and are responsible for the quality and unique characteristics of the wine produced. Of the yeast species that are present in juice, *Saccharomyces cerevisiae* tends to dominate by the end of spontaneous fermentations (Frezier and Dubourdieu, 1992), and is the species which is most often used as a starter culture in inoculated fermentations. *S. cerevisiae* is also widely used as a model eukaryote for molecular and cellular biology, and as such global analysis tools such as DNA microarrays for transcriptomic analysis of *S. cerevisiae* are well developed and easily available (Lashkari et al., 1997).

The most important bacterial activity in wine relates to malolactic fermentation (MLF), which is most commonly associated with the species *Oenococcus oeni*, a lactic acid bacterium. Other species that may conduct MLF include *Lactobacillus plantarum* which may be important in high pH wines (Versari et al., 1999; Du Toit et al., 2010; Lerm et al., 2010). MLF is a secondary fermentation whereby malic acid, naturally present in grape must, is converted to lactic acid by decarboxylation. MLF is important from an oenological perspective as it decreases wine acidity, enhances microbial stability and improves the aroma, flavour and mouthfeel attributes of the wine (Rodriguez and Amberg, 1990; Avedovech et al., 1992; Bartowsky et al., 2002). MLF traditionally occurs shortly after the end of primary fermentation, but current winemaking trends have seen mounting interest in using co-inoculation of yeast and malolactic bacteria at the start of alcoholic fermentation. Possible advantages of this strategy include an increase in fruitiness and balance of the wine, the prevention of off-flavour production by other bacterial strains and a reduction in total fermentation time (Kunkee, 1991; Du Toit et al., 2010; Lerm et al., 2010).

However, little is known about interactions between yeast and bacteria during such co-inoculations, and how such interactions may impact on the fermenting organisms, both in terms of fermentative properties and metabolite production. To investigate such interactions, omic-tools should provide an ideal platform. However, in the case of *O. oeni*, whole genome sequence

* Corresponding author. Tel.: +27 21 8083770.
E-mail addresses: debra@sun.ac.za (D. Rossouw), mdt@sun.ac.za (M. Du Toit), fb2@sun.ac.za (F.F. Bauer).

0740-0020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2011.09.006

information has only recently become available (Mills et al., 2005), and the tools available to study this organism are not as developed as for yeast. Borneman et al. (2010) applied an array-based comparative genome hybridization (aCGH) approach in order to investigate the genomic diversity across ten naturally isolated wine strains of *O. oeni* relative to PSU-1, a commercially available strain whose complete genome was sequenced previously (Mills et al., 2005). Further whole genome transcriptional arrays will potentially provide in depth insights into the effects of environmental influences on *O. oeni* gene expression and behaviour and help to elucidate the metabolic functioning of these organisms during winemaking. Such microarrays, though standard in yeast research, are yet to be made available for comparative transcriptomic studies of *O. oeni*.

Three different kinds of interactions between microorganisms are described in the literature, including inhibition, stimulation and neutralism. Of those modes of interaction, inhibition of LAB by yeast has been most commonly reported in previous studies (Lemaresquier, 1987; Arnink and Henick-Kling, 2005; Nehme et al., 2008). Such studies have also highlighted the complexity of yeast-bacterial interactions, showing that the same yeast strain may either inhibit or stimulate different bacterial strains in winemaking conditions. Recent research has shown that the synergistic inhibitory effects of ethanol, SO2, fatty acids and reduced nutrient content may partly explain the interaction effects of yeast and LAB but do not clarify it entirely (Nehme et al., 2008).

A few studies have reported on yeast strains producing proteins that are active against *O. oeni* (Comitini et al., 2004; Osborne and Edwards, 2007). On the other hand, certain substances that are released by yeasts, such as amino acids (Fourcassier et al., 1992) and mannoproteins (Guilloux-Benatier et al., 1995), may have a positive impact on bacterial growth and malolactic activity. Other stimulatory molecules may include vitamins, nucleotides and lipids, though these have been poorly studied (Alexandre et al., 2004). Importantly, very little consideration has been given to date on the effect that the bacteria may have on the fermenting yeast in the context of co-inoculated MLF.

From a winemaking perspective a better understanding of the consequences of co-inoculation and its impact on yeast and bacterial strains is clearly essential in order to ensure successful MLF and to fully appreciate the impact on the fermentation derived aroma and flavour-active compounds. Considering the large number of commercial yeast and LAB strains available to industry, systematic screening of all possible yeast-bacteria combinations is not feasible. In order to successfully pair yeast and bacteria, and to guarantee successful MLF, detailed information of the nature of the interactions is needed. Thus far, knowledge of the underlying genetic and molecular mechanisms governing yeast–bacterial interaction is lacking. Here we describe a transcriptome-based approach towards understanding the genetic response of a commercial yeast strain to the presence of co-inoculated bacteria. Transcriptomic analyses of wine yeast strains have been on the rise in recent years due to the wealth of information derived from such studies (Erasmus et al., 2003; Rossignol et al., 2003; Varela et al., 2005; Mendes-Ferreira et al., 2007; Marks et al., 2008; Rossouw et al., 2008; Rossouw et al., 2009). These studies have focussed on gene expression changes occurring at different stages of fermentation, or in response to experimental perturbations related to fermentation temperature or medium composition for example. Gene expression of fermenting yeast in mixed fermentations with other microorganisms is still an unexplored area of research.

To approach this issue experimentally, a commonly used industrial yeast strain was inoculated both alone and together with a commercial bacterial starter culture in a synthetic grape juice. Since gene expression arrays for *O. oeni* were not yet available at the time of these experiments, our point of departure was to view the mixed fermentation from the molecular perspective of the fermenting yeast. Transcriptomic analysis was carried out at two time points during fermentation, and volatile aroma compound concentrations, yeast and bacterial growth as well as malic acid degradation were also determined at key time points. The data show that a significant number of genes are differentially expressed in the yeast in these two conditions. Some of the differentially expressed genes are likely expressed in response to chemical changes in the fermenting must that are brought about by bacterial metabolic activities, whereas others might represent a more direct response by the yeast to the presence of a competing organisms. Interestingly, genes that showed strong differential expression in the early stage of fermentation were mostly non-annotated genes, suggesting that this response may not have been covered by the standard phenotypic screens employed in current system-wide data analysis and phenotyping approaches.

2. Materials and methods

2.1. Strains, media and culture conditions

In this study the popular commercial yeast strain VIN13 (Anchor Yeast, South Africa) and *O. oeni* strain S6 (Lerm et al., 2010) were used. Yeast cells were cultivated at 30 °C in YPD synthetic media 1% yeast extract (Biolab, South Africa), 2% peptone (Fluka, Germany), 2% glucose (Sigma, Germany). *O. oeni* was cultured anaerobically in MRS media (Biolab, South Africa) supplemented with 10% tomato juice (called MRST), pH adjusted to 5.5. Solid media was supplemented with 2% agar (Biolab, South Africa).

2.2. Fermentation media

The medium composition of the synthetic grape juice (pH 3.5) was based on a similar media by Nehme et al. (2008) and consisted of: glucose (100 g/l), fructose (100 g/l), yeast extract (Oxoid) (1 g/l), $(NH_4)_2SO_4$ (2 g/l), citric acid (0.3 g/l), L-malic acid (5 g/l), L-tartaric acid (5 g/l), $MgSO_4$ (0.4 g/l), KH_2PO_4 (5 g/l), NaCl (0.2 g/L), $MnSO_4$ (0.05 g/L).

2.3. Fermentation conditions

All fermentations were carried out under microaerobic conditions in 100 ml glass bottles (containing 80 ml of the medium) sealed with rubber stoppers with a CO_2 outlet. The fermentation temperature was approximately 22 °C. Fermentation bottles were inoculated with yeast in the logarithmic growth phase ($OD_{600} = 1$) to an OD_{600} of 0.1 (i.e. a final cell density of approximately 10^6 cfu ml^{-1}). Dry preparations of *O. oeni* S6 were hydrated in distilled water for 20 min before inoculation into the synthetic must. *O. oeni* was inoculated to a final cell density of approximately 5×10^6 cfu ml^{-1}. The fermentations followed a time course of 13 days and the fermentation bottles were weighed regularly to assess the progress of alcoholic fermentation. Samples for transcriptomic and volatile aroma analysis were taken at days 3 and 7 of fermentation. Fermentations of control (yeast-only) and mixed (yeast and O. oeni) cultures were performed in triplicate.

2.4. Growth measurement

Cell proliferation (i.e. growth) was determined spectrophotometrically ($Powerwave_x$, Bio-Tek Instruments) by measuring the optical density (at 600 nm) of 200 μl samples of the suspensions over the 13 day experimental period. At days 0, 3, 7 and 13 viable cell counts were determined by plating onto selective media. For