Phenotypic Investigation of Biofilm Formation and Transcriptional Analysis of Invasive Growth of Commercial Wine *Saccharomyces cerevisiae*

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Contents

Nomenclature

Term	Description
Biofilm	Surface-attached multicellular communities with an extracellular matrix including any related biofilm-forming ability tests such as mat formation and plastic adhesion
Mat	Thin layer of yeast biomass on low-density agar that resembles a film
Filamentous growth	Interchangeable with pseudohyphal growth, a form of growth as a colony that has a filamentous shape, usually contains chains of elongated cells
Filamentous mat	A mat that has a filamentous periphery
Invasive growth	A form of growth that penetrates agar
'Hub and spokes' mat	A flat mat that has raised cables radiating from the hub

NB Filamentous growth and invasive growth are not ploidy-specific unless specified.

Abstract

This study investigated the morphological properties, environmental effects on and gene expression of biofilms, more specifically referred to as mats, formed by laboratory and commercial wine strains of *Saccharomyces cerevisiae*. Two morphological assays were conducted: mat formation and plastic adhesion. Mat features varied between strains and included various architectures, cellular morphologies, and incidence of invasive growth. One commercial strain, L2056, formed mats where a sector produced a distinctive mat morphology, which was retained when subcultured. In considering the role of biofilms in winery conditions, mat formation assays were also performed with grape pulp and adhesion to the soft plastic of common winery hoses. All strains grew invasively on all agar media and appeared to conduct fermentation on the grape-pulp mat assay. Some strains also had the ability to adhere to winery hose plastic. When only limited nitrogen was available, both laboratory and commercial wine strains formed mats with a subpopulation of cells that switched to filamentous and invasive growth. Such invasive growth was influenced by nitrogen concentration, the presence of a neighbouring mat, and by the addition of yeast metabolites. Ethanol and hydrogen sulfide were found to enhance invasive growth of cells within mats exposed to low levels of nitrogen whereas tryptophol and 2-phenylethanol suppressed this enhancement. Sulfite was found to delay overall mat growth. In an effort to understand the cellular decision to switch morphology, changes in the transcriptome of invasively growing cells were studied. In this analysis, 272 genes were identified to be upregulated and 84 genes were downregulated in invasively growing cells. Of the ten largest differentially expressed genes, four were genes encoding hexose transporters (*HXT3*, *HXT4*, *HXT6* and *HXT7*) which had an increase in transcript abundance up to 13-fold. One hypothetical gene (AWRI796_5153) with a 6-fold increase in transcript abundance, has translation sequence homologous to an amidase domain. Following differential expression and Gene Ontology analysis, five GO categories represented the 37 significantly enriched GO terms in the upregulated gene set of invasively growing cells, these being glucose import, carbohydrate metabolic process, fungal-type cell wall organisation, medium-chain fatty acid biosynthetic process and cellular water homeostasis. Since cellular water homeostasis has not previously been associated with invasive growth, and four out of five genes in this group were found to be significantly upregulated in the invasively growing cells, further analysis of deletion mutants of each of these confirmed that *FPS1*, encoding the glycerol export protein, is required for invasive growth of yeast mats in low nitrogen conditions. In summary, this work reports the phenotypic properties of commercial wine yeast biofilms in

environments of both rich nutrient and low nitrogen, either in typical laboratory type agar media or in conditions simulating that of a grape or wine hose. The ability of these yeasts to form complex morphologies, grow invasively into grape solids and attach to winery hose plastic may confer their residency and survival in the vineyard and winery. The influence of different yeast metabolites and transcriptional changes in invasively growing cells provide further understanding of this morphogenetic program.

Declaration statement

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Thesis overview and structure

Purposefully inoculated fermentations using commercial wine yeast are broadly implemented due to their success in completing fermentation efficiently and producing quality wine. Many commercial wine strains, usually *Saccharomyces cerevisiae*, were originally isolated from indigenous microflora of successful fermentations. These strains are now produced commercially and are widely available. Evidence suggests that the use of commercial strains leads to their presence and survival in the vineyard and winery. This could potentially lead to an alteration in native microflora in must and subsequently influence the regional character of wine. The mechanism of how these commercial yeasts remain in the winery environment is barely understood.

Biofilm formation is considered a survival strategy for many fungi and bacteria under harsh conditions. *S. cerevisiae* has been reported to be able to form biofilms, evidenced by the ability to grow into a mat and to adhere to plastic. These abilities have been investigated in not only laboratory strains, but also some clinical yeasts and yeasts isolated from wine grapes and must. It is likely that commercial wine yeasts also possess the ability to form biofilms which could drive their residency in the wine making environment. Current knowledge on yeast biofilms has focussed on the laboratory strain Σ1278b which is not directly applicable to the understanding of wine yeast biofilms since they are substantially genetically different. The study of biofilm formation by commercial wine yeast and their characteristics is therefore warranted.

Given that biofilms are a multicellular growth form, and nitrogen is known to be essential for yeast proliferation, biofilm formation could be affected by nitrogen availability. Lack of nitrogen has been shown to induce a pseudohyphal (filamentous) and invasive growth response in yeast. Filamentous mats can be formed when cells are starved for glucose but the mat formation response to low nitrogen has not been reported. Filamentous and invasive growth responses can be manipulated by other environmental triggers and putative signalling molecules such as temperature, pH, atmosphere, preservatives and fusel alcohols. Studies have shown that cell-cell communication can occur in biofilm formation, but the involvement of this system in yeast, especially *S. cerevisiae* biofilms is poorly understood.

In a quest to understand wine yeast biofilms in greater details, the present study has three aims:

1. Investigation of commercial wine yeast biofilm-forming ability and characteristics;
2. Exploration of mat formation in low nitrogen conditions and the influence of putative quorum sensing or signalling molecules; and
3. Study the genetic regulation of wine yeast biofilms or related phenotypes.

This dissertation has been organised in several chapters to present background information, reports of studies to answer each of the aims, and a conclusion. Chapter 1 establishes the field of knowledge and summarises critical gaps for the present study. Areas of discussion include yeast biofilms and related morphological phenotypes, influence of nitrogen availability, and potential signalling molecules. Chapter 2 presents mat characteristics and plastic adhesion properties of commercial wine yeast strains which addresses Aim 1. Chapter 3 addresses Aim 2 where data involving mat formation of commercial wine yeast strains in a low nitrogen environment and the response to potential signalling molecules is presented. Based on the findings from Chapter 3 that describe invasive growth as the primary observation in low-nitrogen, Chapter 4 presents the investigation of transcriptional changes of invasively growing wine yeast. Chapter 5 is a summary of the main findings including how this work contributes to current knowledge, limitations and future directions of the work.

Chapters 2–4 are presented as unsubmitted work prepared in manuscript style. For consistency, the typing, format, and referencing styles have been adjusted. Numbering of figures and tables has also been modified according to the order in the dissertation. References from all chapters can be found in the Bibliography.