

YEAST: THE WINE BUILDER

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ABSTRACT

“A good wine is “built” in the vineyard” – notwithstanding the glamorous stature of many winemakers (and the relative obscurity of many viticulture practitioners), this sentence passes for truth when issues of wine and of wine quality are discussed. A derivative version of this statement contends that a winemaker may well be in a position to destroy an excellent harvest, but will always fail to make a “good” wine from “bad” grapes. This argument clearly has strong support within the wine making community. But is it necessarily true? What tools may a winemaker use to build a wine in the cellar? Working with a biological system, the most direct and technically least interventionist response to this question may be found in a winemaker’s ability to modulate the incredible complexity and variability of microorganisms that contribute to wine elaboration during and sometimes after fermentation. Without doubt, the most important of these organisms is the wine yeast, as it is responsible for the majority - and for the most important - of the chemical transformations that a grape juice undergoes to become a wine. In this paper, our current understanding of how these transformations occur, how they are regulated, how they impact on the final product, and how different yeast can be used to achieve very different outcomes will be reviewed.

ABSTRACT

“Ein guter Wein wird im Wingerd gemacht” – obgleich der relativ hohen Popularität vieler Kellermeister (zumindest wenn verglichen mit dem Bekanntheitsgrad vieler Wingerdmanager) wird dieser Satz häufig als Wahrheit akzeptiert wenn Weinqualität zur Diskussion steht. Eine etwas andere Version dieses Satzes besagt dass ein Kellermeister zwar durchaus schlechten Wein von einem guten Saft machen kann, aber niemals einen guten Wein von schlechten Trauben produzieren wird. Dieses Argument findet viel Anklang in der Weinwirtschaft – aber ist es unbedingt wahr? Welche Werkzeuge hat ein Kellermeister zur Verfügung um einen Wein im Keller aufzubauen? Da der Gärungsprozess ein gänzlich mikrobiologischer Prozess ist, sollte die direkte, und technisch einfachste Antwort zu dieser Frage in einer besseren Nutzung der natürlichen mikrobiologischen Diversität liegen. Die Weinhefe ist ohne Zweifel der wichtigste der Mikroorganismen im Wein, denn sie ist für die meisten und auch wichtigsten chemischen Prozesse die den Traubensaft in Wein verwandeln verantwortlich. In diesem Artikel wird unser gegenseitliches Verständniss dieser Transformation diskutiert. Spezifische Fragen die beantwortet werden beziehen sich auf die Regulation des Prozesses durch Umwelteinflüsse, und wie verschiedene Hefen besser genutzt werden können um verschiedene und bessere Resultate zu erzielen.

INTRODUCTION

It is somewhat trite to state that there are many different ways that vines can be grown and that wine can be made, and that indeed as a consequence an incredible range of different wines and styles of wine are produced and sold globally. Each of these wines has its own specific character, notwithstanding a frequent claim that winemaking and the product “wine” may have become more standardised over the past decades as a consequence of the pressures exerted by the global wine market. The character of an individual wine is

dependent on an incredible number of factors, starting with the genetic potential (defined by the varietal and clonal origin) of the grapevine, the interaction of this grapevine with its specific environment (natural environmental and human intervention such as planting patterns, trellising, soil treatments, and irrigation), the processing practices during and directly after harvest (selection of grapes, pressing, maceration etc) and the winemaking strategy adopted by the winemaker. Finally, different maturation, ageing and packaging strategies will continue to change the character of the product before it reaches the consumer.

The basic wine sciences of viticulture and oenology have been trying to improve our understanding of this baffling complexity through what can broadly be described as descriptive approaches, based mostly on establishing statistically meaningful correlations between measurable parameters that impact on or are part of the wine production chain. Such parameters can fall into different categories such as environmental or biological, natural or human-induced. Many viticultural approaches for example may correlate specific vineyard management practices with the ripening of berries and the composition of grape juice, while oenological projects may investigate the impact of physical or biological interventions in the cellar on wine composition and perhaps quality. However, in most of these studies, no causal relationship between the investigated factors and the observed outcomes can usually be established. In large part as a consequence, the wine elaboration process today continues to be almost entirely guided by empirical evidence and personal experience, or simply by the gut-feel and the individual taste buds of the producer.

While this state of affairs is certainly in line with the public image of wine as a nature-based and highly individualised product, it does present a challenge for the global wine industry. In the globalised economic system, wine is a product whose economic success is primarily measured in comparison with those of competing beverages such as beer or soft drinks, and less in terms of whether wines from one country or another have lost or won market share – however important such data may be for individual producers. One fundamental difference in this regard is that most products competing with wine can rely on reasonably product identity outputs, allowing branding and the consistent development of customer loyalty for each offering. Importantly, providing an identifiable product does not equal reducing diversity – it refers at least as much to being able to produce a wine that reflects all the particularities of its origin.

In this presentation, current and future approaches to improving our ability to predict fermentation outcomes will be discussed, in particular with regard to the impact of yeast on wine character and quality. The importance of new analysis tools and methodologies, such as those derived from the field of systems biology, on our scientific efforts to better understand the complex interactions of yeast strains with the environment and to better control the fermentation process are highlighted. The driving force behind such approaches is to be able to match specific grape juice composition with specific microbial systems and environmental interventions. The presentation will also reflect a shift in wine research away from improving individual aspects of winemaking and wine yeast strains to providing a holistic understanding of the entire process to better exploit the genetic potential of the natural wine microfauna.

THE WINE MAKING PROCESS AND YEAST

Alcoholic fermentation of grape juice is a complex process resulting from the combined metabolic activities of various species of mainly yeast and bacteria. The metabolic activities of these microorganisms collectively transform the juice into a fermented product, and are in large part responsible for the quality and unique characteristics of the wine produced. The process of wine making and the nature of microorganisms involved have been reviewed extensively in the past (Pretorius, 2000; Swiegers et al. 2005). In short, several yeast species may be dominant at the beginning of fermentation, depending on the specific microflora present in the vineyard, on the grapes or in the cellar. Indeed, several such species may

successively dominate the early stages of fermentation (Le Jeune et al. 2006). Of the yeast species that are present in juice, species of the genus *Saccharomyces* tend to dominate by the end of spontaneous fermentations (Frezier & Dubourdieu, 1992). Such species are also applied as starter cultures when grape juice is inoculated.

Glycolysis, the biochemical pathway of the conversion of hexoses (in the case of wine of glucose and fructose in close to equimolar amounts) to ethanol, CO₂ and chemical energy is probably one of the best characterised, described and modelled of all metabolic pathways (Snoep 2005, Teusink et al. 2000). While this pathway alone profoundly transforms and changes the chemical and sensorial matrix of grape juice, quality of wine is more profoundly affected by all the other metabolic activities of the yeast during and after fermentation. Indeed, few, if any of the major components of grape juice are left untouched, including many varietal metabolites that are seen as signatures for specific wines (Swiegers and Pretorius, 2007).

YEAST METABOLIC ACTIVITY AND IMPACT ON WINE CHARACTER

As any good builder, wine yeast strains first and foremost provide a scaffold or matrix around which to construct the individual character of a wine. Indeed, the quantitatively most prominent compounds produced by alcoholic fermentation, ethanol and CO₂, are also the most obvious feature identifying the products “wine” or “sparkling wine”, and those compounds also provide the backbone for the sensorial perception of all other flavour and aroma active compounds in wine (Fig. 1). While ethanol in particular has intrinsic flavour and aroma active properties, described sometimes as sweetness or as hotness, the compound is clearly more relevant in terms of its impact on the perception of other flavour and aroma compounds than for its own activity (Yu and Pickering, 2008). Similarly CO₂ is for obvious reasons the defining element in sparkling wine. A third compound that can be added to this list of matrix elements is glycerol, since its impact in wine also appears more related to providing a matrix than to its intrinsic flavour or aroma activity (Nieuwoudt et al. 2002). By producing those three compounds, the yeast establishes a scaffold which will indirectly affect all other flavour and aroma compounds that contribute to the specific characteristics of wine. Finally, another yeast-derived product that might be added the list of matrix elements are certain yeast mannoproteins that have been shown to significantly contribute to the overall mouthfeel of wine (Brown et al. 2007, Gonzalez-Ramos and Gonzalez, 2006).

Beyond the matrix, a large number of flavour active compounds impacting on taste are produced. Of the five basic tastes, sweetness and acidity are the most dominant and relevant in wine, and both are profoundly altered through microbial metabolic activity during fermentation. Most obviously, the amount of residual sugar in wine is usually the main cause of sweetness, and is a direct result of the fermentation properties of the yeast strain (at least in the absence of direct intervention by the winemaker to stop fermentation prematurely). Interestingly certain yeast proteins such as Hsp12 have been shown to significantly contribute to the overall perception of sweetness (Marchal et al. 2011).

The perception of acidity in wine, on the other hand, is a direct result of the concentration and combination of organic acids that are both grape and yeast derived. While grape-derived tartaric acid is found in the highest concentration in both grapes and wine, and is not significantly affected by the fermenting microflora, all other relevant organic acids undergo significant concentration changes during fermentation. Malic acid, the second most important grape acid, is usually reduced during alcoholic and more obviously malolactic fermentation, while other acids such as succinic, pyruvic and lactic acid can be produced in significant amounts by wine yeast strains and bacteria. The impact of the balance of individual organic acids in wine on wine sensory perception has received little attention in the past, although each acid has a different sensorial impact, and different ratios of organic acids should result in different sensory properties of wine, even if the case of identical total acidity. Other tastes,

such as bitterness, can be affected by the microflora, sometimes linked to the presence of spoilage organisms, and will not be further discussed here.

While the wine matrix and the flavour compounds provide structure, wine is probably most prominently defined by its aromatic properties, and the wine microflora completely modifies the volatile aroma of grape juice during fermentation. The impact of wine microorganisms, and again most prominently of wine yeast strains, is most obvious in terms of the *de novo* production of compounds such as esters, higher alcohols, fatty acids and aldehydes (Swiegers et al. 2005, Lilly et al. 2006). These compounds, sometimes referred to as secondary metabolites, are produced by a complex network of metabolic pathways, and their production has been shown to be strongly dependent on yeast strain and the composition of the grape juice, in particular regarding the availability of precursors such as amino acids. Furthermore, yeast supports the release of aroma active compounds from grape-derived precursors, as is for example the case for many monoterpenes and volatile thiols (Gamero et al. 2011, Swiegers and Pretorius 2007). This release of aroma active compounds may be dependent on the secretion of hydrolytic enzymes by yeast, or due to enzymatic activities within the yeast, a process sometimes referred to as biotransformation.

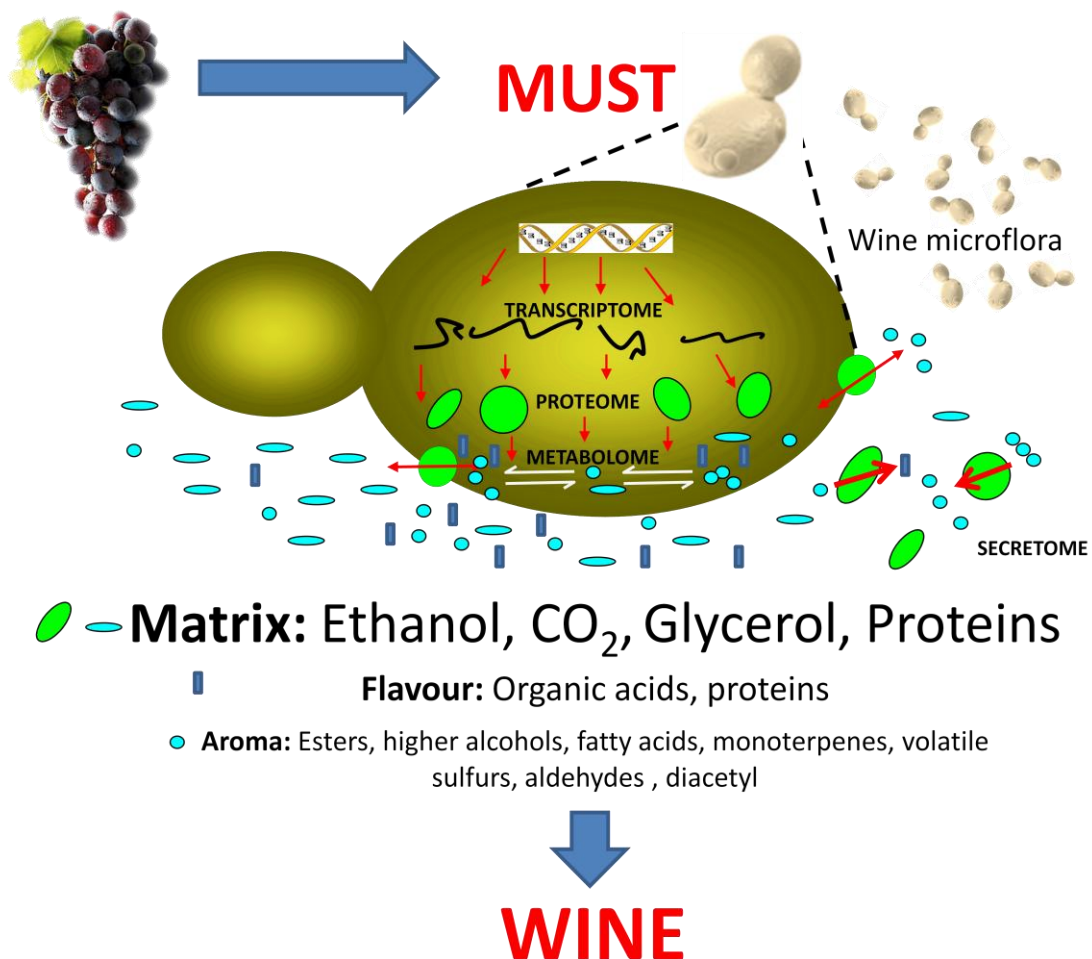


Fig.1. General diagram of the impact of yeast in wine fermentation. The three types of oenologically relevant metabolites that are produced by yeast – general matrix, flavour and aroma compounds - are indicated, as are the most relevant and studied levels of the biological information transfer system (Genome, transcriptome, proteome, metabolome and secretome) whose regulation will define fermentation progress and the production of flavour and aroma active impact compounds. The secretome (all secreted proteins) has many wide-ranging impacts such as enzymatic activities, mouthfeel, haze protection and flavour activities

Finally, the wine microflora also impacts significantly on other sensory aspects of wine, such as visual appearance and mouthfeel. In this regard, protein secretion in the form of hydrolytic enzymes by yeast plays a dominant role, as does autolysis of yeast at the end of fermentation (

Considering the wide ranging impacts, it appears clear that the global wine industry is far from using to the full the genetic potential of the wine-associated microbial diversity. This is most easily demonstrated by highlighting our limited understanding of even the best studied of the biological systems contributing to the building of wine, the currently available commercial wine yeast strains.

WINE YEAST STRAIN DIVERSITY

Our ability to optimally exploit microbial diversity to build a desired wine depends to a large degree on our knowledge and understanding of the genetic potential of the large number of microorganisms that impact on wine. Of these organisms, yeast has been by far the most studied and is the best understood. The genus *Saccharomyces* can be divided into two major groups, *sensu stricto* and *sensu lato* (Barnett, 1992). The *sensu stricto* yeasts include *S. bayanus*, *S. cerevisiae*, *S. paradoxus*, and *S. pastorianus* (Kurtzman & Robnett), but *S. cerevisiae* is the species that is most widely used in the fermentation industry (oenology, bread-making and brewing). *S. cerevisiae* is also widely used as a model eukaryote for molecular and cellular biology, and as such global analysis tools such availability of several genome sequences and DNA microarrays for transcriptomic analysis of this yeast are well developed and easily available. *S. cerevisiae* has been studied at the genetic level since the 1930's. Most of these studies were carried out using only a handful of strains (Mortimer *et al.*, 1957; Mortimer and Johnston 1986) that were selected for their ease of use in laboratory conditions. Thus the knowledge regarding the genetics and molecular biology of *S. cerevisiae* is mostly based on a geno- and phenotypically narrow range of strains, while studies of natural populations and industrial strains of *S. cerevisiae* have been lagging behind (Mortimer 2000).

In contrast to the 'laboratory' yeast strains, industrial yeast strains are geno- and phenotypically highly diversified (Frazier & Dubourdiou, 1992; Schütz & Gafner, 1994; Rossouw *et al.*, 2009). In the wine industry, a large number of such strains are commercially produced. Most of the strains were originally isolated from spontaneous wine fermentations and are of the species *S. cerevisiae*, but other closely related species such as *S. bayanus* or *S. paradoxus* have also been used. These and other closely related species provide a wide range of geno- and phenotypic profiles, which thus far has not been exploited to its full extent. It can be ventured without taking many scientific risks that many new or modified styles of wine can still be invented by making use of this potential. There is clearly also significant potential in further pushing the evolutionary development of win yeast strains. Although the original or natural ecological niche of the species *S. cerevisiae* is still subject to conjecture, industrial environments have certainly provided much of the evolutionary framework for the strains that are currently used in industry, providing an excellent opportunity to conduct comparative studies to investigate evolutionary relationships and the molecular mechanisms underlying phenotypic differentiation. In this regard, it has for example been suggested that functional binding sites of transcription factors and the transcription factors themselves may be the primary evolutionary targets of diversification in yeast (Zheng *et al.* 2010).

APPROACHES TO UNDERSTAND AND EXPLOIT WINE YEAST GENETIC DIVERSITY

Past and present

Traditionally, strains isolated from spontaneous fermentations were produced commercially and were selected and studied for their phenotypic characters. With the increased popularity of inoculation, specific strain development programs were undertaken based on hybridisation of wine yeast and mutagenesis. A number of specific breeding programs were implemented to improve certain characteristics of wine yeast strain (Johnston et al. 2000; Mortimer & Polsinelli, 1999). While breeding of new yeast strains continues to this day, and has more recently included the generation of a range of interspecific hybrids (Bellon et al. 2011), in the past decade more focus has been placed on the targeted genetic improvement of yeast through directed evolution and genetic engineering (Cardiere et al. 2011, Pretorius and Bauer 2002, Dequin 2001). Many individual traits have been targeted through such approaches focused on exploring the genetic constitution of wine yeast strains to improve specific individual characters through targeted breeding and genetic engineering (for review see Pretorius and Bauer 2002; Dequin 2001). Examples of specific targets of genetic engineering approaches include (i) the generation of acidifying or de-acidifying yeast strains (Dequin et al, 1999, Volschenk et al, 1997), (ii) strains with modified aroma production potential (Lilly et al. 2006) or modified cell wall characteristics (Govender et al. 2009), and (iii) strains with increased release of haze protecting proteins (Brown et al. 2007, Gonzalez-Ramos and Gonzalez, 2006). Several attempts were also made to generate strains with lower ethanol yield. The results indicate that to achieve such metabolic targets, the combined overexpression and deletion of several genes is required (Ehsani et al. 2009). These studies highlight the complexity of biological systems. Indeed, biochemical pathways are interconnected and form complex networks. Any specific modification of individual parts of this network will have repercussions elsewhere, and will frequently result in unexpected side effects.

The future

More recently the availability of high quality genome sequence information (Novo et al. 2009) has offered new opportunities for global transcriptomic, proteomic and metabolomic studies. Such approaches are frequently referred to as “system-wide approaches” or “systems biology” and can correlate differences in fermentation phenotypes to gene expression, protein abundance and metabolic regulation (Borneman et al. 2008, 2011, Erasmus et al. 2003, Rossignol et al. 2003, 2009, Rossouw et al. 2008, 2009, 2010). Such studies, combining the tools of genomic sequence analysis, genome-wide gene expression analysis, various proteomic tools and metabolomic profiling have opened new insights into the complex nature of genetic and metabolic regulations.

Combined with advances in data analysis tools, in particular Multi Variate Data Analysis (MVDA) and bioinformatics, such approaches are revolutionising the biological sciences, and have a tremendous impact on our ability to correlate and establish causal relationships between multiple parameters in complex systems (Borneman et al 2007, Rossouw and Bauer 2009). For example, it has become possible in the past few years to better evaluate and predict the impact of grape must nutrient composition on wine aroma development (Ugliano et al. 2009, 2010, Schmidt et al. 2011), the impact of genome wide transcription and genetic regulation on aroma production (Rossouw et al. 2008, 2009) and the correlations between transcriptome, proteome and flavour active metabolites (Rossignol et al. 2009, Rossouw et al. 2010). Such studies open the way to a full understanding of the combinatorial impact of grape juice composition, natural microflora, oenological interventions (nutrients, enzymes, temperature, and oxygen) and the genetic and metabolic regulation of individual yeast strains.

CONCLUSION

New technologies allow for the first time to conduct holistic, system-wide analyses of the process of wine making. While the complexity of the system remains at this stage beyond our data analytical abilities, many of the currently generated data sets manage to establish causal relationships between multiple factors and parameters such as grape juice composition, yeast genetic profile and aroma production. With analytical technologies, including on-line measurements, becoming more powerful and more available to wine producers, and grape juice composition being analysed in more depth even in industrial settings, it will be possible in future to match specific grape juices with the genetic potential of individual wine yeast strains, while making use of a wider variety of genetic backgrounds to achieve predicted outcomes in terms of the flavour and aroma profiles of wine.

REFERENCES

- Barnett JA. 1992. The taxonomy of the genus *Saccharomyces*: a short review for non-taxonomist. *Yeast* 8: 1–23.
- Bellon JR, Eglinton JM, Siebert TE, Pollnitz AP, Rose L, de Barros Lopez M, Chambers PJ. 2011. Newly generated interspecific wine yeast hybrids introduce flavour and aroma diversity to wines. *Appl Microbiol Biotechnol* doi: 10.1007/s00253-011-3294-3
- Borneman AR, Desany BA, Riches D, Affourtit JP, Forgan AH, Pretorius IS, Egholm M, Chambers PJ. 2011. Whole-genome comparison reveals novel genetic elements that characterize the genome of industrial strains of *Saccharomyces cerevisiae*. *PLoS Genet* 7:e1001287
- Borneman AR, Forgan AH, Pretorius IS, Chambers PJ. 2008 Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. *FEMS Yeast Res* 8:1185-1195..
- Borneman AR, Chambers PJ, Pretorius IS. 2007 Yeast systems biology: modelling the winemaker's art. *Trends Biotechnol* 25:349-355.
- Brown SL, Stockdale VJ, Pettolino F, Pocock KF, de Barros Lopes M, Williams PJ, Bacic A, Fincher GB, Høj PB, Waters EJ. 2007. Reducing haziness in white wine by overexpression of *Saccharomyces cerevisiae* genes YOL155c and YDR055w. *Appl Microbiol Biotechnol* 273:1363-76.
- Cadière A, Ortiz-Julien A, Camarasa C, Dequin S. 2011. Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased in vivo flux through the pentose phosphate pathway. *Metab Eng* 13:263-271.
- Dequin S, Baptista E, Barre P. 1999. Acidification of Grape Musts by *Saccharomyces cerevisiae* Wine Yeast Strains Genetically Engineered to Produce Lactic Acid. *Am. J. Enol Vitic* 50:1:45-50
- Dequin S. 2001. The potential of genetic engineering for improving brewing, wine-making and baking yeasts. *Appl Microbiol Biotechnol* 56: 577-588,

Ehsani M, Fernández MR, Biosca JA, Julien A, Dequin S. 2009 Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 75:3196-2205.

Erasmus DJ, van der Merwe GK, van Vuuren HJ. 2003. Genome-wide expression analyses: Metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. *FEMS Yeast Res* 3:375-399.

Frezier V, Dubourdiou D. 1992. Ecology of yeast strain *Saccharomyces cerevisiae* during spontaneous fermentation in a Bordeaux winery. *Am J Enol Vitic* 43:375-380.

Gamero A, Manzanares P, Querol A, Belloch C. 2011. Monoterpene alcohols release and bioconversion by *Saccharomyces species* and hybrids. *Int J Food Microbiol* 145:92-97.

Gonzalez-Ramos D, Gonzalez R. 2006. Genetic determinants of the release of mannoproteins of enological interest by *Saccharomyces cerevisiae*. *J Agric Food Chem* 54:9411-9416.

Govender P, Bester MC, Bauer FF. 2010. *FLO* gene dependent phenotypes in industrial wine yeast strains. *Appl Microbiol Biotechnol* 86:931-945.

Kurtzman C. P. and C. J. Robnett, 1991 Phylogenetic relationship among species of *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces* and *Schwanniomyces* determined from partial ribosomal RNA sequences. *Yeast* 7: 61–72.

Le Jeune C, Erny C, Demuyter C, Lollier M. 2006. Evolution of the population of *Saccharomyces cerevisiae* from grape to wine in a spontaneous fermentation. *Food Microbiol* 23:709-716.

Lilly M, Bauer FF, Styger G, Lambrechts MG, Pretorius IS. 2006. The effect of increased branched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. *FEMS Yeast Res* 6:726-743

Marchal A, Marullo P, Moine V, Dubourdiou D. 2011. Influence of yeast macromolecules on sweetness in dry wines: Role of the *Saccharomyces cerevisiae* protein Hsp12. *J Agric Food Chem* 59:2004-1010

Masneuf I, Hansen J, Groth C, Piskur J, Dubourdiou D. 2001 New hybrids between *Saccharomyces sensu stricto* yeast species found among wine and cider production strains. *Appl Microbiol Biotechnol* 56:577-88.

Mortimer RK. 2000, Evolution and variation of the yeast (*Saccharomyces*) genome. *Genome Res.* 10: 403–409.

Mortimer RK, Johnston J. 1986. Genealogy of principal strains of the Yeast Genetics Stock

Mortimer RK, Lerner R, Barr J. 1957 Ultraviolet-induced biochemical mutants of *Saccharomyces cerevisiae*. *U.S. Afon.Energy. Comm. Doc. UCRL 3746*: 1–10.

Mortimer R, Polsinelli M. 1999. On the origins of wine yeast. *Res Microbiol* 150:199-204.

Nieuwoudt HH, Prior BA, I.S. Pretorius IS, Bauer FF. 2002. Glycerol in South African table wines: an assessment of its contribution to wine quality. *S Afr J Enol Vitic* 23:22-30.

Novo M, Bigey F, Beyne E, Galeote V, Gavory F, Mallet S, Cambon B, Legras JL, Wincker P, Casaregola S, Dequin S. 2009 Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. *Proc Natl Acad Sci USA* 106:16333-16338.

Pretorius IS. 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast* 16:675-729.

Pretorius, I.S. & F.F. Bauer. 2002. Meeting the consumer challenge through genetically customised wine yeast strains. *Trends Biotechnol* 20:426-432.

Renault P, Miot-Sertier C, Marullo P, Hernández-Orte P, Lagarrigue L, Lonvaud-Funel A, Bely M. 2009. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. *Int J Food Microbiol* 134:201-210.

Rossignol T, Dulau L, Julien A, Blondin B. 2003. Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. *Yeast* 20:1369-1385.

Rossignol T, Kobi D, Jacquet-Gutfreund L, Blondin B. 2009 The proteome of a wine yeast strain during fermentation, correlation with the transcriptome. *J Appl Microbiol* 107:47-55.

Rossouw D, Bauer FF. 2009. Wine science in the omics era: The impact of systems biology on the future of wine research. *SA J Enol Vitic* 30:101-110.

Rossouw D, Naes T, Bauer FF. 2008. Linking gene regulation and the exo-metabolome: A comparative transcriptomics approach to identify genes that impact on the production of volatile aroma compounds in yeast. *BMC Genomics* 9:530.

Rossouw D, Olivares-Hernandes R, Nielsen J, Bauer FF. 2009. A comparative 'omics' approach to investigate differences in wine yeast physiology and metabolism during fermentation. *Appl Environ Microbiol* 75:6600-6012.

Rossouw D., van den Dool AH, Jacobson D, Bauer FF. 2010. Comparative Transcriptomic and Proteomic Profiling of Industrial Wine Yeast Strains. *Appl Environ Microbiol* 76:3911-3923.

Schmidt SA, Dillon S, Kolouchova R, Henschke PA, Chambers PJ. 2011 Impacts of variations in elemental nutrient concentration of Chardonnay musts on *Saccharomyces cerevisiae* fermentation kinetics and wine composition. *Appl Microbiol Biotechnol*. DOI 10.1007/s00253-011-3197-3

Schütz M, Gafner J. 1994 Dynamics of the yeast strain population during spontaneous alcoholic fermentation determined by CHEF gel electrophoresis. *Lett Appl Microbiol* 19: 253–257.

Snoep JL. 2005. The Silicon Cell initiative: working towards a detailed kinetic description at the cellular level. *Curr Opin Biotechnol* 16:336-43.

Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Aust J Grape Wine Res* 11:139-173.

Swiegers JH, Pretorius IS. 2007. Modulation of volatile sulfur compounds by wine yeast. *Appl Microbiol Biotechnol* 74:954-960.

Teusink B, Passarge J, Reijenga CA, Esgalhado E, van der Weijden CC, Schepper M, Walsh MC, Bakker BM, van Dam K, Westerhoff HV, Snoep JL. 2000. Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? Testing biochemistry. *Eur J Biochem* 267:5313-5329.

Ugliano M, Travis B, Francis IL, Henschke PA. 2010. Volatile Composition and Sensory Properties of Shiraz Wines As Affected by Nitrogen Supplementation and Yeast Species: Rationalizing Nitrogen Modulation of Wine Aroma. *J Agric Food Chem* 58:12417–12425

Ugliano M, Fedrizzi B, Siebert T, Travis B, Magno F, Versini G, Henschke PA. 2009. Effect of nitrogen supplementation and *Saccharomyces* species on hydrogen sulfide and other volatile sulfur compounds in shiraz fermentation and wine. *J Agric Food Chem* 57:4948-4955.

Vilanova M, Ugliano M, Varela C, Siebert T, Pretorius IS, Henschke PA. 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Appl Microbiol Biotechnol* 77:145-157.

Yu P Pickering, GJ 2008. Ethanol Difference Thresholds in Wine and the Influence of Mode of Evaluation and Wine Style. *Am J Enol Vitic* 59:2:146-152.

Zheng W, Zhao H, Mancera E, Steinmetz L, Snyder M. 2010 Genetic analysis of variation in transcription factor binding in yeast. *Nature* 464: 1187-1191.