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Genetics of Yeast Impacting Wine Quality

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Key Words

Saccharomyces, yeast, wine, flavor, allele

Abstract

The availability of the sequence of the *Saccharomyces* genome in combination with the development of chemical analytical technologies with dynamic ranges sensitive enough to detect volatile aromatic compounds has generated a renewed interest in defining the role of yeast in the generation of wine aroma and flavor. Genetic differences among wine strains are well documented and aroma profiles also appear to vary, implying that specific allelic alterations may exist and impact the production of compounds associated with flavor. Partial or complete sequencing data on several wine strains are available and reveal underlying genetic differences across strains in key genes implicated in flavor formation. This review discusses the current understanding of the roles of *Saccharomyces* in wine flavor with an emphasis on positive contributions to flavor and highlights the discoveries of the underlying enzymatic and metabolic mechanisms responsible for the yeast contribution to wine quality.

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INTRODUCTION

The yeast *Saccharomyces cerevisiae* and related species are the agents of fermentation converting grape juice into wine and, as a consequence, have a significant influence on the sensory profile and therefore the perceived quality of wine. Yeast activity can impact all of the sensory attributes of a wine: aroma, taste, color, clarity, mouthfeel, oral tactile sensations such as astringency, mucosal trigeminal nerve detection of effervescence, and chemically induced perceptions of heat or cold. Yeast metabolites can also detract from wine quality as *Saccharomyces* is responsible for the production of several off-characters in wine, such as the sulfur-containing volatiles, higher alcohols, and aldehydes. The overall role of *Saccharomyces* in the development of wine flavor is complex and only recently becoming fully appreciated. Several comprehensive reviews on the varied effects of *Saccharomyces* on wine composition have appeared (Bartowsky & Pretorius 2009; Dubourdieu et al. 2006; Hazelwood et al. 2008; Landaud et al. 2008; Marchand et al. 2000; Moreno-Arribas & Polo 2005; Polaskova et al. 2008; Rauhut 1993, 2009; Rapp & Mandery 1986; Romano et al. 2003; Saerens et al. 2009; Swiegers et al. 2005; Swiegers & Pretorius 2007; Ugliano & Henschke 2009). Identification of the genetic bases of flavor formation in yeast will permit manipulation of the sensory characteristics of wine and thereby allow the winemaker greater control over wine flavor.

Flavor is an all-encompassing term used to describe the totality of the sensory attributes of a product. The flavor-active compounds in wine can derive from the grape itself, from microbial activity, from extraction of cooperage such as oak during fermentation or aging, or from oxygen-dependent and -independent chemical reactions that occur during aging. Wine aroma is dependent upon the profile of volatile compounds present in the wine. There are several classes of volatile compounds found in wine: alcohols, aliphatics, benzene derivatives, carbonyls, esters, organic acids, S-containing compounds, shikimic acid derivatives, polyols, isoprenoid-derived terpenes and C13 norisoprenoids, methoxypyrazines, vinyl, and volatile phenols (Baumes 2009; Ferreira et al. 1998; Ferreira & Cacho 2009; Guth 1997a,b; Lee et al. 2007; Mendes-Pinto 2009; Nykanen 1986; Polaskova et al. 2008; Preston et al. 2008; Rapp & Mandery 1986; Tominaga et al. 1998, 2000a,b; Vilanova et al. 2007; Zhang et al. 2007). Volatile compounds may be synthesized or extracted directly or be present in the form of a nonvolatile precursor requiring chemical or biochemical activity to liberate the odor-active component. An odor-active compound is a volatile compound that contributes to the overall aroma. In contrast, an odor-impact compound is one that possesses a distinctive aroma that can be specifically detected in the aroma profile of the wine. Odor-active compounds can affect the ability to detect odor-impact components. Yeast metabolites have been characterized as forming a generic wine aroma that functions as a buffer diminishing the ability to detect odor-impact compounds (Ferreira & Cacho 2009).

The development of more sensitive technologies for the analysis and identification of aroma compounds in wine, such as solid phase microextraction (SPME) (Ferreira et al. 2001, 2002, 2009; Guth 1997a,b; Jelen 2006; Lopez et al. 1999; Siebert et al. 2005), has allowed researchers to more clearly define the origins of compounds produced. Chemical mechanisms of synthesis, however, do not entirely account for the levels of many compounds that are found in wine (Escudero et al. 2007, Ferreira et al. 1998, Kotseridis & Baumes 2000), underscoring the more important role of biological synthesis and conversion. Deconstruction experiments, the identification of all aroma compounds in a wine with subsequent reconstruction or recreation of a model wine containing all of those chemical constituents at the same concentrations at which they were originally found (Aznar et al. 2001; Escudero et al. 2004, 2007; Ferreira et al. 1998, 2002, 2009), have identified the important enhancer role played by yeast metabolites as well as the capacity of yeast compounds to buffer or diminish perception of specific odors.

Strategies Used to Investigate the Genetics of Flavor Formation

Several approaches have been taken to identify genes that are important in the formation of flavor compounds by *Saccharomyces*. These approaches include mutation and overexpression. Mutation of a gene impacting a specific flavor is expected to lead to a loss of the compound required for that flavor. Overexpression of a gene and therefore of the enzymatic activity required for a specific flavor would likewise be expected to lead to the formation of higher levels of the specific flavor compound. The main problem with these approaches is the lack of a selection for identifying the mutants. Screening isolates for loss of an aroma compound can be challenging. Alternatively, genomic analyses can be utilized to identify genes or proteins that increase in expression under conditions of formation of high levels of a specific compound and that are present with reduced levels of expression when the compound is not observed.

These approaches often require some knowledge of the compounds and pathways required for specific flavor attributes. Defining the specific genes responsible for specific wine flavors is challenging. Often the proteins encoded by these genes perform some other function within the cell and may not be constitutively expressed. There is also a strong influence of grape composition on the formation of flavor-active compounds by *Saccharomyces*. In many cases, the role of yeast metabolism is in the liberation of an aromatic compound from a nonvolatile precursor molecule (Baumes 2009; Chassagne et al. 2005; Daenen et al. 2007; Darriet et al. 1988; Dubourdieu et al. 2006; Dubourdieu & Tominaga 2009; Fernandez-Gonzalez et al. 2002; Fernandez-Gonzalez & Di Stefano 2003; Howell et al. 2005; Huynh-Ba et al. 2003; Maicas & Mateo 2005; Marchand et al. 2000; Murat et al. 2001a,b; Ugliano et al. 2006). For example, the release of yeast cell wall mannoprotein components modifies both wine tannin structure and wine color through binding of both grape tannins and pigments (Ugliano & Henschke 2009). Dissecting the myriad of possible interactive effects on flavor is difficult, but is greatly assisted by the availability of mutants lacking specific activities.

A complicating factor in these analyses is the sequence diversity of wine strains of *Saccharomyces*. *S. cerevisiae* and *S. bayanus* display roughly 80% identity of coding and 74% identity of noncoding sequences (Cliften et al. 2003). Significant genetic diversity exists among wine strains of *S. cerevisiae* (Ambrona et al. 2005; Baleiras Couto et al. 1996; Briones et al. 1996; Cavalieri et al. 2000; Dunn et al. 2005; Gallego et al. 2005; Gresham et al. 2006; Infante et al. 2003; Izquierdo Canas et al. 1997; Johnston et al. 2000; Khan et al. 2000; Legras et al. 2007; Liti et al. 2006, 2009; Lopes et al. 2002; Sabate et al. 1998; Schuller et al. 2005; Schutz & Gafner 1993; Valero et al. 2006; Van Der Westhuizen et al. 2000a,b; Versavaud et al. 1995; Winzeler et al. 2003). The greatest numbers of genotypes in these studies are generally represented by a single isolate, indicating that the true extent of the diversity present in the wild is extensive. Analyses of the presence of single nucleotide polymorphisms (SNPs) suggest that they exist across populations of *Saccharomyces* with a frequency of approximately 2.8 SNPs per kilobase of DNA (Schacherer et al. 2007, 2009) to 7 SNPs per kilobase of DNA (Borneman et al. 2008). Deletions of genetic material also occur (Schacherer et al. 2009) but are found at a very low frequency in the essential genes. In addition to the demonstration of allelic differences in coding regions, the genetic diversity of wine yeasts has also been documented by analysis of transcript profiles under identical growth conditions (Fay et al. 2004, Townsend et al. 2003). There is the potential for significant variation in gene expression profiles as a consequence of underlying genetic differences across strains, making comparisons of strains grown under different conditions challenging, but also enabling identification of naturally arising genetic changes impacting flavor formation.

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Other Factors Important in Flavor Production

The genetic profile of *Saccharomyces* is of obvious importance in the formation of metabolites that confer specific flavors to wine. However, several other factors also impact the spectrum of compounds formed. Many of the *Saccharomyces* metabolites derive from nitrogen metabolism so the nature and level of nitrogen sources present will affect the spectrum of compounds produced. The activity of some of the pathways involved in flavor formation, such as the sulfur-containing volatiles, is regulated by stress factors and the level of stress of the culture will influence the spectrum of compounds produced. The level of stress is impacted by the composition of the grapes at harvest. Other factors, such as the rate of fermentation and temperature released will impact flavor compound retention during the fermentation. Rapid, warm fermentations will lose more aromatic volatiles than cooler, slow fermentations. Yeast may also produce enzymes such as esterases that will hydrolyze aromatic compounds leading to their loss.

In addition to *Saccharomyces*, several other yeast genera can be found in wine that can also contribute to the flavor profile of the wine. The non-*Saccharomyces* yeasts found on the surface of the grape, members of the *Hanseniaspora*, *Metschnikowia*, *Candida*, and *Pichia* genera, often make higher levels of esters and other aroma components than does *Saccharomyces* (Fleet 1993b, Fleet & Heard 1993, Fleet et al. 2002, Gil et al. 1996, Howell et al. 2006, Kunkee & Bisson 1993, Swiegers et al. 2005). *Saccharomyces* can indirectly impact the activities of these microbes via direct competition for nutrients or via creation of conditions not conducive to the growth of these other species. Spoilage organisms are also important and to the extent that *Saccharomyces* metabolism reduces their ability to grow, yeast can impact the stability of wine. This review presents a summary of our current understanding of the role of *Saccharomyces* in wine flavor and focuses on the genetic determinants underlying the production of flavor-active compounds in this yeast.

ROLES OF *SACCHAROMYCES* IN WINE FLAVOR

There are multiple ways in which the biological activity of *Saccharomyces* can impact wine flavor. Yeast biochemical activity may lead to the de novo biosynthesis of flavor-active compounds and yeast enzymatic activity may convert grape components into volatile odor-active forms. Yeast cellular components, such as breakdown products of cell wall mannoproteins, may modify flavor of wine and impact wine clarity (Pozo-Bayon & Reineccius 2009, Ugliano & Henschke 2009). Metabolites produced by yeast can partake in the chemical reactions that occur in wine during aging (Cullere et al. 2007, Cutzach et al. 2009, Jarauta et al. 2005, Spillman et al. 1998). Such modification may either create novel flavor-active components or lead to the loss of flavors derived from other sources. In addition, yeast metabolites may act in a more complex fashion in the enhancement of the perception of existing flavors or in the masking or buffering of odor-active components (Escudero et al. 2007; Ferreira et al. 1998, 2009; Ferreira & Cacho 2009). If the wine is aged on the yeast sediment or lees, the release of enzymatic activities during cell lysis or autolysis can impact wine flavor, especially mouthfeel (Alexandre & Guilloux-Benatier 2008; Feuillat & Charpentier 1982; Francioli et al. 1999, 2003; Leroy et al. 1990). Yeast enzymatic activities have been shown to be active for years during sparkling wine aging (Alexandre & Guilloux-Benatier 2008, Feuillat & Charpentier 1982, Leroy et al. 1990). These activities can modify grape components releasing bound volatile compounds or may hydrolyze existing volatile compounds or their precursors. These effects have been best studied in Champagne production, where the contribution of lees aging has a major effect on wine composition (Alexandre & Guilloux-Benatier 2008, Feuillat & Charpentier 1982, Leroy et al. 1990). Finally, yeast may impact flavor dramatically via biological inhibition of the growth of other microbes that would themselves otherwise impact

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wine characteristics (Bisson & Joseph 2009, Boulton et al. 1996, Fleet 1993b, Fleet et al. 2002, Howell et al. 2006, Romano et al. 2003).

A common myth in wine production is that autochthonous yeast coevolved with grapes in vineyards to generate strains with optimized flavor production from the fruit of that vineyard (Thorngate 1998). Flavor-active compounds are generally yeast end products, side products, or coproducts of metabolism or alternately serve to detoxify compounds in the environment to which the cell is permeable. Some aromatic compounds are insect attractants, and it is likely that selection for production was also driven by the selective spread of specific genomes via insect vectors. Aroma compound production therefore evolved to assure optimal metabolic activities of the yeast to allow survival, persistence, and dominance of their native environment. In many instances, the biochemical modification of flavor components is generally conducted by metabolic enzymes engaged in fundamental cellular processes not dedicated to flavor compound formation.

Several studies have examined the impact of different strains of *Saccharomyces* on wine flavor (Carrau et al. 2008, Chatonnet et al. 1993, Clemente-Jimenez et al. 2004, Daenen et al. 2007, Howell et al. 2004, Lee et al. 2004, Miller et al. 2007, Nykanen & Nykanen 1997, Rossouw et al. 2008, Swiegers et al. 2005, Swiegers & Pretorious 2007, Ugliano et al. 2006, Valero et al. 2006). In general, differences can be observed in the production of aromatic compounds, but these variations often do not necessarily lead to detectable changes in the sensory profile of the wine (Thorngate 1998). There are two main reasons likely explaining the lack of a dramatic impact of yeast on wine aroma. First, the characters made by yeast tend to be described sensorially as generic or nondescript fruity or floral aromas but not as specific odor-impact compounds, with the notable exception of the S-containing off-odors (Bartowsky & Pretorius 2009, Ferreira et al. 2009, Ugliano & Henschke 2009). Therefore, the impact of different concentrations of these positive compounds may be masked by the more dominant varietal characters. Secondly, the flavor-active compounds obtained, whether as a consequence of direct synthesis or indirect liberation or conversion, are largely dependent upon the starting concentrations in the grape juice and on processing decisions employed, such as the use of nutrient additions, juice treatments to remove the sources of precursor compounds, fermentation temperature, and pH adjustment, which serve to mitigate the effect of yeast metabolism (Godard et al. 2007, Jimenez-Marti et al. 2007, Jimenez-Marti & del Olmo 2008, Lee et al. 2004, Masneuf-Pomarede et al. 2006, Mendes-Ferreira et al. 2007, Miller et al. 2007, Molina et al. 2007, Moreira et al. 2002, Plata et al. 2004, Saerens et al. 2008, Subileau et al. 2008, Thibon et al. 2008, Thurston et al. 1982, Younis & Stewart 1998). Finally, many of the yeast components continue to undergo chemical reactions during aging so that although significant differences may be detectable immediately following fermentation, as the wine ages the fermentation bouquet becomes less important (Cullere et al. 2007, Cutzach et al. 2009, Jarauta et al. 2005). The various impacts of *Saccharomyces* on wine flavor are summarized in **Table 1**.

A FLAVOR-ACTIVE COMPOUNDS PRODUCED BY *SACCHAROMYCES*

Saccharomyces is capable of producing a wide array of aromatic compounds that have been detected in wine (Bardi et al. 1998; Bartowsky & Pretorius 2009; Dickinson et al. 2003; Dubourdieu et al. 2006; Fernandez-Gonzalez & Di Stefano 2003; Ferreira et al. 2009; Ferreira & Cacho 2009; Hazelwood et al. 2008; Howell et al. 2005, 2006; Landaud et al. 2008; Kotseridis & Baumes 2000; Marchand et al. 2000; Mason & Dufour 2000; Miller et al. 2007; Molina et al. 2007; Moreira et al. 2002; Murat et al. 2001a; Nykanen 1986; Parkkinen & Suomalainen 1982; Rahut 1993, 2009; Romano et al. 2003; Rossouw et al. 2008; Saerens et al. 2009; Schoondermark-Stolk et al. 2006a,b; Spiropoulos et al. 2000; Suomalainen 1981; Swiegers et al. 2005; Swiegers & Pretorius 2007; Thorngate 1998; Thurston et al. 1982; Tominaga et al. 2000a,b; Ugliano

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Table 1 Effects of *Saccharomyces* on wine flavor

Precursor	Yeast action	Flavor-active component
Sugar catabolism	De novo biosynthesis	Ethanol, acetaldehyde, organic acids, diacetyl
Nitrogen catabolism	De novo biosynthesis	Esters, fusel alcohols, fusel acids, fusel aldehydes
Sulfur catabolism	De novo biosynthesis	S-containing volatiles
Fatty acid biosynthesis	De novo biosynthesis	Esters, free fatty acids, fatty acid aldehydes
Bound glycosides	β-glucosidase activity	Terpenes, norisoprenoids, alcohols, phenolic acids
Cysteinyl/glutathiyl conjugates	Carbon sulfur lyase activity	Volatile thiols
Phenolic acids	Oxidation/reduction	Modified phenolics
Macromolecular components	Hydrolysis/degradation	Lees aging/autolysis characters

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et al. 2006; Ugliano & Henschke 2009; Verstrepen et al. 2003; Vilanova et al. 2007; Yoshimoto et al. 2002). The major classes of compounds formed are acids, alcohols, carbonyl compounds, esters, and S-containing volatiles (**Table 2**). These compounds are often found in levels above the threshold of detection (Ferreira et al. 2009). **Table 2** also includes the common descriptive terms given to these compounds. Many are classified simply as fruity, meaning there is a perception of fruit character but not of a distinctive type of fruit. The fruity yeast esters serve an important role in amplifying the perception of the fruit identifier compounds of the specific wine variety.

In addition to analysis of the volatile compounds produced during fermentation, research to identify the genes underlying the synthesis of these compounds has also been undertaken (Alexander et al. 2003; Borneman et al. 2008; Cordente et al. 2007; Daenen et al. 2007; Darriet et al. 1988; Dickinson & Norte 1993; Eden et al. 2001; Fujii et al. 1994, 1996; Fujiwara et al. 1999; Howell et al. 2005; Larroy et al. 2002; Rossouw et al. 2008; Saerens et al. 2006; Saint-Prix et al. 2004; Schoondermark-Stolk et al. 2006b; Spiropoulos & Bisson 2000; Verstrepen et al. 2003; Vuralhan et al. 2003). Several different approaches have been taken to identify the flavor-impact genes. Where the biosynthetic pathways have been well characterized, the known members of the pathway have been investigated for roles in aroma production. In most cases, the role of the known pathway in production of target volatiles has been confirmed, both by showing the absence of the compound in null mutants not expressing the pathway and by showing higher levels of the compound in strains overexpressing the activity (Alexander et al. 2003; Cordente et al. 2007; Delneri et al. 1999; Dickinson et al. 2003; Howell et al. 2005; Iraqui et al. 1999; Larroy et al. 2002; Lilly et al. 2006a,b; Saerens et al. 2006; Saint-Prix et al. 2004; Subileau et al. 2008; Thibon et al. 2008; Verstrepen et al. 2003; Vuralhan et al. 2003).

Genomic analyses have also been employed to identify the genes impacting volatile formation. Two types of analyses have been performed, taking advantage of genomic tools. In the first type of analysis, changes in expression patterns of a single strain are investigated for conditions under which the aroma compounds are produced. These transcript profiles are then compared with those of growth conditions under which these compounds are not found (Jimenez-Marti et al. 2007, Schoondermark-Stolk et al. 2006a). The second type of transcriptome analyses compares mRNA profiles of strains with differing levels of aroma compound production under the same growth condition. Rossouw et al. (2008) investigated the correlation between the transcriptome and the exo-metabolome, the pattern of metabolites, of five different wine strains in synthetic juice media. These researchers used multivariate statistical analyses and selected five genes that showed changes in expression consistent with the levels of aromatic compounds produced by the different strains, *AAD10*, *AAD14*, *ACS1*, *BAT1*, and YMR201W to assess the impact of overexpression of their gene products. Overexpression of all but YMR210W showed an impact on the aroma profile. Thus,

Table 2 Common flavor-active compounds produced by *Saccharomyces*

Compound class	Compound[a]	Aroma descriptor[b]
Alcohols	**Ethanol**	Alcohol, hot
	Propanol	Solvent, pungent
	Butanol	Alcohol, solvent
	2-Methylpropanol	Solvent, pungent
	2-Methylbutanol	Solvent, pungent
	3-Methylbutanol	Solvent, pungent
	Hexanol	Cut grass
	Phenylethanol	Floral, rose
Acids	**Acetate**	Pungent
	Propanoate	Vinegar
	Butyrate	Cheese, rancid
	2-Methylpropanoate	Cheese, rancid
	2-Methylbutanoate	Cheese, sweaty, pungent
	3-Methylbutanoate	Strong cheese
	Hexanoate	Cheese, sweaty
	Octanoate	Rancid
	Decanoate	Rancid fat
Esters	**Ethyl butyrate**	Floral
Ethyl	Ethyl hydroxybutyrate	Burnt marshmallow
	Ethyl hexanoate	Green apple
	Ethyl octanoate	Soap
	Ethyl decanoate	Soap
	Ethyl propanoate	Fruity
	Ethyl 2-methylpropanoate	Fruity, pineapple
	Ethyl 2-methylbutyrate	Fruity
	Ethyl 3-methylbutytrate	Berry
Acetate	**Ethyl acetate**	Fruity, vinegar, solvent
	Hexylacetate	Perfume
	2-Methylpropylacetate	Fruity, banana
	2-Methylbutylacetate	Fruity, banana
	3-Methylbutylacetate	Banana
	Phenethylacetate	Floral, rose
Carbonyls	**Acetaldehyde**	Oxidized apple, sherry
	Diacetyl	Butter, rancid
	Acetoin	Butter
S-containing volatiles	Hydrogen sulfide	Rotten egg
	Methanethiol	Cooked cabbage
	Ethanethiol	Onion, rubber
	Dimethylsulfide	Cabbage, cooked corn
	Dimethyldisulfide	Cabbage, onion

(*Continued*)

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Table 2 (*Continued*)

Compound class	Compound[a]	Aroma descriptor[b]
	Dimethyltrisulfide	Cabbage
	Diethylsulfide	Garlic
	Diethyldisulfide	Strong onion
	Methionol	Cooked cabbage, garlic
	Methional	Cereal, potato
Thiols	**4-Mercapto-4-methylpentan 2-one**	Citrus zest, cat urine, box tree
	3-mercaptohexan-1-ol	Box tree, passion fruit
	3-mercaptohexylaceate	Box tree, passion fruit
	Fururylthiol	Roasted coffee, meat

[a]Taken from Ferreira et al. 2009; compounds in bold are found near or above their sensory limits of detection.
[b]Descriptors from Bartowsky & Pretorius 2009, Ferreira et al. 2009, Ugliano & Henschke 2009.

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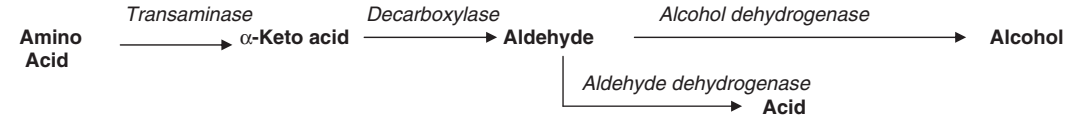
it may be possible to genetically manipulate aroma production in wine strains to tailor the flavor profile of the wine to match the composition desired by the winemaker. In general, the genomic analyses have identified some of the known pathway components as altering in expression under conditions in which the aromatic compound is observed, but more often changes in the expression patterns of genes known to be required for synthesis given the mutational analyses are not altered. These observations suggest that the simple presence or absence of the catalytic activity is not the driving force behind aroma production.

Although multiple compounds with aromatic or flavor impacts have been detected in wine (Bartowsky & Pretorius 2009, Ugliano & Henschke 2009), there are three classes of flavor-impact compounds produced by yeast that are commonly found at levels above their thresholds of detection: fusel alcohols, esters, and S-containing volatiles (Ferreira et al. 2009). These compounds have an impact on the flavor of wine and manipulation of their levels may allow microbial enhancement of wine composition.

Alcohols. *Saccharomyces* produces several alcohols that can impact wine aroma and flavor. Ethanol, the principal end product of sugar catabolism, plays several roles in flavor (Escudero et al. 2007, Ferreira & Cacho 2009). Ethanol itself can have an aroma, and it can be perceived chemically as hot in high enough concentrations (Bartowsky & Pretorius 2009). Ethanol serves to balance the acidity of wine, thereby playing an important role in taste perception. Another effect of ethanol is in the buffering of the detection of other aromatic compounds (Escudero et al. 2007). In this case, the ability to detect compounds present in concentrations above their thresholds of detection is diminished. Several mechanisms by which ethanol may diminish detection of other compounds have been proposed (Escudero et al. 2007, Pozo-Bayon & Reineccius 2009).

In addition to ethanol, other aromatic alcohols are also commonly found in wines. These alcohols are commonly referred to as the higher alcohols to denote that they contain more than two carbon atoms. The higher alcohols produced from amino acid catabolism have been termed fusel oils because of the objectionable aromas attributed to these compounds in distillates (Dickinson et al. 2003, Hazelwood et al. 2008). There are three classes of amino acid–derived fusel alcohols: branched chain, sulfur containing, and aromatic (**Figure 1**). Fusel alcohols are formed via the well-characterized Ehrlich pathway. Amino acids are first deaminated by specific transaminases to form an α-keto acid, which is then decarboxylated to form an aldehyde. The aldehyde can then be either reduced to form the fusel alcohol or oxidized to form a fusel acid. Both the fusel alcohols and acids



Branched Chain:				
Leucine	α-Ketoisocaproate	3-methylbutanal	**3-methylbutanoate**	**3-methylbutanol**
Isoleucine	α-Ketomethylvalerate	2-methylbutanal	**2-methylbutanoate**	2-methylbutanol
Valine	α-ketoisovalerate	2-methylpropanal	**2-methylpropanoate**	**2-methypropanol**
S-Containing:				
Methionine	α-keto(methylthio)butyrate	methional	3-(methylthio)propanoate	**methionol**
Aromatic:				
Phenylalanine	3-phenylpyrvate	2-phenylacetaldehyde	2-phenethanoate	**phenylethanol**
Tryptophan	3-indolpyrvate	3-indoleacetaldehyde	2-indolethanoate	tryptophol
Tyrosine	hydroxyphenylpyruvate	hydroxyphenylacetaldehyde	hydroxyphenylethanoate	tyrosol

Figure 1

Fusel compound synthesis in *Saccharomyces cerevisiae*. The intermediates in the production of fusel alcohols can also be found in wines. The compounds listed in bold have been detected in wine.

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can be found in wines (Bartowsky & Pretorius 2009, Ferreira et al. 2009, Ugliano & Henschke 2009). Fusel aldehydes can be found in aged wines as a result of the reoxidation of the alcohols during aging (Cullere et al. 2007). The ratio of fusel acids to fusel alcohols is largely dictated by the redox status of the cell and whether reduced or oxidized cofactors are in short supply. The intermediate keto acids can play another important role in wine quality. These compounds have been shown to bind to the anthocyanins, red wine pigments, forming pyranoanthocyanins, a colored form more stable against oxidative loss (Ugliano & Henschke 2009). Leucine, isoleucine, and valine degradation lead to the branched chain fusel compounds. All three aromatic amino acids, phenylalanine, tyrosine, and tryptophan, are also catabolized via the Ehrlich pathway (Hazelwood et al. 2008).

The specific enzymes required for the conversion of different amino acid species to the corresponding fusel alcohols have been identified (**Table 3**). *BAT1* and *BAT2* encode the amino transaminases for the branched chain amino acids, and *ARO8* and *ARO9* are the transaminases utilized for the aromatic amino acids and methionine (Dickinson & Norte 1993; Eden et al. 2001; Hazelwood et al. 2008; Lilly et al. 2006a,b; Ugliano & Henschke 2009).

Five decarboxylases participate in the Ehrlich pathway: *PDC1*, *PDC5*, *PDC6*, *ARO10*, and *THI3* (Dickinson et al. 2003, Hazelwood et al. 2008, Vuralhan et al. 2003). *PDC1*, *PDC5*, and *PDC6* encode pyruvate decarboxylase enzymes used in carbon catabolism. Interestingly, the decarboxylases display some substrate specificity (Hazelwood et al. 2008). The main decarboxylase for the leucine pathway is Thi3p. (Dickinson et al. 1997). However, Thi3p plays a regulatory role in thiamine biosynthesis, so it is not clear if the requirement of this gene for the leucine pathway reflects a catalytic or regulatory role (Hazelwood et al. 2008, Nosaka et al. 2005). Any one of the three pyruvate decarboxylases is required for the valine pathway, and each of the five decarboxylases can be used in isoleucine degradation. With the exception of *THI3*, any of the other decarboxylases can function in the aromatic amino acid degradation pathway. *ARO10* encodes a broad specificity decarboxylase expressed during stationary phase (Hazelwood et al. 2008).

Any of the seven alcohol dehydrogenases (*ADH1*, *ADH2*, *ADH3*, *ADH4*, *ADH5*, *ADH6*, and *ADH7*) or *SFA1* can serve as the final reductase in fusel oil formation regardless of the amino acid (Delneri et al. 1999, Hazelwood et al. 2008). The aldehyde dehydrogenases (*ALD1*, *ALD2*, *ALD3*, *ALD4*, *ALD5*, and *ALD6*) similarly have been shown to play a role in fusel acid formation

Table 3 Genes implicated in flavor-active compound formation

Class of compound	Enzymatic activity	Genes identified
Fusel oil (higher alcohol)	Branched chain amino acid transferase	*BAT1*, *BAT2*
	Aromatic amino acid transferase	*ARO8*, *ARO9*
	Decarboxylase	*ARO10*, *PDC1*, *PDC5*, *PDC6*, *THI3*
	Alcohol dehydrogenase	*ADH1*, *ADH2*, *ADH3*, *ADH4*, *ADH5*, *ADH6*, *ADH7*, *SFA1*
Volatile organic acids	Aldehyde dehydrogenase	*ALD2*, *ALD3*, *ALD4*, *ALD5*, *ALD6*
	Fusel acid transport	*PDR12*
Esters	Alcohol acetyl transferase	*ATF1*, *ATF2*
	Acyl transferase	*EEB1*, *EHT1*, *YMR210W*
	Esterase	*IAH2*, *EEB1*, *EHT1*
Sulfur-containing volatiles	Sulfur amino acid biosynthesis	*CYS3*, *CYS4*, *MET5*, *MET6*, *MET8*, *MET10*, *MET17*, *STR2*, *STR3*
	Cysteine degradation	*CYS3*
	Glutathione degradation	*DUG1*, *DUG2*, *DUG3*, *ECM38*
Carbonyl compounds	Aryl alcohol dehydrogenase	*AAD3*, *AAD4*, *AAD6*, *AAD10*, *AAD14*, *AAD15*, *AAD16*
	Acetolactate synthesase	*ILV2*
	Butanediol dehydrogenase	*BDH1*, *BDH2*
Volatile aglycones	β-glucosidase	*BGL2*, *EXG1*, *EXG2*
Cys-conjugates	Cyseinyl lyase	*BNA3*, *CYS3*, *GLO1*, *STR3*, *ICR7*
Transcriptome analysis		*ACS1*

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(Hazelwood et al. 2008). The alcohol and aldehyde dehydrogenases catalyze reversible reactions, which may also serve to further balance the NAD^+/NADH ratio within the cell.

The fusel alcohols are thought to simply diffuse from the cell to the surrounding medium. Export of the fusel acids requires a specific transporter encoded by the *PDR12* gene (Hazelwood et al. 2006, 2008). The fusel alcohols are also less toxic than the fusel acids, and their formation is favored under anaerobic fermentation conditions (Hazelwood et al. 2008). Analysis of the source of the fusel oils in wine production suggests that they derive from internally synthesized amino acids rather than via the degradation of the amino acids present in the juice (Hazelwood et al. 2008). It is likely that the initial amino acids are consumed in protein synthesis during growth as that amino acid and other more preferred nitrogen sources are catabolized early on. As cells enter stationary phase and there is enhanced amino acid turnover, it is of the newly synthesized amino acids.

Analysis of the factors enabling the morphogenic switch from single cells to pseudohyphae in *Saccharomyces* demonstrated a novel role of two of the aromatic fusel oils, phenylethanol and tryptophol, as signaling molecules (Chen & Fink 2006). When added externally, both of these compounds induce the expression of *FLO11*, a key gene required for filamentous growth. The presence of both fusel oils simultaneously leads to an even greater increase than either one alone, implying an additive effect and two, at least partially independent, mechanisms for induction. The aromatic amino acids are generally present in low concentrations within the cell and are not readily interconverted into other nitrogen compounds. Therefore, the degradation of these compounds as nitrogen sources would indeed be a signal of severe nitrogen limitation. The combination of externally added fusel oils and the ability to produce the compounds internally results in the highest level of induction of *FLO11* (Chen & Fink 2006). This observation suggests that these

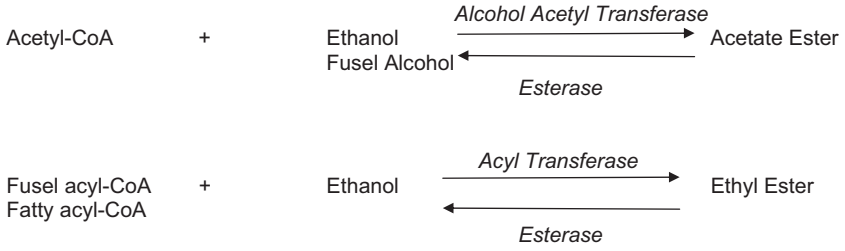

Figure 2

Synthesis of esters in *Saccharomyces cerevisiae*. Two classes of esters are formed by *Saccharomyces*: those that contain acetate as the acyl compound and those that contain ethanol as the alcohol moiety.

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compounds could additionally play a role in quorum sensing and allow a biphasic response to both external and internal signals of nitrogen limitation.

A transcriptome analysis of actively growing cells following aromatic fusel oil addition indicated that approximately 150 genes were upregulated with approximately 70% of these genes associated with entry into stationary phase (Chen & Fink 2006). The nonaromatic fusel oils had no effect on the transition to filamentous growth. However, they may play important roles in other signaling processes. Isoamyl alcohol was also shown to induce molecular changes in yeast cells (Kern et al. 2004), consistent with a signaling role for these molecules in general.

Esters. Esters are formed from the condensation reaction between an alcohol and an acyl group carried by coenzyme A (**Figure 2**). There are two major classes of ester compounds produced by *Saccharomyces*: the ethyl esters and the acetate esters (Bartowsky & Pretorius 2009; Fujii et al. 1994, 1996; Mason & Dufour 2000; Nykanen 1986; Parkkinen & Suomalainenn 1982; Saerens et al. 2009; Suomalainen 1981; Ugliano & Henschke 2009; Verstrepen et al. 2003). Ethanol serves as the alcohol coreactant for the ethyl esters. The acyl moiety of the ethyl esters can derive from carbon or nitrogen metabolism, but the sensorially important ethyl esters are generated during fatty acid biosynthesis (**Table 2**). Factors that stimulate fatty acid biosynthesis also stimulate fatty acid ester formation (Bardi et al. 1998; Lilly et al. 2006a,b; Saerens et al. 2008, 2009). Mutations that block fatty acid biosynthesis also block ester formation. This suggests that esters derive from fatty acid biosynthesis, not degradation (Saerens et al. 2009, Thurston et al. 1982).

Acetyl-CoA serves as the acyl donor for the acetate esters (**Figure 2**). The alcohol coreactant can be ethanol, an alcohol generated from amino acid catabolism or any other aldehyde detoxification process. The main aromatic acetate esters found in wine derive from reactions either with ethanol or with fusel oils.

The levels of esters found in wine are a consequence of synthesis, enzymatic or spontaneous hydrolysis, and levels of substrate molecules (Fukuda et al. 1998, Spillman et al. 1998). In some aged wine styles, esters make no contribution to the aroma profile because of the completeness of hydrolysis, whereas in others they are important contributors to wine flavor.

Genes involved in ester formation have been identified (**Table 3**). Ethyl ester formation is catalyzed by the acyl transferases encoded by *EEB1*, *EHT1*, and YMR210W, and acetate esters are formed by the action of the alcohol transferases *ATF1* and *ATF2* (Fujii et al. 1994, 1996; Fujiwara et al. 1999; Lilly et al. 2006a,b; Saerens et al. 2006, 2008, 2009; Verstrepen et al. 2003). Mutational analysis of these genes suggests that other unknown transferases may also be involved in ester synthesis. *Saccharomyces* also possesses ester hydrolases. The principal hydrolase is encoded by the *IAH1* gene. Both *EEB1* and *EHT1* also have esterase activity. Mutations that affect fatty acid

biosynthesis or fusel oil formation will also negatively affect ester formation via the elimination of coreactants (Saerens et al. 2009). Overexpression of the carnitine acetyl transferase enzymes reduces ester formation, suggesting that ester biosynthesis may be substrate driven (Cordente et al. 2007). Carnitine acetyl transferases function to shuttle active acetyl groups to the mitochondrion. There are three carnitine acetyltransferases in yeast, *CAT2*, *YAT1*, and *YAT2* (**Table 3**).

Ester formation is quite variable across yeast strains and cultivation conditions (Bardi et al. 1998; Carrau et al. 2008; Fujiwara et al. 1999; Fukuda et al. 1998; Jimenez-Marti et al. 2007; Miller et al. 2007; Molina et al. 2007; Nykanen & Nykanen 1997; Romano et al. 2003; Saerens et al. 2008, 2009; Thurston et al. 1982; Younis & Stewart 1998). The temperature of fermentation has a striking impact on ester formation. Esters are formed during midfermentation. Nitrogen levels also impact ester formation. Factors inhibiting the Ehrlich pathway decrease ester formation, again confirmation that the process is substrate driven. Lower temperature fermentations tend to have higher ester contents (Saerens et al. 2009). Research has shown that this effect is not simply due to retention of volatile esters or inhibition of esterase activity but due to the gene expression patterns under low temperature conditions (Beltran et al. 2006, Molina et al. 2007).

Several hypotheses have been proposed for the formation of esters (Saerens et al. 2009). Esters may be formed to serve regeneration of coenzyme A from acyl-bound forms when fatty acid biosynthesis is abruptly terminated. Ester formation may also be an important detoxification process as esters are far less toxic than either alcohols or acids. Esters may also play an important role in signaling similarly to the aromatic fusel oils. The formation of the fatty acid esters would signal a termination of fatty acid biosynthesis and a lack of molecular oxygen during growth. Esters may also serve as insect attractants, and ester production during stationary phase may have been selected for by virtue of the fact that those yeasts releasing such compounds attracted insects and were then spread.

As a class of aromatics, the esters have received much attention as potential targets for improving the quality of wine. Esters are generally described as fruity or floral with the longer chain fatty acid esters characterized as soapy or perfume-like. These notes are all positive in wine and are particularly important in white and rose wines consumed at a young age. The rate of ester hydrolysis at low pH means they are mostly dissipated in aged red wines. Given that most esters have what are described as generic notes they are not odor-impact compounds, meaning they do not confer a single identifiable scent to the wine (Ferreira et al. 2009). However, the generic fruitiness serves to boost the perception of the other fruit components of the wine, amplifying the varietal character. Esters also comprise what has been termed a flavor family meaning that additive effects are observed with mixtures of esters (Escudero et al. 2007). Each individual ester may be present below its threshold of detection, but the mixture in total is above the detection threshold. There are esters that, if present at a high enough concentration, will confer a negative component to the wine, such as the cloying rose aroma of phenethylacetate and the solvent character of ethyl acetate.

S-containing volatiles. Several sulfur-containing volatile compounds are also produced by *Saccharomyces* during grape juice fermentation. Sulfur volatiles can be generated from the reduction of sulfate for amino acid biosynthesis, from the catabolism of the sulfur containing amino acids, methionine, and cysteine, from the degradation of the tripeptide glutathione or its adduct, S-adenosylmethionine, and from the degradation of the S-containing vitamins biotin and thiamine (Rauhut 1993, 2009). In contrast to the esters, the S-volatiles can confer distinctive and often negative aromas to wine (Rauhut 1993, 2009). The S-volatiles are problematic because of their low thresholds of detection, chemical reactivity, and difficulty in removal (Rauhut 1993, 2009). Several classes of sulfur compounds are formed by *Saccharomyces* during wine production. Sulfides, thiols (mercaptans), thioethers, and thioacetic acid esters are the most commonly found

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compounds (Rauhut 1993). These primary metabolite compounds can react with a host of other compounds forming a wide diversity of S-volatiles (Rauhut 1993, 2009). Several detailed reviews of S-volatile formation have been published (Bartowsky & Pretorius 2009; Landaud et al. 2008; Mendes-Ferreira et al. 2002; Moreira et al. 2002; Rauhut 1993, 2009; Swiegers & Pretorius 2007; Ugliano & Henschke 2009), and only a brief summary of the most common S-volatiles will be presented here.

The main volatile sulfur compound formed is hydrogen sulfide (H_2S). H_2S is formed from the reduction of sulfate during sulfate assimilation and has an odor of rotten eggs. The sulfite reductase enzyme complex reduces sulfite to sulfide. The reduced sulfide can be released from the enzyme as H_2S. Multiple factors including strain genetic background influence the level and timing of production of H_2S and have also been reviewed in detail (Landaud et al. 2008; Linderholm et al. 2006; Rauhut 1993, 2009; Spiropoulos et al. 2000). H_2S can also be formed from cysteine via cysteine desulfhydrolase. H_2S is chemically active and can react with acetaldehyde or ethanol to form ethanedithiol, a volatile compound with a strong rubber note (Rauhut 2009). H_2S can also interact with a variety of wine components during aging. The sulfur chemistry of wine aging is poorly understood and the impacts of sulfide formation difficult to predict (Rauhut 2009).

Many of the sulfur volatiles derive from the biosynthesis of sulfur-containing amino acids or from their degradation (**Table 3**). Mutations of genes encoding components of sulfite reductase and other enzymes in the pathway have been shown to impact levels of hydrogen sulfide (reviewed in Ugliano & Henschke 2009). Methionine degradation leads to the formation of methional and methionol, using the same enzymes, Aro8p and Aro9p, implicated in fusel compound formation (Rauhut 2009).

Ethanethiol and methanethiol are also yeast metabolites thought to be derived from the degradation of methionine found in wine that are highly reactive, forming diethylsulfide, diethyldisulfide, and dimethydisulfide. These two thiols can also lead to the formation of the thioacetates, S-methylthioacetate and S-ethylthioacetate (Bartowsky & Pretorius 2009; Rauhut 1993, 2009). Methanethiol esters can be found in wine, such as S-methylthioacetate, S-methylproonanoate, and S-methylthiobutanoate (Bartowsky & Pretorius 2009; Rauhut 1993, 2009).

The degradation of cysteine and of the cysteine-containing tripeptide also leads to the formation of sulfur volatiles, but the pathways and enzymes involved have not been fully elucidated (Rauhut 2009). At low concentrations, below the recognition threshold, dimethyl sulfide has been shown to have a positive impact on wine aroma (Segurel et al. 2004). Glutathione is required for maintenance of the cellular redox status and is a tripeptide composed of cysteine, glutamate, and glycine. Glutathione is reactive and can form bonds via the sulfur moiety to a variety of compounds that are then subsequently degraded in the yeast cell, generating again a wide spectrum of volatile compounds. There are two pathways for the degradation of glutathione, the *DUG1*, *DUG2*, and *DUG3* complex and *ECM38* (**Table 3**). Although rare, volatile degradation products of biotin, thiamine, and S-adenosylmethionine have also been found in wine, generally at levels below the threshold of detection (Rauhut 1993, 2009).

Modification of Grape Flavor Components by *Saccharomyces*

Saccharomyces also plays an important role in the generation of wine aroma compounds from precursors present in the grape juice. The spectrum of compounds formed is dependent upon the variety of grape and the composition of the juice at harvest (Baumes 2009). Most juices have faint aromas that become stronger during fermentation. There are four major types of modifications of grape components that are catalyzed by yeast bioactivity. Yeast glucosidases are capable of releasing the aromatic moiety from nonvolatile glycoside precursors. Similarly,

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yeast cysteinyl lyases can release volatile thiol compounds from cysteinyl, and glutathyl-bound precursor molecules are also important in varietal aroma. Yeast oxidases and reductases can convert volatile grape aroma compounds into different species. Yeast can modify aromatic compounds in other ways not involving oxidation or reduction, changing the volatility and nature of the aroma associated with the compound (Bartowsky & Pretorius 2009, Ugliano & Henschke 2009). The genes associated with these activities are listed in **Table 3**. A variety of other types of grape volatile compound modifications have been described in yeast and previously reviewed (Ugliano & Henschke 2009). In some cases, the specific enzymes involved in each of these processes have been identified, but in others the mechanism of modification remains unknown.

Four classes of glycoconjugates have been found in wine (Maicas & Mateo 2005). The simplest class is the monosaccharide glycosides that contain a β-D-glucose unit. There are three types of disaccharide glycosides that contain in addition to the β-D-glucose unit either an arabinose, rhamnose, or apiose sugar (Maicas & Mateo 2005). Several types of aromatic compounds are present as gylcosides: terpenes, C_{13}-norisoprenoids, alcohols, volatile phenols (Bartowsky & Pretorius 2009, Maicas & Mateo 2005, Ugliano et al. 2006, Ugliano & Henschke 2009). Research has shown that *Saccharomyces* possesses broad spectrum β-glucosidase activity capable of cleaving a variety of glycoconjugates (Ugliano et al. 2006). The disaccharide precursors are degraded in a two-step process. First, the terminal sugar is released. This is followed by removal of the glucose unit. *Saccharomyces* seems to not possess enzymes capable of cleaving the terminal sugar groups but does contain B-glucosidase activity (Mateo & Di Stefano 1998, Ugliano et al. 2006). However, the precise enzyme(s) involved has not yet been identified.

Non-volatile S-(L-cysteine) conjugates are found in wines that upon hydrolysis release aromatic thiols (Dubourdieu et al. 2006; Tominaga et al. 1998, 2000a,b). Three such thiols have been identified as important for the varietal character of Sauvignon Blanc wines: 4-mercapto-4-methylpentan-2-one, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexanol (Dubourdieu & Tominaga 2009). Other thiols have also been identified in both red and white wines (Dubourdieu & Tominaga 2009). A genetic analysis of thiol release identified four genes impacting cysteine-conjugate cleavage: YJL060W (*BNA3*), YAL012W (*CYS3*), YFR055W (*ICR7*), and YML004C (*GLO1*) (Howell et al. 2005). Volatile thiol release is repressed in the presence of ammonia and is under general nitrogen repression control (Subileau et al. 2008, Thibon et al. 2008), suggesting that the lyase activity is expressed under poor nitrogen conditions.

GENETIC ANALYSIS OF FLAVOR FORMATION

A summary of the known genes involved in either de novo flavor-impact compound formation or modification of grape precursor molecules is presented in **Table 3**. Partial or complete genomic sequence information is available for nine wine strains: commercial isolate AWRI1631 (Borneman et al. 2008), native vineyard isolate RM11–1a (Brem et al. 2002), and seven strains (Y55, L1374, L1528, BC187, DBVPG1106, DBVPG6040, Yllc17-E5) that were sequenced as part of a comprehensive analysis of diversity across *S. cerevisiae* (Liti et al. 2009). Analysis of nonconservative allele variation for a subset of genes involved in aroma formation as compared with the laboratory strain S288C is presented in **Table 4**. Four genes representative of fusel oil synthesis, *ARO8*, *ARO9*, *ARO10*, and *BAT1*, four genes from ester formation, *ATF2*, *EEB1*, *EHT1*, and *IAH1*, and four genes involved in volatile thiol release, *BNA3*, *CYS3*, *GLO1*, and *STR3*, were selected for analysis of allele variation across the population of nine wine strains. The *ARO8*, *EEB1*, and *IAH1* genes were conserved across all strains. Only one strain displayed a nonconservative allele change for three additional genes, *BAT1* (Y55), *CYS3* (Y55), and *STR3* (RM11–1a) suggesting these genes are also highly conserved. The *ARO10* gene showed a single

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Table 4 Allele variation in flavor-impact genes

Gene	Allele	Wine strains with allele
BAT1	T68I	Y55
ARO8	N/A	All match S288C
ARO9	T12A	BC187, DBVPG6040, RM11–1a, YIIc17-E5, Y55
	N79D	BC187, DBVPG6040, YIIc17-E5, Y55
	S374C	Y55
ARO10	I374T	L1528, RM11–1a
ATF2	G6S	BC187, DBVPG1106, L1374, L1528, RM11–1a, YIIc17-E5
	K214Q	Y55
	S320G	Y55
	E406K	Y55
	E435Q	All strains
	G530S	AWRI1631
EEB1	N/A	All match S288C
EHT1	E117K	AWRI1631, BC187, DBVPG1106, L1374, L1528, RM11–1a, Y55
	R384Q	AWRI1631, BC187, DBVPG1106, DBVPG6040, L1374, L1528, RM11–1a
IAH1	N/A	All match S288C
BNA3	M84I	AWRI1631, BC187, DBVPG1106, L1374, L1528, RM11–1a
	N318K	AWRI1631
CYS3	N266I	Y55
GLO1	T36A	AWRI1631, BC187, DBVPG1106, DBVPG6040, L1374, L1528, RM11–1a, YIIc17-E5
	G156D	AWRI1631
	H322Y	AWRI1631, BC187, DBVPG1106, L1374, L1528, RM11–1a, YIIc17-E5
STR3	C362G	RM11–1a
	L373S	RM11–1a

allele difference shared by two unrelated strains, L1528 and RM11–1a. The other genes display multiple nonconservative changes across several of the strains. Thus, some of the genes that underlie flavor formation show high conservation across the population of wine strains that have been analyzed. Other genes are much less conserved, consistent with previous observations for genes of the sulfate reduction pathway (Linderholm et al. 2006). Three of the strains, AWRI1631, RM11–1a, and Y55, show several unique alleles compared with the rest of the set of wine strains. This is also consistent with previous analyses of the differences in strains with respect to alleles of the sulfate reduction pathway (Linderholm et al. 2006). It is interesting that the end products of these pathways have been implicated in signaling processes. One can imagine environments under which the generation of a false signal and the inhibition of other members of the population would be genetically advantageous. Alternatively, in other environments hyper-responsiveness to population signals may be more beneficial to survival. The impact of these differences on enzymatic activity and flavor formation is unknown, but this analysis indicates that sequence divergence in these key genes involved in flavor formation exists in the wild.

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USE OF GENETIC MANIPULATION TO ENHANCE FLAVOR

Given the growing importance of *Saccharomyces* metabolites to wine flavor, it is not surprising that researchers are exploring the feasibility of genetic manipulation of flavor production. There are two approaches to the manipulation of flavor formation, the alteration of existing genes in *Saccharomyces* and the introduction of novel pathways and activities. The former approach can involve classical breeding or genetic engineering. The introduction of foreign genes would require designation of the strains as a genetically modified organism (GMO). Currently neither consumers nor producers are interested in wines made with GMO organisms. However, that may change in the future.

Genetic modification of existing traits may involve the in vitro creation of a targeted allele change or the isolation of a naturally occurring specific allele from another strain or different species of *Saccharomyces* followed by allele replacement in the intended strain. Alternately, alteration of promoter or terminator sequences to change the pattern of expression or regulation of the gene or protein can be utilized to manipulate flavor compound formation. The construction of a novel hybrid protein that will display an altered activity or post-translational regulation may also be used to manipulate metabolite production. In some cases, a null allele may be desired to eliminate an unwanted activity. However, null alleles may impose a strong selective pressure for suppression or other compensating alterations of the genome and may lead to a strain that is not competitive against the wild strains of *Saccharomyces* that will be present in the winery. Many of these approaches have been discussed above.

Yeast strains that express enzymatic activities enhancing grape flavor and aroma characters are also being developed and tested. These strains express higher levels of innate activities impacting flavor compound production or contain mutations of native genes leading to the release of metabolic intermediates that possess an aromatic character (Bardi et al. 1998, 1999; Cebollero et al. 2005; Fukuda et al. 1998; Ganga et al. 1999; Lilly et al. 2000, 2006a, 2006b). For example, mutation of the *ERG20* gene, which is involved in sterol biosynthesis, leads to the release of terpenes by yeast (Javelot et al. 1991). Terpenes are responsible for the characteristic odors of Muscat grapes.

In addition to manipulation of native genes, there are several enzymatic activities that may enhance flavor if expressed in wine yeast. There are three goals of the creation of more flavorful yeast: the use of yeast to enhance the natural grape flavors via the expression of grape genes, the creation of strains that produce novel flavors not currently found in the grape, and the construction of yeast strains that will carry out some of the important reactions associated with the malolactic fermentation. Yeast strains expressing a fungal β(1–4) endoglucanase have been generated (Perez-Gonzalez et al. 1993; Van Rensburg et al. 1996, 1997, 1998). These strains result in the release of more intense fruity aglycone characters in the wine. However, the use of these strains may impact the long-term aging potential of the wine and lead to the loss of too many characters too quickly. The expression of foreign genes in *Saccharomyces* with the express purpose of flavor manipulation is in its infancy. It is clear that this yeast may indeed be modified to produce novel flavors and aroma, but what is not clear is whether or not these yeasts will be accepted as tools for winemaking.

CONCLUSIONS

The yeast *Saccharomyces* plays an integral and varied role in the evolution of wine flavor. Genes known to be involved in the synthesis of aromatic compounds by this yeast show nonconservative changes in sequence across a population of wine strains suggesting that naturally arising variation in sequence of flavor-impact genes may underlie the observed differences in volatile formation of wine strains. Further, the presence of naturally arising allele variation suggests that the wine yeast genome may be exploited to generate novel strains with enhanced flavor abilities.

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SUMMARY POINTS

1. *Saccharomyces* has an important influence on wine composition and flavor and therefore perceived quality.
2. Several yeast derived flavor compounds are typically found at levels higher than their thresholds of detection.
3. Compounds produced by *Saccharomyces* tend to enhance or detract from grape flavor components and the effects of yeast metabolism are difficult to predict.
4. Several flavor-impact genes in *Saccharomyces* have been identified.
5. Analysis of the sequences of nine wine or wine-associated strains indicates the existence of allele variation among the flavor-impact genes.

FUTURE ISSUES

1. The continued analysis of flavor-impact compounds and the role of yeast metabolism in the evolution of wine flavor will reveal novel roles for this yeast in flavor evolution.
2. The identification of yeast flavor-impact gene allele diversity in *Saccharomyces* suggests that gene manipulation can be used to enhance desired flavors without resorting to genetically modified organism (GMO) technology.
3. In the future, the genetic basis of differences in flavor compound formation across wine strains of *Saccharomyces* will be elucidated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

Alexander NJ, McCormick SP, Hohn TM. 2003. The identification of the *Saccharomyces cerevisiae* gene *AYT1* (ORF-YLL063C) encoding an acetyltransferase. *Yeast* 19:1425–30

Alexandre H, Guilloux-Benatier M. 2008. Yeast autolysis in sparkling wines—a review. *Aus. J. Grape Wine Res.* 12:119–27

Ambrona J, Vinagre A, Ramirez M. 2005. Rapid asymmetrical evolution of *Saccharomyces cerevisiae* wine yeasts. *Yeast* 22:1299–1306

Aznar M, Lopez R, Cacho JF, Ferreira V. 2001. Identification and quantification of impact odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem.* 49:2924–29

Baleiras Couto MM, Eijsma B, Hofstra H, Huis in't Veld JHJ, vander Vossen JMBM. 1996. Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. *Appl. Environ. Microbiol.* 62:41–46

Bardi L, Crivelli C, Marzona M. 1998. Esterase activity and release of ethyl esters of medium-chain fatty acids by *Saccharomyces cerevisiae* during anaerobic growth. *Can. J. Microbiol.* 44:1171–76

Bardi L, Cocito C, Marzona M. 1999. *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids. *Int. J. Food Microbiol.* 47:133–40

Bartowsky EJ, Pretorius IS. 2009. Microbial formation and modification of flavor and off-flavor compounds in wine. See Konig et al. 2009, pp. 209–32

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
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Baumes R. 2009. Wine aroma precursors. See Moreno-Arribas & Polo 2009, pp. 251–74
Beltran G, Novo M, Leberre V, Sokol S, Labourdette D, et al. 2006. Integration of transcriptomic and metabolic analysis for understanding the global responses of low-temperature winemaking fermentations. *FEMS Yeast Res.* 6:1167–83
Bisson FL, Joseph CML. 2009. Yeasts. See Konig et al. 2009, pp. 47–60
Brem RB, Yvert G, Clinton R, Kruglyak L. 2002. Genetic dissection of transcriptional regulation in budding yeast. *Science* 296:752–55
Borneman AR, Forgan AH, Pretorius IS, Chambers PJ. 2008. Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. *FEMS Yeast Res.* 8:1185–95
Boulton RB, Singleton VL, Bisson LF, Kunkee RE. 1996. *Principles and Practices of Winemaking*. New York: Chapman & Hall. 604 pp.
Briones AI, Ubeda J, Grando MS. 1996. Differentiation of *Saccharomyces cerevisiae* strains isolated from fermenting musts according to karyotype patterns. *Int. J. Food Microbiol.* 28:369–77
Carrau FM, Medina K, Farina L, Boido E, Henschke PA, et al. 2008. Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Res.* 8:1196–207
Cavalieri D, Townsend JP, Hartl DL. 2000. Manifold anomalies in gene expression in a vineyard isolate of *Saccharomyces cerevisiae* revealed by DNA microarray analysis. *Proc. Natl. Acad. Sci. USA* 97:12369–74
Cebollero E, Martinez-Rodriquez M, Carrascosa AV, Gonzalez R. 2005. Overexpression of *csc1–1*. A plausible strategy to obtain wine yeast strains that undergo accelerated autolysis. *FEMS Microbiol. Lett.* 246:1–9
Chassagne D, Vernizeau S, Nedjma M, Alexandre H. 2005. Hydrolysis and sorption by *Saccharomyces cerevisiae* strains of Chardonnay grape must glycosides during fermentation. *Enzyme Microb. Technol.* 37:212–17
Chatonnet P, Dubourdieu D, Boidron JN, Lavigne V. 1993. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J. Sci. Food Agric.* 62:191–202
Chen H, Fink GR. 2006. Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes Dev.* 20:1150–61
Clemente-Jimenez JM, Mingorance-Carzola L, Martinez-Rodriguez S, Las Heras-Vazquez FJ, Rodriguez-Vico F. 2004. Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. *Food Microbiol.* 21:149–55
Cliften P, Sudarsanam P, Desikan A, Fulton L, Fulton B, et al. 2003. Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. *Science* 301:71–76
Cordente AG, Swiegers JH, Hegardt FG, Pretorius IS. 2007. Modulating aroma compounds during wine fermentation by manipulating carnitine acetyltransferase in *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* 267:159–66
Cullere L, Cacho J, Ferreira V. 2007. An assessment of the role played by some oxidation-related aldehydes in wine aroma. *J. Agric. Food Chem.* 55:876–81
Cutzach I, Chatonnet P, Dubourdieu D. 2009. Influence of storage conditions on the formation of some volatile compounds in white fortified wines (vins doux naturels) during the aging process. *J. Agric. Food Chem.* 48:2340–45
Daenen L, Saison D, Sterckx F, Delvaux FR, Verachtert H, et al. 2007. Screening and evaluation of the glucoside hydrolase activity in *Saccharomyces* and *Brettanomyces*. *J. Appl. Microbiol.* 104:478–88
Darriet P, Boidron JN, Dubourdieu D. 1988. L'hydrolyse des heterosides terpeniques du Muscat a Petit Grains por les enzymes periplasmiques de *Saccharomyces cerevisiae*. *Conn. Vigne Vin.* 22:189–95
Delneri D, Gardner DCJ, Bruschi CV, Oliver SG. 1999. Disruption of seven hypothetical aryl alcohol dehydrogenase genes from *Saccharomyces cerevisiae* and construction of a multiple knock-out strain. *Yeast* 15:1681–89
Dickinson JR, Norte V. 1993. A study of branched-chain amino acid aminotransferase and isolation of mutations affecting the catabolism of branched-chain amino acids in *Saccharomyces cerevisiae*. *FEBS Lett.* 326:24–32
Dickinson JR, Lanterman MM, Danner DJ, Pearson BM, Sanz P, et al. 1997. A ^{13}C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 272:26871–78

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
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Dickinson JR, Eshantha L, Salgado J, Hewlins MJE. 2003. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 278:8028–34

Dubourdieu D, Tominaga T, Masneuf I, Peyrot des Gachons C, Murat ML. 2006. The role of yeasts in grape flavor development during fermentation: the example of Sauvignon blanc. *Am. J. Enol. Vitic.* 57:81–88

Dubourdieu D, Tominaga T. 2009. Polyfunctional thiol compounds. See Moreno-Arribas & Polo 2009, pp. 276–294

Dunn B, Levine RP, Sherlock G. 2005. Microarray karyotyping of commercial wine yeast strains reveals shared, as well as unique, genomic signatures. *BMC Genomics* 6:1–21

Eden A, Van Nedervelde L, Drukker M, Benvenisty N, Deboug A. 2001. Involvement of branched-chain amino acid aminotransferases in the production of fusel alcohols during fermentation in yeast. *Appl. Microbiol. Biotechnol.* 55:296–300

Escudero A, Gogorza B, Melus MA, Ortin N, Cacho J, et al. 2004. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* 52:3516–24

Escudero A, Campo E, Farina L, Cacho J, Ferreira V. 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of frutiness of wines. *J. Agric. Food Chem.* 55:4501–10

Fay JC, McCullough HL, Sniegowski PD, Eisen MB. 2004. Population genetic variation in gene expression is associated with phenotypic variation in *Saccharomyces cerevisiae*. *Genome Biol.* 5:R26.1–R26.14

Fernandez-Gonzalez M, Di Stefano R, Briones A. 2002. Hydrolysis and transformation of terpene glycosides from muscat by different yeast species. *Food Microbiol.* 20:35–41

Fernandez-Gonzalez M, Di Stefano R. 2003. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces cerevisiae*. *Lebensm-Wiss. Technol.* 37:467–73

Ferreira V, Lopez R, Escudero A, Cacho JF. 1998. The aroma of Grenache red wine: hierarchy and nature of its main odorants. *J. Sci. Food Agric.* 77:259–67

Ferreira V, Aznar M, Lopez R, Cacho J. 2001. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. *J. Agric. Food Chem.* 49:4818–24

Ferreira V, Ortin N, Escudero A, Lopez R, Cacho J. 2002. Chemical characterization of the aroma of Grenache rose wines: aroma extract dilution analysis, quantitative determination and sensory reconstitution studies. *J. Agric. Food Chem.* 50:4048–54

Ferreira V, Cacho J. 2009. Identification of impact odorants of wines. See Moreno-Arribas & Polo 2009, pp. 393–416

Ferreira V, Lopez R, Aznar M. 2009. Olfactometry and aroma extract dilution analysis of wines. In *Analysis of Taste and Aroma*, ed. JF Jackson, HF Linskens, pp. 89–122. Berlin Heidelberg: Springer-Verlag. 269 pp.

Feuillat M, Charpentier C. 1982. Autolysis of yeast in Champagne. *Am. J. Enol. Vitic.* 33:5–13

Fleet GH, ed. 1993a. *Wine Microbiology and Biotechnology*. Camberwell, Victoria, Australia: Harwood Academic. 510 pp.

Fleet GH. 1993b. The microorganisms of winemaking—isolation, enumeration and identification. See Fleet 1993a, pp. 1–26

Fleet GH, Heard GM. 1993. Yeasts—Growth during fermentation. See Fleet 1993a, pp. 27–54

Fleet GH, Prakitchaiwattana C, Beh AL, Heard GM. 2002. The yeast ecology of wine grapes. In *Biodiversity and Biotechnology of Wine Yeasts*, ed. M Ciani, pp. 1–17. Kerala, India: Research Signpost. 123 pp.

Francioli S, Guerra M, Lopez-Tamames E, Guadayoi JM, Caixach J. 1999. Aroma of sparking wines by headspace/solid phase microextraction and gas chromatography/mass spectrometry. *Am. J. Enol. Vitic.* 50:404–8

Francioli S, Torens J, Riiu-Aumatell M, Lopez-Tamames E, Buxaderas S. 2003. Volatile compounds of SPME-GC as age markers of sparkling wines. *Am. J. Enol. Vitic.* 54:158–62

Fujii T, Nagasawa N, Iwamatsu A, Bogaki T, Tamai W, et al. 1994. Molecular cloning, sequence analysis, and expression of the yeast alcohol acetyltransferase gene. *Appl. Environ. Microbiol.* 60:2786–92

Fujii T, Yoshimoto H, Tamai Y. 1996. Acetate ester production by *Saccharomyces cerevisiae* lacking the *ATF1* gene encoding the alcohol acetyltransferase. *J. Ferment. Bioeng.* 81:538–42

Fujiwara D, Kobayashi O, Yoshimoto H, Harashima S, Tamai Y. 1999. Molecular mechanism of the multiple regulation of the *Saccharomyces cerevisiae ATF1* gene encoding alcohol acetyltransferase. *Yeast* 15:1183–97

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
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Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
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Fukuda K, Yamamoto N, Kiyokawa Y, Yanagiuchi T, Wakai Y, et al. 1998. Balance of activities of alcohol acetyltransferase and esterase in *Saccharomyces cerevisiae* is important for production of isoamyl acetate. *Appl. Environ. Microbiol.* 64:4076–78

Gallego FJ, Perez MA, Nunez Y, Hildago P. 2005. Comparison of RAPDs, AFLPs and SSR marker for genetic analysis of yeast strains of *Saccharomyces cerevisiae*. *Food Microbiol.* 22:561–68

Ganga MA, Pinaga F, Valles S, Ramon D, Querol A. 1999. Aroma improving microvinification processes by use of a recombinant yeast strain expressing the *Aspergillus nidulans xlnA* gene. *Int. J. Food Microbiol.* 47:171–78

Gil JV, Mateo JJ, Jimenez M, Pastor A, Huerta T. 1996. Aroma compounds in wine as influenced by apiculate yeasts. *J. Food Sci.* 61:1247–49

Godard P, Urrestarazu A, Vissers S, Kontos K, Bontempi G, et al. 2007. Effect of 21 different nitrogen sources on global gene expression in the yeast *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 27:3065–86

Gresham D, Ruderfer DM, Pratt SC, Schacherer J, Dunham MJ, et al. 2006. Genome-wide detection of polymorphisms at nucleotide resolution with a single DNA microarray. *Science* 311:1932–36

Guth H. 1997a. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* 45:3022–26

Guth H. 1997b. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* 45:3027–32

Hazelwood LA, Tai SI, Boer VM, de Winde JH, Pronk JT, et al. 2006. A new physiological role for Pdr12p in *Saccharomyces cerevisiae* export of aromatic and branched chain organic acids produced in amino acid catabolism. *FEMS Yeast Res.* 6:937–45

Hazelwood LA, Daran JM, van Maris AJA, Pronk JT, Dickinson JR. 2008. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Appl. Environ. Microbiol.* 74:2259–66

Howell KS, Swiegers JH, Elsey GM, Siebert TE, Bartowsky EJ, et al. 2004. Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett.* 240:125–129

Howell KS, Klein M, Swiegers JH, Hayasaki Y, Elsey GM, et al. 2005. Genetic determinants of volatile thiol release by *Saccharomyces cerevisiae* during wine fermentation. *Appl. Environ. Microbiol.* 71:5420–26

Howell KS, Cozzolino, Bartowsky EJ, Fleet GH, Henschke PA. 2006. Metabolic profiling as a tool for revealing *Saccharomyces* interactions during wine fermentation. *FEMS Yeast Res.* 6:91–101

Huynh-Ba T, Matthey-Doret W, Fay LB, Rhlid RB. 2003. Generation of thiols by biotransformation of cysteine-aldehyde conjugates with baker's yeast. *J. Agric. Food Chem.* 51:3629–35

Infante JJ, Dombek KM, Rebordinos L, Cantoral JM, Young ET. 2003. Genome-wide amplifications caused by chromosomal rearrangements play a major role in the adaptive evolution of natural yeast. *Genetics* 165:1745–59

Iraqui I, Vissers S, Andre B, Urrestarazu A. 1999. Transcriptional induction by aromatic amino acids in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 19:3360–71

Izquierdo Canas PM, Ubeda Iranzo JF, Briones Perez AI. 1997. Study of the karyotype of wine yeasts isolated in the region of Valdepenas in two consecutive vintages. *Food Microbiol.* 14:221–25

Jarauta I, Cacho J, Ferreira V. 2005. Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study. *J. Agric. Food Chem.* 53:4166–77

Javelot C, Griard P, Colonna-Ceccaldi B, Vladescu B. 1991. Introduction of terpene-producing ability in a wine strain of *Saccharomyces cerevisiae*. *J. Biotechnol.* 21:239–52

Jelen HH. 2006. Solid-phase microextraction in the analysis of food taints and off-flavors. *J. Chromatogr. Sci.* 44:399–415

Jimenez-Marti E, Aranda A, Mendes-Ferreira A, Mendes-Faia A, del Olmo M. 2007. The nature of the nitrogen source added to nitrogen depleted vinifications conducted by a *Saccharomyces cerevisiae* strain in synthetic must affects gene expression and the levels of several volatile compounds. *Antonie von Leeuwenhock* 92:61–75

Jimenez-Marti E, del Olmo M. 2008. Addition of ammonia or amino acids to a nitrogen-depleted medium affects gene expression patterns in yeast cells during alcoholic fermentation. *FEMS Yeast Res.* 8:245–56

Johnston JR, Baccari C, Mortimer RK. 2000. Genotypic characterization of strains of commercial wine yeasts by tetrad analysis. *Res. Microbiol.* 151:583–90
Kern K, Nunn CD, Pichova A, Dickinson JR. 2004. Isoamyl alcohol-induced morphological change in *Saccharomyces cerevisiae* invokes increases in mitochondria and cell wall chitin content. *FEMS Yeast Res.* 5:43–49
Khan W, Augustyn OPH, Van Der Westhuizen TJ, Lambrechts MG, Pretorius IS. 2000. Geographic distribution and evaluation of *Saccharomyces cerevisiae* strains isolated from vineyards in the warmer inland regions of the Western Cape in South Africa. *S. Afr. J. Enol. Vitic.* 21:17–31
Konig H, Unden G, Frohlich J, eds. 2009. *Biology of Microorganisms on Grapes, in Musts and in Wine*. Berlin Heidelberg: Springer-Verlag. 522 pp.
Kotseridis Y, Baumes R. 2000. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J. Agric. Food Chem.* 48:400–6
Kunkee RE, Bisson LF. 1993. Winemaking yeasts. In: *The Yeasts: Yeast Technology*, ed. AH Rose, JS Harrison, pp. 69–126. London: Academic
Landaud S, Helinck S, Bonnarme P. 2008. Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented foods. *Appl. Microbiol. Biotechnol.* 77:1191–205
Larroy C, Pares X, Biosca JA. 2002. Characterization of a *Saccharomyces cerevisiae* NADP(H)-dependent alcohol dehydrogenase (ADHVII), a member of the cinnamyl alcohol dehydrogenase family. *Eur. J. Biochem.* 269:5738–45
Lee SJ, Rathbone D, Asimont S, Adden R, Ebeler SE. 2004. Dynamic changes in ester formation during Chardonnay juice fermentations with different yeast inoculation and initial Brix conditions. *Am. J. Enol. Vitic.* 55:346–54
Lee SH, Seo M, Riu M, Cotta JP, Block DE, et al. 2007. Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wines. *Am. J. Enol. Vitic.* 58:291–301
Legras JL, Merdinoglu D, Cornuet JM, Karst F. 2007. Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Mol. Ecol.* 16:2091–102
Leroy MJ, Charpentier M, Duteurtre B, Feuillat M, Charpentier C. 1990. Yeast autolysis during Champagne aging. *Am. J. Enol. Vitic.* 41:21–28
Lilly M, Lambrechts MG, Pretorius IS. 2000. Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. *App. Environ. Microbiol.* 66:744–53
Lilly M, Bauer FF, Lambrechts MG, Swiegers JH, Cozzolino D, et al. 2006a. The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. *Yeast* 23:641–59
Lilly M, Bauer FF, Styger G, Lambrechts MG, Pretorius IS. 2006b. The effect of increased branch-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.* 6:726–43
Linderholm AL, Olineka TL, Hong Y, Bisson LF. 2006. Allele diversity among genes of the sulfate reduction pathway in wine strains of *Saccharomyces cerevisiae*. *Am. J. Enol. Vitic.* 57:431–40
Liti G, Barton DBH, Louis EJ. 2006. Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. *Genetics* 174:839–50
Liti G, Carter DM, Moses AM, Warringer J, Parts L, et al. 2009. Population genomics of domestic and wild yeast. *Nature* 458:337–41
Lopes CA, van Groock M, Querol A, Caballero AC. 2002. *Saccharomyces cerevisiae* wine yeast populations in a cold region in Argentinian Patagonia: a study at different fermentation scales. *J. Appl. Microbiol.* 93:608–15
Lopez R, Ferreira V, Hernandez P, Cacho JF. 1999. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *J. Sci. Food Agric.* 79:1461–67
Maicas S, Mateo JJ. 2005. Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. *Appl. Microbiol. Biotechnol.* 67:322–35
Marchand S, de Revel G, Bertrand A. 2000. Approaches to wine aroma: release of aroma compounds from reactions between cysteine and carbonyl compounds in wine. *J. Agric. Food Chem.* 48:4890–95
Masneuf-Pomarede I, Le Jeune C, Durrens P, Lollier M, Aigle M, et al. 2006. Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines. *Int. J. Food Microbiol.* 108:385–90
Mason AB, Dufour JP. 2000. Alcohol acetyltransferases and the significance of ester synthesis in yeast. *Yeast* 16:1287–98

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
by Rowan University on 01/02/12. For personal use only.

Mateo JJ, Di Stefano R. 1998. Enological properties of β-glucosidase in wine yeasts. *Food Microbiol.* 14:583–91
Mendes-Ferreira A, Mendes-Faia A, Leao C. 2002. Survey of hydrogen sulphide production by wine yeasts. *J. Food Prot.* 65:1033–37
Mendes-Ferreira A, del Olmo M, Garcia-Martinez J, Jimenez-Marti E, Mendes-Faia A, et al. 2007. Transcriptional response of *Saccharomyces cerevisiae* to different nitrogen concentrations during alcoholic fermentation. *Appl. Environ. Microbiol.* 73:3049–60
Mendes-Pinto MM. 2009. Carotenoid breakdown products the—norisoprenoids—in wine aroma. *Arch. Biochem. Biophys.* 483:236–45
Miller AC, Wolff SR, Bisson LF, Ebeler SE. 2007. Yeast strain and nitrogen supplementation: dynamics of volatile ester formation in Chardonnay juice fermentations. *Am. J. Enol. Vitic.* 58:470–83
Molina AM, Swiegers JH, Varela C, Pretorius IS, Agosin E. 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Appl. Microbiol. Biotechnol.* 77:675–87
Moreira N, Mendes F, Pereira O, Guedes de Pinho P, Hogg T, et al. 2002. Volatile sulphur compounds in wines related to yeast metabolism and nitrogen composition of grape musts. *Anal. Chim. Acta* 458:157–67
Moreno-Arribas MV, Polo MC. 2005. Winemaking biochemistry and microbiology: current knowledge and future trends. *Crit. Rev. Food Sci. Nutr.* 45:265–86
Moreno-Arribas MV, Polo MC, eds. 2009. *Wine Chemistry and Biochemistry*. New York: Springer Science + Business Media. 735 pp.
Murat ML, Masneuf I, Darriet P, Lavigne V, Tominaga T, et al. 2001a. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *Am. J. Enol. Vitic.* 52:136–9
Murat ML, Tominaga T, Dubourdieu D. 2001b. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. *J. Agric. Food Chem.* 49:5412–17
Nosaka K, Onozuka M, Konno H, Kawasaki Y, Nishimura H, et al. 2005. Genetic regulation mediated by thiamine pyrophosphate-binding motif in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 58:467–79
Nykanen L. 1986. Formation and occurrence of flavor compounds in wine and distilled beverages. *Am. J. Enol. Vitic.* 37:84–96
Nykanen L, Nykanen I. 1997. Production of esters by different yeast strains in sugar fermentation. *J. Inst. Brew.* 83:30–31
Parkkinen E, Suomalainen H. 1982. Esterases of baker's yeast. II. Substrate specificities towards esters formed during sugar fermentations. *J. Inst. Brew.* 88:34–38
Perez-Gonzalez JA, Gonzalez R, Querol A, Sendra J, Ramon D. 1993. Construction of a recombinant wine yeast strain expressing β-(1,4)-endoglucanase and its use in microvinification processes. *App. Environ. Microbiol.* 59:2801–6
Plata C, Mauricio JC, Millan C, Ortega JM. 2004. Influence of glucose and oxygen on the production of ethylacetate by a *Saccharomyces cerevisiae* strain during alcoholic fermentation. *World J. Microbiol. Biotechnol.* 21:115–21
Polaskova P, Herszag J, Ebeler E. 2008. Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* 37:2478–89
Pozo-Bayon MA, Reineccius G. 2009. Interactions between wine matrix macro-components and aroma compounds. See Moreno-Arribas & Polo 2009, pp. 417–36
Preston LD, Block DE, Heymann H, Soleas G, Noble AC, et al. 2008. Defining vegetal aromas in Cabernet Sauvignon using sensory and chemical evaluations. *Am. J. Enol. Vitic.* 59:137–45
Rauhut D. 1993. Yeasts—production of sulfur compounds. See Fleet 1993a, pp. 183–224
Rauhut D. 2009. Usage and formation of sulphur compounds. See Konig et al. 2009, pp. 181–208
Rapp A, Mandery H. 1986. Wine aroma. *Experientia* 42:873–84
Romano P, Fiore C, Paraggio M, Caruso M, Capece A. 2003. Function of yeast species and strains in wine flavor. *Int. J. Food Microbiol.* 86:169–80
Rossouw D, Naes T, Bauer F. 2008. Linking gene regulation and the exo-metabolome: a comparative transcriptomics approach to identify genes that impact the production of volatile aroma compounds in yeast. *BMC Genomics* 9:530
Sabate J, Cano J, Querol A, Guillamon JM. 1998. Diversity of *Saccharomyces* strains in wine fermentations: analysis for two consecutive years. *Lett. Appl. Microbiol.* 26:452–55

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
by Rowan University on 01/02/12. For personal use only.

Saerens SMG, Verstrepen KJ, Van Laere SDM, Voet ARD, Van Dijck P, et al. 2006. The *Saccharomyces cerevisiae EHT1* and *EEB1* genes encode novel enzymes with medium chain fatty acid ethyl ester synthesis and hydrolysis capacity. *J. Biol. Chem.* 281:4446–56

Saerens SM, Delvaux F, Verstrepen KJ, Van Dijck P, Thevelein JM, et al. 2008. Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Appl. Environ. Microbiol.* 74:454–61

Saerens SM, Delvaux FR, Verstrepen KJ, Thevelein JM. 2009. Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microb. Biotechnol.* In press; doi:10.1111/j.1751-7915.2009.00106

Saint-Prix F, Bonquist L, Dequin S. 2004. Functional analysis of the ALD gene family of *Saccharomyces cerevisiae* during anaerobic growth on glucose: the $NADP^+$-dependent Ald6p and Ald5p isoforms play a major role in acetate formation. *Microbiology* 150:2209–20

Schacherer J, Ruderfer DM, Gresham D, Dolinski K, Botstein D, et al. 2007. Genome-wide analysis of nucleotide-level variation in commonly used *Saccharomyces cerevisiae* strains. *PLoS One* 3:e322

Schacherer J, Shapiro JA, Ruderfer DM, Kruglyak L. 2009. Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature* 458:342–45

Schoondermark-Stolk SA, Jansen M, Verkleij AJ, Verrips CT, Euvernink GJW, et al. 2006a. Genome-wide transcription survey on flavour production in *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* 22:1347–56

Schoondermark-Stolk SA, Jansen M, Veurink JH, Verkleij AJ, Verrips CT, et al. 2006b. Rapid identification of target genes for 3-methyl-1-butanol production in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 70:237–46

Schuller D, Alves H, Dequin S, Casal M. 2005. Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde region of Portugal. *FEMS Microbiol. Ecol.* 51:167–77

Schutz M, Gafner J. 1993. Analysis of yeast diversity during spontaneous and induced alcoholic fermentations. *J. Appl. Microbiol.* 75:551–58

Segurel MA, Razungles AJ, Riou C, Salles M, Baumes RL. 2004. Contribution of dimethyl sulfide to the aroma of syrah and Grenache noir wines and estimation of its potential in grapes of these varieties. *J. Agric. Food Chem.* 52:7084–93

Siebert TE, Smyth HE, Capone DL, Neuwohner C, Pardon KH, et al. 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal. Bioanal. Chem.* 381:937–47

Spillman PJ, Pollnitz AP, Liacopoulos D, Pardon KH, Sefton MA. 1998. Formation and degradation of furfuryl alcohol, 5-methylfurfurly alcohol, vanillyl alcohol and their ethyl esters in barrel-aged wines. *J. Agric. Food Chem.* 46:657–63

Spiropoulos A, Bisson LF. 2000. *MET17* and hydrogen sulfide formation in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 66:4421–26

Spiropoulos A, Tanaka J, Flerianos I, Bisson LF. 2000. Characterization of hydrogen sulfide formation in commercial and natural wine isolates of *Saccharomyces*. *Am. J. Enol. Vitic.* 51:233–48

Subileau M, Schneider R, Salmon JM, Degryse E. 2008. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon Blanc at the level of precursor transport. *FEMS Yeast Res.* 8:771–80

Suomalainen H. 1981. Yeast esterases and aroma esters in alcoholic beverages. *J. Inst. Brew.* 87:296–300

Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacteria modulation of wine aroma and flavor. *Aus. J. Grape Wine Res.* 11:139–173

Swiegers JH, Pretorius IS. 2007. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* 74:954–60

Thibon C, Marullo P, Claisse O, Cullin C, Dubourdieu D, et al. 2008. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. *FEMS Yeast Res.* 8:1076–86

Thorngate JH. 1998. Yeast strain and wine flavor: nature or nurture? In *Chemistry of Wine Flavor*, ed. AL Waterhouse, SE Ebeler, pp. 66–80. Washington, DC: Am. Chem. Soc. 245 pp.

Thurston PA, Quain DE, Tibb RS. 1982. Lipid metabolism and the regulation of volatile ester synthesis in *Saccharomyces cerevisiae*. *J. Inst. Brew.* 88:90–94

Tominaga T, Peyrot des Gachons C, Dubourdieu D. 1998. A new type of flavour precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: S-cysteine conjugates. *J. Agric. Food Chem.* 46:5215–19

Annu. Rev. Food Sci. Technol. 2010.1:139-162. Downloaded from www.annualreviews.org
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Tominaga T, Baltenweck Guyot R, Peyrot des Gachons C, Dubourdieu D. 2000. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am. J. Enol. Vitic.* 51:178–81

Tominaga T, Blanchard L, Darriet P, Dubourdieu D. 2000. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* 48:1799–802

Townsend JP, Cavalieri D, Hartl DL. 2003. Population genetic variation in genome-wide gene expression. *Molec. Biol. Evol.* 20:955–63

Ugliano M, Bartowsky EJ, McCarthy J, Moio L, Henschke PA. 2006. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* 54:6322–31

Ugliano M, Henschke P. 2009. Yeasts and wine flavour. See Moreno-Arribas 2009, pp. 313–392

Valero E, Cambon B, Schuller D, Casal M, Dequin S. 2006. Biodiversity of *Saccharomyces* yeast strains from grape berries of wine producing areas using starter commercial yeasts. *FEMS Yeast Res.* 7:317–29

Van Der Westhuizen TJ, Augustyn OHP, Pretorius IS. 2000a. Geographical distribution of indigenous *Saccharomyces cerevisiae* strains isolated from vineyards in the coastal regions of the Western Cape in South Africa. *S. Afr. J. Enol. Vitic.* 21:3–9

Van Der Westhuizen TJ, Augustyn OHP, Kahn W, Pretorius IS. 2000b. Seasonal variation of indigenous *Saccharomyces cerevisiae* strains isolated from vineyards of the Western Cape in South Africa. *S. Afr. J. Enol. Vitic.* 21:10–16

Van Rensburg P, Van Zyl W, Pretorius IS. 1996. Co-expression of a *Phanerochaete chrysosporium* cellobiohydrolase gene and a *Butyrivibrio fibrisolvens* endo-β-1,4-glucanase gene in *Saccharomyces cerevisiae*. *Curr. Genet.* 30:246–50

Van Rensburg P, Van Zyl WH, Pretorius IS. 1997. Over-expression of the *Saccharomyces cerevisiae* exo-β-1,3-glucanase gene together with the *Bacillus subtilis* endo-β-1,3-1,4 glucanase gene and the *Butyrivibrio fibrisolvens* endo-β-1,4-glucanase gene in yeast. *J. Biotech.* 55:43–53

Van Rensburg P, Van Zyl W, Pretorius IS. 1998. Engineering yeast for efficient cellulose degradation. *Yeast* 14:67–76

Versavaud A, Courcoux P, Roulland C, Dulau L, Hallet JN. 1995. Genetic diversity and geographical distribution of wild *Saccharomyces cerevisiae* strains from the wine-producing area of Charentes, France. *Appl. Environ. Microbiol.* 61:3521–29

Verstrepen KJ, Van Laere SDM, Vanderhaegen BMP, Derdelinckx G, Dufour JP, et al. 2003. Expression levels of the yeast alcohol acetyltransferase genes *ATF1*, *Lg-ATF1*, and *ATF2* control the formation of a broad range of volatile esters. *Appl. Environ. Microbiol.* 69:5228–37

Vilanova M, Cortes S, Santiago JL, Martinez C, Fernandez E. 2007. Aromatic compounds in wines produced during fermentation: effect of three red cultivars. *Int. J. Food Prop.* 10:867–75

Vuralhan Z, Moais MA, Tai SL, Piper MDW, Pronk JT. 2003. Identification and characterization of phenylpyruvate decarboxylase genes in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 69:4534–41

Winzeler EA, Castillo-Davis CI, Oshiro G, Liang D, Richards DR, et al. 2003. Genetic diversity in yeast assessed with whole-genome oligonucleotide arrays. *Genetics* 163:79–89

Yoshimoto H, Fukushige T, Yonezawa T, Sone H. 2002. Genetical and physiological analysis of branched-chain alcohols and isoamyl acetate production in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 59:501–8

Younis OS, Stewart GG. 1998. Sugar uptake and subsequent ester and higher alcohol production by *Saccharomyces cerevisiae*. *J. Inst. Brew.* 104:255–64

Zhang M, Xu Q, Duan C, Qu W, Wu Y. 2007. Comparative study of aromatic compounds in young red wines from Cabernet Sauvignon, Cabernet Franc and Cabernet Gernischet varieties in China. *J. Food Sci.* 72:248–52

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