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Review article

Yeast interactions and wine flavour

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Abstract

Wine is the product of complex interactions between fungi, yeasts and bacteria that commence in the vineyard and continue throughout the fermentation process until packaging. Although grape cultivar and cultivation provide the foundations of wine flavour, microorganisms, especially yeasts, impact on the subtlety and individuality of the flavour response. Consequently, it is important to identify and understand the ecological interactions that occur between the different microbial groups, species and strains. These interactions encompass yeast–yeast, yeast–filamentous fungi and yeast–bacteria responses. The surface of healthy grapes has a predominance of *Aureobasidium pullulans*, *Metschnikowia*, *Hanseniaspora* (*Kloeckera*), *Cryptococcus* and *Rhodotorula* species depending on stage of maturity. This microflora moderates the growth of spoilage and mycotoxigenic fungi on grapes, the species and strains of yeasts that contribute to alcoholic fermentation, and the bacteria that contribute to malolactic fermentation. Damaged grapes have increased populations of lactic and acetic acid bacteria that impact on yeasts during alcoholic fermentation. Alcoholic fermentation is characterised by the successional growth of various yeast species and strains, where yeast–yeast interactions determine the ecology. Through yeast–bacterial interactions, this ecology can determine progression of the malolactic fermentation, and potential growth of spoilage bacteria in the final product. The mechanisms by which one species/strain impacts on another in grape-wine ecosystems include: production of lytic enzymes, ethanol, sulphur dioxide and killer toxin/bacteriocin like peptides; nutrient depletion including removal of oxygen, and production of carbon dioxide; and release of cell autolytic components. Cell–cell communication through quorum sensing molecules needs investigation.

Keywords: Wine; Yeast; Bacteria; Fungi; Interactions; Ecology

1. Introduction

The flavour of wine is a sensory perception that varies with the individual, the context of the consumer experience and the chemical composition of the product. The final response is the outcome of complex chemosensory interactions that are difficult to predict because of the influences of many variables. Nevertheless, research on many fronts is gradually providing an understanding of these influences (Thorngate, 1997). The chemical composition of wine is the foundation of the sensory response and is determined by many factors. These include the grape variety, the geographical and viticultural conditions of grape cultivation, the microbial ecology of the grape and fermentation processes, and winemaking practices (Cole and Noble, 1997).

Microorganisms have a prominent role in determining the chemical composition of wine. They affect

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the quality of the grape prior to harvest and, during fermentation, they metabolise grape sugars and other components into ethanol, carbon dioxide and hundreds of secondary end-products that, collectively, contribute to the subtlety and individuality of wine character (Nykänen, 1986; Lambrechts and Pretorius, 2000). Yeasts, bacteria and filamentous fungi all contribute to the microbial ecology of wine production and the chemical composition of wine, although yeasts have the dominating influence because of their role in conducting the alcoholic fermentation (Fleet, 1993; Fugelsang, 1997). Many factors affect the microbial ecology of wine production, of which the chemical composition of the grape juice and the fermentation processes are most significant. In complex microbial ecosystems, containing mixtures of different species and strains, there is the possibility that interactions will occur between individual microorganisms, themselves, and this will also determine the final ecology. Wine production presents such an ecosystem where, in relation to yeasts, there is the potential for yeast–yeast interactions, yeast–filamentous fungi interactions, and yeast–bacteria interactions. The diversity and significance of these interactions in relation to wine production have not been thoroughly considered in the literature, and are the focus of this discussion.

2. Interaction mechanisms

Ecological theory describes an array of interactive associations between microorganisms (Boddy and Wimpenny, 1992). These are neutralism, commensalism, mutualism or synergism, amensalism or antagonism, predation or parasitism, and competition. From the perspective of practical winemaking, the relevant outcomes of these interactions are whether or not they enhance or inhibit the growth of any particular species or strain. Within the wine ecosystem, there are numerous mechanisms whereby one yeast may influence the growth of another yeast or a species within the bacterial or fungal groups. Examples of these mechanisms will be presented in the following sections, but some broad concepts will be mentioned here.

Early growth of yeasts in grape juice decreases or strips it of nutrients, making the resultant wine less favourable as an environment for any further microbial growth. Moreover, such growth generates an array of metabolites, some of which will be toxic to other species. The inhibitory effects of ethanol and short chain fatty acids on some microorganisms are well documented (Bisson, 1999; Bauer and Pretorius, 2000; Fleet, 2001). Carbon dioxide production and purging of the juice/wine strips it of oxygen, thereby limiting the growth of aerobic species. Some species may produce inhibitory peptides, proteins or glycoprotein, such as killer toxins, and enzymes that destroy other species by lysis of their cell walls. However, there are also mechanisms which lead to enhanced microbial growth. The large amount of yeast biomass produced during fermentation will die and autolyse, releasing amino acids and vitamins that may encourage the growth of other species later in the process (Fleet, 2001). Moreover, this biomass could function as a bioadsorbent to remove toxic substances (e.g. metal ions, grape phenols), from the ecosystem. Proteolytic and pectolytic yeast species may hydrolyse the proteins and pectins of grape juice to produce substrates for subsequent growth of other species (Charoenchai et al., 1997).

The concept of quorum sensing as a mechanism by which microbial cells communicate with each other and regulate population growth has yet to be considered in the context of wine microbiology (Bisson, 1999). Recently, it was shown that bacterial populations regulate their behaviour and expression of certain properties through the production of low-molecular-mass signalling molecules (Whitehead et al., 2001). These molecules, called quorum sensing signals, are produced throughout growth and, when their concentration reaches a certain threshold, they activate or inhibit gene expression to modify the behaviour of the whole population. In the case of Gram-negative bacteria, *N*-acyl-homoserine lactones have been identified as signalling molecules and they can regulate properties such as biofilm formation, production of exo-polymers and production of extracellular hydrolytic enzymes. Some amino acids and small peptides have similar functions in Gram-positive bacteria. In the case of yeasts, there is evidence that biocarbonate (Ohkuni et al., 1998), acetaldehyde (Richard et al., 1996) and ammonia (Palkova et al., 1997) may act as cell communicating molecules. Recently, farnesol has been identified as a quorum

sensing molecule that inhibits the transition of *Candida albicans* from the yeast to the hyphal stage and its ability to form biofilms (Hornby et al., 2001; Ramage et al., 2002). Possibly, quorum sensing may be a factor in the ability of *Pseudomonas aeruginosa* to kill *C. albicans* (Hogan and Kolter, 2002), and this analogy could be extended to other yeast–bacteria interactions. Quorum sensing represents a new concept in microbial ecology and research is needed to explore its significance in wine production.

3. Interactions between yeasts and filamentous fungi

Filamentous fungi impact on wine production at several stages during the chain of operations (Table 1). The main influence occurs during grape cultivation, when they cause grape spoilage (Fugelsang, 1997; Fleet, 1998; Stummer et al., 2003) and, also, have the potential to produce mycotoxins such as ochratoxin A (Tateo and Bononi, 2001; Markaki et al., 2001; Stander and Steyn, 2002; Sage et al., 2002; Hocking et al., 2003). A diversity of fungi can infect grapes prior to harvest, the principal ones being species of *Botrytis*, *Uncinula*, *Alternaria*, *Plasmopara*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Oidium* and *Cladosporum* (Emmett et al., 1988; Fugelsang, 1997; Fleet, 2001). At this stage, there are several possibilities for yeast–filamentous fungi interactions and potential influence on wine flavour. Fungal growth on grapes can produce various metabolites and conditions that may disturb the ecology and growth of yeasts during alcoholic fermentation. There are reports that *Botrytis cinerea*, *Aspergillus* spp. and *Penicillium* spp. produce metabolites that retard the growth of yeasts during fermentation (Lafon-Lafourcade, 1984; Ribéreau-Gayon, 1985; Reed and Nagodawithana, 1988; Doneche, 1993). The chemical identity of such metabolites and the specificity of their effect on yeasts require investigation. However, Ribéreau-Gayon (1985) has indicated that the anti-yeast factor of *B. cinerea* is possibly a mannoprotein This fungus also produces significant quantities of β-glucans that, indirectly, could affect yeast activity (Doneche, 1993). In contrast, Reed and Nagodawithana (1988) mention early work that shows a stimulatory effect of some grape fungi on the alcoholic fermentation. Fungal growth on grapes may lead to conditions that favour the growth of acetic acid bacteria (Ribéreau-Gayon, 1985). These bacteria produce elevated levels of acetic acid and other substances that retard yeast growth during fermentation (Drysdale and Fleet, 1988).

Table 1
Mechanisms by which filamentous fungi impact on wine flavour and quality

- Spoilage of grapes in the vineyard
- Specific contribution to the production of botrytized or "noble rot" wines
- Production of mycotoxins in grapes and their transfer to wines
- Production of metabolites that enhance or inhibit the growth of wine yeasts and malolactic bacteria
- Cause of earthy, corky taints in wines after growth in grapes, corks and wine barrels

Control of fungal growth on grapes is largely achieved by routine application of chemical fungicides. Various fungicides are used, some of which may influence the ecology and performance of yeasts during fermentation, but more research is needed to define the specificity of such influences (Cabras et al., 1999; Cabras and Angioni, 2000). Consumer and market pressures to decrease the use of agrichemicals in wine production are leading to new and alternative strategies to control fungal growth on grapes. One such initiative involves the use of yeasts as natural biocontrol agents (Chalutz and Droby, 1998). This concept has emerged from observations that some yeast species, normally associated with the surfaces of fruits, including grapes, have strong antifungal activity (reviewed in Fleet, 2003; Suzzi et al., 1995; Guinebretiere et al., 2000). Such yeasts include *Metschnikowia pulcherrima*, various species of *Candida*, *Pichia*, *Cryptococcus*, and some *Saccharomyces* and *Zygosaccharomyces* species. One species, *Candida oleophila*, is registered for commercial use (Droby et al., 1998). Some of these yeasts produce 1,3-β-glucanases that destroy fungal walls. The possibility that the killer toxins of yeasts may also inhibit filamentous fungi needs more exploration (Walker et al., 1995).

Corky, mouldy, earthy taints occasionally occur in wines, and significantly depreciate their quality and acceptability to the consumer. The main component responsible for this problem is 2,4,6-trichloroanisole, but other anisoles, guaiacol, geosmin and pyrizines

may also be involved (Lee and Simpson, 1993). Generally, these substances have extremely low flavour thresholds. They are produced by microbial metabolism during production and storage of corks used for bottling the wine. Subsequently, they are leached from the cork into the wine, to cause the flavour taint. The microbial ecology and biochemistry of this problem are quite complex and not well understood (Silva Pereira et al., 2000). Filamentous fungi that grow within the lenticels of the cork as part of the natural microflora or as contaminants are mainly responsible for the problem, but yeasts and bacteria have also been implicated (Lee and Simpson, 1993; Jager et al., 1996; Silva Pereira et al., 2000; Alvarez-Rodriguez et al., 2002). Yeasts isolated from corks include species of *Rhodotorula*, *Cryptococcus* and *Candida*. It would be useful to screen the yeast flora of corks, to identify species that could be used as natural antagonists of the main taint-producing fungi.

Another interesting dimension of yeast–fungal interaction involves the deactivation of fungal metabolites by yeasts during alcoholic fermentation. Mushroom flavour taints produced on grapes by the powdery mildew fungus, *Uncinula necator*, are diminished during alcoholic fermentation, and appears to be due to the ability of *Saccharomyces cerevisiae* to convert 1-octen-3-one to the less offensive 3-octanone (Darriet et al., 2002; Stummer et al., 2003). Yeast cell wall components, particularly the 1,3-β-glucans, have the ability to adsorb mycotoxins (Lyons, 2002). Toxins produced by mould contamination of grapes are likely to be adsorbed to the yeast surface and removed by the process of racking wine at the completion of alcoholic fermentation.

4. Yeast–yeast interactions

Yeasts are the prominent organisms of wine production and determine wine flavour and other qualities by a range of mechanisms and activities (Table 2). While *S. cerevisiae* is the main species of wine production, other species have significant roles. Interactions between the different species will occur at various stages throughout production. The diversity of these interactions and their impacts on process efficiency and product quality need to be identified and evaluated. It is well established that, within *S. cerevisiae*, different strains have different effects on wine flavour (e.g. variation in production of glycerol, acetic acid, hydrogen sulfide) (Henschke, 1997). Similarly, strain variation will occur in other species. Accordingly, interactive behaviour needs to be considered at both the species and strain level.

Table 2
Mechanisms by which yeasts impact on wine flavour

- Affect grape quality before harvest; biocontrol of moulds
- Conduct alcoholic fermentation of grape juice into wine
- Biocatalyse transformation of flavour neutral, grape components into flavour active components
- Impact on wine flavour and other properties through autolysis
- Bioadsorb components of grape juice
- Cause spoilage during bulk storage of wine in the cellar and after packaging
- Influence growth of malolactic and spoilage bacteria

4.1. Yeast ecology of grapes

Grapes are a primary source of yeasts that enter the winery environment. Consequently, the ecological interactions that occur on grapes will contribute to the species diversity in the winery. Information on the yeast ecology of grapes is neither complete nor conclusive (Martini et al., 1996; Mortimer and Polsinelli, 1999; reviewed in Fleet et al., 2002). Generally, very few yeasts ($10-10^3$ cfu/g) are detected on immature grape berries, but they increase to populations of 10^4-10^6 cfu/g as the grapes mature to harvest. During ripening, sugars leach or diffuse from the inner tissues of the grape to the surface, thereby encouraging yeast growth. Unripe grapes harbour a predominance of *Rhodotorula*, *Cryptococcus* and *Candida* species, along with the yeast-like fungus *Aureobasidium pullulans*. Most of these species are also isolated from mature, ripe grapes but, at this stage, species of the apiculate yeasts, *Hanseniaspora* (anamorph *Kloeckera*) and *Metschnikowia*, are mostly predominant. Damage to the skin and surface of grapes increases the availability of nutrients for microbial growth, and encourages a greater population ($>10^6$ cfu/g) and diversity of yeasts that need to co-exist with various filamentous fungi, acetic acid bacteria and lactic acid bacteria that also develop under these conditions (Fleet et al., 2002). Damaged grapes have increased incidence of *Hanseniaspora* (*Kloeckera*), *Candida* and *Metschnikowia* species, as well as spe-

cies of *Saccharomyces* and *Zygosaccharomyces*. It is not uncommon to find damaged grapes in vineyards, but the impact of this factor on the yeast ecology of wine production has been underestimated in earlier literature. Obviously, the extent of this impact will be determined by the relative proportions of damaged to undamaged fruits.

The principal wine yeast, *S. cerevisiae*, is not prevalent on wine grapes. Generally, it occurs at populations less than 10–100 cfu/g and is best isolated by enrichment culture than direct agar plating (Fleet et al., 2002; Mannazzu et al., 2002). Some researchers have not been able to isolate this species from healthy, mature grapes, and these observations have raised speculations and controversies as to its origins in wine production (Martini et al., 1996; Mortimer and Polsinelli, 1999).

There are many unanswered questions as to why certain yeast species (e.g. *Hanseniaspora*/*Kloeckera*, *Metschnikowia*) predominate on wine grapes, and others (e.g. *S. cerevisiae*) are absent. Influencing factors include: physiological and biochemical compatibility of the species with the surface chemistry of the grape (e.g. adhesion to grape surface, metabolise available nutrients); tolerance of the natural stresses of temperature, sunlight, irradiation, periodic desiccation; tolerance to chemical inhibitors, from the grape itself and from the application of agrichemicals; and interactions with other species (yeasts, bacteria, filamentous fungi) (Fleet et al., 2002; Andrews and Buck, 2002). Yeast–yeast interactions could be important, but these require investigation. There are suggestions that *M. pulcherrima*, commonly found on grapes, is inhibitory to a range of other yeasts, including *S. cerevisiae* (Nguyen and Panon, 1998).

4.2. Alcoholic fermentation

The alcoholic fermentation is the main activity by which yeasts make a positive contribution to wine flavour (Henschke, 1997). They do this by several mechanisms: (i) utilising grape juice constituents, (ii) producing ethanol and other solvents that help to extract flavour components from grape solids, (iii) producing enzymes that transform neutral grape compounds into flavour active compounds, (iv) producing many hundreds of flavour active, secondary metabolites (e.g. acids, alcohols, esters, polyols, aldehydes, ketones, volatile sulphur compounds), and (v) autolytic degradation of dead yeast cells (Cole and Noble, 1997; Lambrechts and Pretorius, 2000). These reactions, especially the production of secondary metabolites, vary with the species and strain of yeast. Tables comparing the diversity of metabolite production by different yeasts may be found in Fleet (1992), Lema et al. (1996), Romano (1997), Heard (1999), and Lambrechts and Pretorius (2000). Thus, the uniqueness and individuality of the flavour contribution by yeasts depends on the species and strain ecology of fermentation and the many factors that determine this ecology (Fleet and Heard, 1993; Fleet, 2001).

In recent years, there have been major advances in understanding the yeast ecology of wine fermentation (Fleet, 2001). Grape juice fermentation presents a complex ecosystem that involves the interactive growth and biochemical activities of a mixture of yeast species and strains. These yeasts originate from (i) the flora of the grapes, (ii) the flora associated with the surfaces of winery equipment and the winery environment (e.g. air, insects), and (iii) added starter cultures, if used. Generally, species of *Hanseniaspora* (*Kloeckera*), *Candida* and *Metschnikowia* initiate the fermentation, and largely originate from the grapes. Sometimes, species of *Pichia*, *Issatchenkia* and *Kluyveromyces* may also grow at this stage. These yeasts grow to about 10^6–10^7 cfu/ml but, by mid-fermentation, begin to decline and die off. At this time, *S. cerevisiae* becomes predominant (10^7–10^8 cfu/ml) and continues the fermentation until its completion. Occasionally, the fermentation may not proceed to completion and unacceptably high amounts (>2–5 g/l) of unfermented sugars remain in the wine. These fermentations are often termed sluggish or stuck and present major practical problems to the winemaker (Bisson, 1999). In addition to the successional growth of different yeast species throughout the course of fermentation, there is an underlying successional development of strains within each species. This latter revelation became most evident with the use of molecular techniques that enabled strain differentiation and recognition (Pretorius, 2000; Fleet, 2001). Up to five or more strains of *S. cerevisiae* have been found in the one ferment, and similar findings have been reported for some non-*Saccharomyces* species (Schulz and Gaffner, 1993; Henick-Kling et al., 1998; Sabate et al., 1998; reviewed in Fleet, 2001).

Many factors affect the occurrence and growth of yeasts during alcoholic fermentation. These include the initial population and diversity of species and strains in the grape juice, inoculation of the juice with selected starter cultures, chemical composition of juice including any fungicide/pesticide residues, processing conditions such as concentration of sulphur dioxide addition and temperature of fermentation, and interactions between the different yeast species and strains (Fleet and Heard, 1993; Bisson, 1999; Fleet, 2001). The following sections focus on the significance of interactions in determining the ecological outcome.

Ethanol production by *S. cerevisiae* is considered to be a major factor that governs the growth and influence of non-*Saccharomyces* species during fermentation. Generally, the species of *Hanseniaspora*, *Candida*, *Pichia*, *Kluyveromyces*, *Metschnikowia* and *Issatchenkia* found in grape juice are not tolerant of ethanol concentrations exceeding 5–7%, and this explains their decline and death as the fermentation progresses beyond the mid-stage (Heard and Fleet, 1988; Gao and Fleet, 1988). However, low temperatures decrease the sensitivity of these species to ethanol and, consequently, wine fermentations conducted at temperatures less than 15–20 °C may show a greater contribution from *Hanseniaspora* and *Candida* species. On such occasions, these species may equal *S. cerevisiae* as the predominant species at the end of fermentation and, accordingly, would have a greater impact on wine flavour (Heard and Fleet, 1988; Erten, 2002). There are recent reports of some strains of *Candida* species that may have ethanol tolerances similar to *S. cerevisiae* (Cocolin et al., 2001; Mills et al., 2002). Strains of *Candida stellata* fall into this category and have been used in co-culture with *S. cerevisiae* to enhance the glycerol content and other flavour characteristics of wines (Ciani and Ferraro, 1998; Soden et al., 2000).

Schizosaccharomyces pombe, *Zygosaccharomyces bailii* and *Zygosaccharomyces fermentati* are well known for their tolerance of high ethanol concentrations (>10%) and occur in winery environments (Fleet, 2000; Romano and Suzzi, 1993). Surprisingly, they are rarely reported as contributors to grape juice fermentations and the reasons for this require investigation. Possibly, they grow slower than other wine yeasts and, consequently, are out-competed, or they may be inhibited by factors produced by these yeasts. All three species have the ability to utilise malic acid, which is a positive attribute in many winemaking instances. While some strains of these species are known to produce off-flavours, a program of selection and evaluation could reveal strains with desirable flavour attributes.

The increase in ethanol concentration during alcoholic fermentation could also explain the sequential growth of strains within a species. Strains of *S. cerevisiae*, as well as those of other species, vary in their tolerance to ethanol stress (Fleet, 1992; Bauer and Pretorius, 2000; Bisson and Block, 2002). Strains with higher ethanol tolerance are more likely to dominate at later, rather than earlier stages of fermentation. This behaviour has been demonstrated experimentally, along with the interactive effect of fermentation temperature (Torija et al., 2002), and becomes an important consideration in designing mixtures of yeast strains (oligo-strains) for use as cultures to enhance the complexity of wine flavour (Grossman et al., 1996).

Short- to medium-chain fatty acids, such as hexanoic, octanoic and decanoic acids, are produced during alcoholic fermentation and, above certain thresholds, become inhibitory to *S. cerevisiae* and, probably, to other species (Viegas et al., 1989; Edwards et al., 1990; Bisson, 1999). Production of these acids varies significantly with yeast species and strain (Lema et al., 1996; Lambrechts and Pretorius, 2000) and could influence the sequential growth of yeasts during fermentation. However, further research is needed to assess the full impact of these acids on the conduct of alcoholic fermentation.

Nutrient availability and nutrient limitation are likely factors that modulate the yeast ecology of fermentation, as one yeast species or strain produces or utilises a nutrient relevant to another species or strain. Evidence for these types of interactions is scant, but various possibilities could be proposed. The non-*Saccharomyces* yeasts appear to be less tolerant of very low oxygen availability than *S. cerevisiae*. Removal of residual oxygen from fermentating grape juice by the vigorous growth of *S. cerevisiae* could contribute to the early death of these non-*Saccharomyces* species (Hansen et al., 2001). Non-*Saccharomyces* species growing early in the fermentation could utilise amino acids and vitamins, and limit the subsequent growth of strains of *S. cerevisiae*. There are

reports that *Kloeckera apiculata* could strip the grape juice of thiamine and other micronutrients, leading to deficient growth of *S. cerevisiae* (Bisson, 1999; Mortimer, 2000). However, some non-*Saccharomyces* species, such as *Kl. apiculata* and *M. pulcherrima* are significantly proteolytic (Charoenchai et al., 1997; Dizzy and Bisson, 2000) and could generate amino acids for use by *S. cerevisiae*. The early death and autolysis of these non-*Saccharomyces* yeasts (Hernawan and Fleet, 1995) is another possible source of nutrients for *S. cerevisiae*, and spoilage yeasts. Cell wall polysaccharides, principally mannoproteins, are also released by yeast autolysis and these could combine with tannins and anthocyanins to impact on wine astringency and colour (Escot et al., 2001). So far, the studies on polysaccharide release relate only to *S. cerevisiae*, and need to be extended to include the non-*Saccharomyces* species.

There is an extensive literature on the isolation of killer toxin producing strains, killer-sensitive strains and killer neutral strains of *S. cerevisiae* from fermenting grape juice (van Vuuren and Jacobs, 1992; Shimizu, 1993; Musmanno et al., 1999; Guriérrez et al., 2001). Although many winemaking variables affect the expression of killer and killer-sensitive phenotypes, there is good evidence that killer interactions may determine species and strain evolution during fermentation. Killer strains of *S. cerevisiae* sometimes predominate at the completion of fermentation, suggesting that they have asserted their killer property and taken over the fermentation. Killer strains have been found within wine isolates of *Candida*, *Pichia* and *Hanseniaspora* and some of these can assert their killer action against wine strains of *S. cerevisiae* (Fleet and Heard, 1993). However, properly designed studies are needed to connect killer interactions and an impact on wine flavour. We have demonstrated how killer interactions between strains of *S. cerevisiae* could be used to manipulate the autolytic response of wine yeasts, and give increased protein content in wine (Todd et al., 2000).

4.3. Yeast spoilage of wines

Yeasts can spoil wines at several stages during production. Unacceptable flavours can be produced if inappropriate yeast species or strains grow during the alcoholic fermentation. These defects include wines with excessive concentrations of hydrogen sulfide and other sulphur volatiles, acetic acid, various esters, and volatile phenols (Sponholz, 1993; Fleet, 1992, 1998; Fugelsang, 1997; Du Toit and Pretorius, 2000). Such occurrences highlight the importance of understanding and managing the yeast ecology of fermentation, as mentioned already.

Bulk storage of wines in tanks and barrels prior to packaging is another critical point where yeast spoilage may develop. Wine that is exposed to air, as in incompletely filled tanks or barrels, quickly develops a surface flora of weakly fermentative or oxidative yeasts, usually species of *Candida* and *Pichia* (e.g. *Pichia membranifaciens*). These species oxidise ethanol, glycerol and acids, giving wines unacceptably high levels of acetaldehyde, esters and acetic acid. Bulk wines, as well as bottled wines, are also spoiled by fermentative species of *Zygosaccharomyces*, *Dekkera* (anomorph *Brettanomyces*), *Saccharomyces* and *Saccharomycodes*. In addition to causing excessive carbonation, sediments and haze, these species produce estery and acid off-flavours (Sponholz, 1993). Species of *Dekkera*/*Brettanomyces* are also associated with the production of unpleasant mousy and medicinal taints, because they can form tetrahydropyridines and volatile phenolic substances such as 4-ethylguaiacol and 4-ethyl phenol (Grbin and Henschke, 2000; Du Toit and Pretorius, 2000). Management of these types of spoilage is generally done by following good manufacturing practice and hygiene. However, some yeast interactive phenomena may be relevant. Yeast autolysis after alcoholic fermentation could be a significant source of micronutrients for the growth of these spoilage species (Charpentier and Feuillat, 1993), especially those of *Dekkera*/*Brettanomyces* (Guilloux-Benatier et al., 2001). Consequently, removal of yeast sediment soon after the completion of alcoholic fermentation may minimise this risk. Some authors have suggested a more proactive control by selecting or engineering strains of *S. cerevisiae*, or other desirable species, with killer activity directed against key spoilage species (Shimizu, 1993; Du Toit and Pretorius, 2000).

5. Yeast–bacteria interactions

Bacteria have both positive and negative influences on wine production (Table 3) and these contributions

Table 3
Mechanisms by which bacteria impact on wine flavour and quality

- Spoilage of grapes in the vineyard
- Potential cause of sluggish or stuck alcoholic fermentation
- Conduct malolactic fermentation
- Spoilage of wine during storage in the cellar and after packaging; production of biogenic amines
- Contribution to corky, earthy taints by growth on corks, wooden barrels

can be moderated by interactions with yeasts. Two groups of bacteria are particularly significant in wine microbiology—lactic acid bacteria and acetic acid bacteria. The pH and ethanol tolerance of species within these groups are main factors that select for their occurrence in winery ecosystems (Fleet, 1993). Within the lactic acid bacteria, species of *Lactobacillus* and *Pediococcus* are most relevant, along with *Oenococcus oeni* (formerly *Leuconostoc oenos*) which conducts the malolactic fermentation (Lonvaud-Funel, 1999). Within the acetic acid bacteria, *Acetobacter aceti*, *Acetobacter pasteurianus* and *Gluconobacter oxydans* are commonly isolated (Drysdale and Fleet, 1988), although *Acetobacter liquefaciens* and *Acetobacter hansenii* have recently been found (Du Toit and Pretorius, 2000).

Grapes, especially if damaged, are a primary source of bacteria in the winery environment (Wibowo et al., 1985; Drysdale and Fleet, 1988). However, definitive studies on the bacterial ecology of grapes are lacking. We have recently found *Bacillus thuringiensis* to be predominant on wine grapes harvested from numerous vineyards in New South Wales, Australia, and correlated its occurrence with the use of this species as a biopesticide during grape cultivation (Bae and Fleet, unpublished data). There will be opportunities for yeast–bacterial interactions at the stage of grape cultivation but, so far, these have not been investigated.

Grape juices produced from healthy, mature grapes have low populations ($<10^3$–10^4 cfu/ml) of bacteria. With a vigorous onset of alcoholic fermentation by yeasts, these bacteria generally show little growth and die off to nondetectable levels (Fleet, 2001). However, if yeast growth is delayed, various species of lactic acid bacteria and acetic acid bacteria may grow, inhibit the growth of yeasts, and cause sluggish or stuck fermentations. Similar outcomes occur if juice is prepared from damaged grape berries where there are elevated populations of lactic and acetic acid bacteria (Ribéreau-Gayon, 1985; Fleet and Heard, 1993; Bisson, 1999; Fleet, 2001). Various species of *Lactobacillus*, including a new species, *Lactobacillus kunkei*, certain strains of *O. oeni*, *A. aceti* and *A. pasteurianus* have been implicated in this problem (Drysdale and Fleet, 1989; Huang et al., 1996; Edwards et al., 1998a,b, 1999). Metabolism of grape juice sugars by these bacteria produces concentrations of acetic acid that become inhibitory to the growth of *S. cerevisiae* (Ludovico et al., 2001). However, additional inhibitory factors are suspected to be involved and further research is needed to establish their identity.

The malolactic fermentation is an important secondary fermentation that occurs in many wines, generally, about 2–3 weeks after completion of the alcoholic fermentation (Lonvaud-Funel, 1999). Lactic acid bacteria, principally *O. oeni*, are responsible for this fermentation. These bacteria are naturally resident in the wine or commercial strains may be inoculated. Growth of *O. oeni* during this fermentation functions to decrease wine acidity by transforming L-malic acid into L-lactic acid, enhance wine flavour and complexity through production of additional metabolites, and to increase subsequent microbiological stability of the wine by removal of residual nutrients and production of bacteriocins (Fleet, 2001). For many winemakers, these are significant benefits. Many factors affect the growth of *O. oeni* in wines and the conduct of the malolactic fermentation. Among these, yeast–bacterial interactions can be very important. A significant body of literature convincingly demonstrates that the strain/s of *S. cerevisiae* responsible for the alcoholic fermentation can inhibit the subsequent growth of *O. oeni* and the malolactic fermentation (Markides, 1993). The relationship is very much strain-dependent, at both the yeast and bacteria levels (Fornachon, 1968). The molecular basis of this interaction requires further study, but could involve yeast production of inhibitory short chain fatty acids (e.g. hexanoic, octanoic, decanoic), sulphur dioxide, peptides and proteins (Fornachon, 1968; Wibowo et al., 1988; Dick et al., 1992; Markides, 1993; Capucho and San Romao, 1994; Eglinton and Henschke, 1996; Lonvaud-Funel et al., 1998; Guilloux-Benatier et al., 1998). However, there is also evidence that, in a general sense, yeasts may stimulate the growth of *O. oeni* and onset of

malolactic fermentation. This behaviour is linked to the autolysis of yeasts after alcoholic fermentation and the release of nutrients favourable to bacterial growth (Fornachon, 1968; Guilloux-Benatier et al., 1993; Patynowski et al., 2002). Some yeast species and strains autolyse faster than others and this may account for the different bacterial responses that have been reported (Fornachon, 1968).

Spoilage species of lactic acid bacteria, acetic acid bacteria and, occasionally, *Bacillus* and *Clostridium* species may grow in wines during storage in the cellar and after bottling (Sponholz, 1993; Fugelsang, 1997; Fleet, 1998; Du Toit and Pretorius, 2000). Their growth is probably encouraged by nutrients released by autolysis of wine yeasts and also *O. oeni* (Crouigneau et al., 2000). Fornachon (1968) reported an interesting inhibitory action of spoilage yeasts (e.g. *Pichia* spp., *Saccharomycodes ludwigii*, *Candida pulcherrima*) on the growth of spoilage lactic acid bacteria (*Lactobacillus hilgardii*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*) and suggested that this may be related to inhibitory concentrations of sulphur dioxide produced by these yeasts. Yeasts and lactic acid bacteria, including species found in wines, are known to co-flocculate, and this phenomenon relates to bacterial affinity for the mannoproteins of yeast cells walls (Peng et al., 2001). However, it needs to be determined if these types of interactions occur in wine ecosystems. Another interesting development is the engineering of recombinant strains of *S. cerevisiae* with genes encoding the production of bacteriocins and lysozyme that are active against wine spoilage bacteria (du Toit and Pretorius, 2000). While such strains may have practical application in controlling wine spoilage, their commercial use will require approval by government regulatory authorities, and general acceptance by consumers (Pretorius and Bauer, 2002).

6. Conclusion

Wine is the product of many diverse interactions between yeasts, fungi and bacteria. These relationships commence in the vineyard and continue throughout the fermentation and storage processes. They determine the ecological profile of the production chain and have positive and negative influences on wine flavour. Their significance in contributing to the quality and efficiency of wine production warrants greater recognition. The biochemical, physiological and molecular bases of these interactions are little understood and require investigation.

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