Yeast Hybrids in Winemaking

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Summary

Aim: There is significant variety in the choice of commercial yeast strains available for wine production. Yeast hybrids represent one class of commercial yeasts. The purpose of this review is to define what yeast hybrids are, to describe how they arise in nature and in the laboratory, and to discuss the features of these yeasts that differentiate them from non-hybrid strains.

Key Themes:

- How does mating occur normally in yeast?
- What is a hybrid yeast strain?
- How do hybrid strains form in nature?
- How are hybrid wine strains constructed in the laboratory?
- Are natural hybrids of wine yeast found in nature?
- What are the features of laboratory-generated wine yeast hybrids?
- What are the impacts of hybrid strains on wine composition?

Impact and Significance: Yeast influence wine aroma and flavor profiles in multiple ways¹. In addition to the formation of ethanol, yeast can directly produce both positive and negative aroma impact compounds, such as esters, higher alcohols, higher aldehydes, and sulfur-volatile compounds. Some yeast strains can produce factors that enhance mouthfeel, and many can modify varietal aroma through enzymatic and chemical mechanisms. Yeast mannoproteins and polysaccharides may also affect wine stability. Last, yeast end products can alter the wine matrix and therefore the sensory expression of wine characters. Yeast hybrids combine traits of *Saccharomyces cerevisiae* and other species in this genus, and thereby expand the metabolic repertoire of the strain. These yeasts can create novel characters in wine as well as conduct fermentation under non-standard conditions.

Key words: *Saccharomyces* species, yeast hybrids

Overview

The yeast *Saccharomyces cerevisiae* is the primary organism responsible for the conversion of grape juice into wine. This species is comprised of many different strains. Strains are individual isolates of the same species that differ in the precise sequence or composition of genes and therefore display nonidentical biological properties. As the genetic composition of different yeast strains varies, so does their impact on wine characteristics. Genetic differences or mutations arise in yeast due to errors in DNA replication or repair. These differences may be benign, lead to loss of function of a gene and protein, alter the function of the protein, create novel functions and activities, or alter the physiological properties of the cell. Other yeast species of the genus *Saccharomyces* have a different array of chemical products and enzymatic activities as compared with *S. cerevisiae*, and these species can also be found in association with wines, wineries, and vineyards². Most of the natural vineyard and winery isolates of the wine yeast *S. cerevisiae* contain only DNA native to this species².

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In microbiology, a species is defined by reproductive isolation, meaning that only the members of the species will mate with each other and produce fertile offspring. Different species within the same genus often share many common genes and properties but display reproductive isolation, meaning that should they mate, fertile offspring are not produced. Occasionally, illicit mating between different species of the same genus will occur and create hybrid strains with novel genetic properties.

Key Themes

How does mating occur normally in yeast? In order to appreciate how hybrids of different species arise in nature, the process of mating within the species of *Saccharomyces* will first be described. The *Saccharomyces* life cycle consists of vegetative growth as either a diploid or a haploid, with diploids being able to sporulate and create haploid spores and cells, and haploid cells being able to mate and re-form the diploid state (Figure 1). This multicomponent life cycle enables genetic exchange between haploid cells and the selection for and against spontaneous mutations that arise in the population. Different genotypes are tested for enhanced fitness in the environment, and those variants that are more fit will dominate. This process allows microorganisms to adapt to new and changing environments.

There are two haploid states, called “a” and “α”, in *Saccharomyces*³. When an “a” haploid cell is next to an “α” haploid cell, the two cells will grow toward each other, fuse, and form a zygote, followed by nuclear fusion and the formation of a diploid (Figure 1). Such mating is common in the yeast kingdom and happens regularly. Following cell-surface merger, the cytoplasm initially contains two independent nuclei that then undergo a fusion event. This fusion event reconstitutes the two sets of chromosomes of a diploid (Figure 1).

The diploid form may undergo the special process of meiosis or sporulation and produce four haploid spores (Figure 1). Meiosis is the process by which gametes are formed in higher eukaryotes, such as plants and animals. During meiosis, the DNA is first replicated to generate a transient 4N state (with four sets of chromosomes) that can then undergo genetic rearrangements with each other, producing chromosomes with unique assortments of DNA when compared with those in the parent strains. Ultimately, four haploid (i.e., 1N) nuclei are formed, and each is packaged into a unique cell or spore. The released spores may then grow vegetatively as haploids or mate to re-form the

diploid state (Figure 1). During this process, the genes in the genome have been reassorted or shuffled, and the spores may express traits not seen in either of the parents⁴. In some cases, the new cells are more fit for their environment, and in other cases, they are less fit and will be lost from the population. Repeated rounds of vegetative growth, accumulation of mutations, sporulation, and remating yield genetic variety across the population of *S. cerevisiae* strains found in nature. Thus, the variability seen in wine characteristics as a consequence of yeast strain choice derives from the natural processes of genome change, exchange, and reassortment.

What is a hybrid yeast strain? Several commercial and native strains of *S. cerevisiae* are genetic hybrids. A hybrid is defined biologically as an organism that contains genomic DNA from two or more hereditarily distinct parents. Hybrids may arise from the mating of two genetically different strains of the same species, and these strains are called intraspecific hybrids. This type of hybrid yields fertile spores. A second class of hybrids are called interspecific hybrids and arise from the mating of two different species or genera. In the case of wine yeast, interspecific hybrids are the product of the fusion of cells from different species of *Saccharomyces*⁴. These hybrids arise naturally in the environment when the two species are present in the same ecological niche under conditions leading to cell-to-cell fusion. Sometimes, hybrid strains may define a unique species. In this case, progenies of hybrid strains

Figure 1 The life cycle of the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* can grow vegetatively by budding either as a haploid (one set of chromosomes) or as a diploid (two sets of chromosomes). Haploids exist as one of two mating types, “a” or “α”, and MAT_a and MAT_α cells can undergo shmoo formation (directional elongated cell growth) and cell and nuclear fusion events leading to the formation of a zygote. The zygote can grow vegetatively as a diploid. Diploids can undergo meiosis, forming four haploid spores, two “a” and two “α” mating types.

are fertile when crossed against other members of the hybrid strain, but not when crossed against other species. A third class of hybrids also exists. These are strains that contain mostly the genome of one species with some stretches of DNA from another species⁵. Since it is not clear whether these strains are derived from interspecific hybrids with loss of foreign DNA or, alternatively, have picked up DNA from the environment, they are referred to as carrying introgressions, which are large regions of DNA foreign to that species. EC1118 is an example of a commercial wine strain that carries introgressions⁶.

The *Saccharomyces* genus contains seven non-hybrid and two hybrid species (Table 1)⁷. The lager yeast *S. pastorianus* is a natural hybrid of two other species, *S. cerevisiae* and *S. eubayanus*, and *S. bayanus* appears to be a hybrid of three species: *S. cerevisiae*, *S. eubayanus*, and *S. uvarum*, and may have arisen from a rare mating of *S. pastorianus* and *S. uvarum*⁸. Thus, yeast hybrids arise commonly in nature.

Hybrids are thought to combine the metabolic properties of the two originating species and therefore may have unique patterns of metabolism and end-product profiles. Often, they can combine desirable properties of the two starting strains, combining in wine yeast a low temperature tolerance of one parent with an array of aroma characters produced by the other. These features would be selected for in the natural environment. For example, a low-temperature-tolerant strain may not produce the array of esters needed to attract insects and disperse the yeast, but a high-ester-producing strain may not be able to grow at low temperature. The hybrid strain would be able to both grow at low temperature and produce insect-attracting ester compounds, and could thus come to dominate the environment.

Table 1 Recognized species in the yeast genus *Saccharomyces*⁷.

Nonhybrid species

S. arboricolus
S. bayanus var. *uvarum*
S. cariocanus
S. cerevisiae
S. kudriavzevii
S. mikatae
S. paradoxus

Hybrid species

S. bayanus var. *bayanus*
S. pastorianus

Hybrids can display the trait of one parent (dominant trait), a mixture of a trait (both traits expressed simultaneously, with neither trait being dominant), or a novel trait (trait not found in either parent). Hybrids may be genetically stable as a hybrid and fully maintain both sets of genomes, or may be unstable and lose some of the DNA from one or both parents.

How do hybrid strains form in nature? Hybrids are generated in the wild when haploid cells of different species mate to form a diploid cell, or if two diploid cells of different species fuse to form a tetraploid cell (4N). Diploid cells normally do not mate with other cells because they already contain two sets of chromosomes. Several naturally arising hybrids of *Saccharomyces* have been isolated from vineyards⁴. These hybrids have been isolated at levels higher than would be predicted from simple rare mistakes in mating in the wild.

A clue to the origins of interspecific hybrids came from an analysis of yeasts associated with wasps⁹. Although normally digested in the wasp intestine, viable strains of *Saccharomyces* can be isolated from wasps, and these wasps are thought to play an important role in dispersal of *Saccharomyces* in the wild⁹. Wasps were fed five genetically different strains of *S. cerevisiae*, each of which carried different genetic mutations, and the wasps were allowed to hibernate in isolation¹⁰. Yeasts were harvested from the wasp intestinal tract and evaluated for the evidence of mating. After four months in the wasps, the yeasts exhibited significant outbreeding—evidence of sporulation of the five different strains and mating of the released spores across the five strains, generating intraspecific hybrids.

Analysis of yeast from social wasps caught in the wild revealed that interspecific mating can occur as well in this environment. Hybrids of *S. cerevisiae* × *S. uvarum* and *S. cerevisiae* × *S. paradoxus* were found¹⁰. This observation could mean either that the wasps prefer feeding on substances that naturally contain yeast hybrids, or that inter- as well as intraspecific mating occurs in the wasp intestinal tract. To differentiate between these two possibilities, the wasps were fed pure strains of *S. cerevisiae* and *S. paradoxus* simultaneously. Over one-third of the wasps developed interspecific hybrids of *S. paradoxus* and *S. cerevisiae*, indicating that illicit as well as licit mating occurs within the insect. Interestingly, both the pure *S. cerevisiae* strains and the pure *S. paradoxus* strains could no longer be isolated from the wasp intestine at the end of the hibernation period, and only the hybrid strains survived¹⁰. Thus, hybrids are better able to adapt to the intestine

than are either of the parental strains. This study identifies for the first time the likely location of formation of hybrid yeast strains in the wild. Further, the diversity of fruit substrates present adjacent to vineyards is expected to impact the diversity of yeasts found in wasps as they visit a variety of different food sources. Regions that have a greater diversity of wasp food sources may show, in turn, a greater diversity of interspecific hybrids on grapes at the point of harvest, and this would be an interesting study to conduct.

How are hybrid wine strains constructed in the laboratory? Hybrid strains can also be directly created in the laboratory through a process known as protoplast fusion. In protoplast fusion, the cell walls of two cells are partially digested using specific enzymes, and the cells are then artificially attached to each other via lectins that link two plasma membranes. The plasma membranes then fuse to each other, and the two cells become one (Figure 2). The cell wall is then repaired, and the hybrid cells can undergo cell division and grow.

Laboratory construction of hybrid strains enables mating of more distantly related species or of species not normally found in the same environmental niche. Partial digestion of the cell wall and formation of a protoplast—a cell with an intact plasma membrane—leads to cells sensitive to the osmotic conditions of the environment and that can easily lyse. A similar pro-

cess of partial digestion of the yeast outer surface may be occurring within the insect intestine, and passage through that intestine may provide sufficient buffering conditions to enable cell fusion and repair⁹.

Triploid and tetraploid strains can also be derived from two different species using protoplast fusion, but are not genetically stable because of the high chromosome number as well as the odd number of sets of chromosomes¹¹. *Saccharomyces* cells normally replicate and maintain 16 individual chromosomes or chromosome pairs. If the number of chromosomes is greater than 16, the yeast has difficulty replicating all of them during a normal cell division, and some chromosomes may be only partially replicated or not replicated at all. This unstable state leads to chromosome loss, which is often random. If the lost genetic information is not essential for growth and survival, the new cell will be able to continue to grow. In addition, there may be recombination events between chromosomes generating novel or chimeric chromosomes not found in either parent. The traits expressed by these hybrid genomes can be dominated by one of the parents and show traits highly similar to that parent or can be true mixtures of traits of both parents. Thus, the products of protoplast fusion are not genetically stable, and growth of these strains will select for viable survivors that generally have a smaller genome size than that present at the original fusion event.

Are natural hybrids of wine yeast found in nature? The first demonstration of the hybrid genetic nature of wine strains was published in 1998¹². The authors used karyotype analyses, partial sequencing, and restriction length polymorphisms to show that some wine isolates were hybrids of *S. cerevisiae* and *S. bayanus*. Aside from *S. cerevisiae*, three species can be found in wine fermentations and winery environments: *S. bayanus*, *S. uvarum*, and occasionally *S. paradoxus*. Hybrids arising from mating events between *S. cerevisiae* and each of these three species have been found among winery isolates¹³. Since these yeasts can occupy the same local environment, and there is no prezygotic barrier to cell fusion, the appearance of hybrids among these yeasts in winery environments is not surprising.

More surprising was the isolation of hybrids of *S. cerevisiae* and *S. kudriavzevii* from wine. *S. kudriavzevii* was isolated originally from decaying leaves in Japan¹⁴. This species had not been found in grape fermentations or in any other type of fermentative process. Hybrids between *S. cerevisiae* and *S. kudriavzevii* also have been reported in Europe¹⁵.

Figure 2 The process of protoplast fusion between species in *Saccharomyces*. The cell wall is gently digested, leaving an intact plasma membrane. Lectins are used to attach protoplasts to each other, and conditions are maintained that support cell fusion and reconstruction of the cell wall, creating a hybrid strain. (Sc = *S. cerevisiae*; Sk = *S. kudriavzevii*; Sck = hybrid of *S. cerevisiae* and *S. kudriavzevii*).

The European parental *S. kudriavzevii* strain was subsequently shown to be present on oak bark in Portugal and was found in the same locations as *S. cerevisiae* and *S. paradoxus*¹⁶. Thus, it is thought that the hybrids between *S. cerevisiae* and *S. kudriavzevii* arose in non-winery or vineyard environments, and these hybrids carry some selective advantages for fermentations. Sequencing of the commercial wine strain VIN7 (a yeast strain isolated in South Africa) demonstrated that it is an allotriploid hybrid, carrying a diploid set of chromosomes from *S. cerevisiae* and a haploid set from *S. kudriavzevii*¹⁷.

The wine isolates of *S. cerevisiae*/*S. kudriavzevii* hybrids have been shown to vary genetically, ranging from diploids and triploids to tetraploids (2N, 3N, and 4N respectively)¹⁸, and to be the products of independent hybridization events and sometimes of multiple hybridization events between the initial hybrids and the original parents¹⁸. Other scientists have reached the same conclusion that hybrid isolates have arisen from rare mating events between *S. cerevisiae* and *S. kudriavzevii*, and that multiple independent hybridizations have occurred, including at least six independent hybridization events for the 26 strains examined in detail¹⁷, and the genetic changes associated with these events include chromosomal rearrangements and gene loss¹⁹.

The observation of multiple independent events giving rise to hybrids between *S. cerevisiae* and the non-wine species *S. kudriavzevii* suggests that the hybrids have some traits that are advantageous in wine fermentations. Several researchers have investigated the fermentation characteristics of hybrids of these two yeasts and compared them with those of their parents²⁰. *S. kudriavzevii* is more cryotolerant than *S. cerevisiae*, growing well at temperatures below the range that supports growth of *S. cerevisiae*²¹. The natural hybrid strains retain this ability to grow at low temperatures, but also express the ethanol and osmotic tolerance associated with *S. cerevisiae*. Thus, these hybrid strains would be selected for during fermentations at low temperatures. *S. kudriavzevii* is not competitive as a species in grape juice fermentations, quickly dying off, although the reason for the rapid decline in this species is not known²².

Analysis of the lipid composition of the *S. cerevisiae*/*S. kudriavzevii* hybrids indicates that their lipid profiles have a hybrid pattern more similar to *S. kudriavzevii*, thus explaining the greater temperature tolerance of the hybrids than that of *S. cerevisiae*²³. Thus, the hybrids in essence contribute the best

of both worlds to cell metabolism and enable a more rapid development of novel phenotypes²³.

Hybrid strains also show novel phenotypes in aroma production and modification. These strains impact both direct synthesis of aroma compounds and the release or modification of varietal aroma components²⁴. The spectrum of compounds produced was shown to be temperature dependent²⁵, such that non-hybrid strains were thought to have a more appealing aroma profile at higher temperatures. Analysis of regulation of aroma production indicated that the hybrids have unique regulatory mechanisms for aroma production not easily predicted from the parental strains²⁶.

What are the features of laboratory-generated wine yeast hybrids? Hybrids of various *Saccharomyces* species can be generated in the laboratory through selection for rare mating events or via the aforementioned protoplast fusion²⁷. In some regions, protoplast fusion is considered a “GMO” technology²⁸, and for that reason, research has focused on mating to generate hybrids in the laboratory. The hybrids initially are simple fusions of the two sets of chromosomes, but selective pressure, especially in grape juice, selects for specific chromosomal loss and rearrangement patterns²⁷.

As with the natural hybrids, these laboratory hybrids show different levels of production of aroma compounds and sometimes display levels similar to those produced by one of the parents, but for other compounds, the level can be higher or lower than those produced by either parent²⁹. Much of this work focuses on the same species found as parents in natural hybrid strains, but one study showed hybrids could be made from *S. cerevisiae* and *S. mikatae* and provide unique traits and characteristics for wine production³⁰. Novel constructed hybrids may also prove useful commercial strains for the wine industry.

What are the impacts of hybrid strains on wine composition? The two main positive traits of hybrid strains for winemaking are their greater temperature tolerance and the broader spectrum of aroma compounds produced, either as de novo yeast aroma characters or in the modification and release of grape-varietal characters³¹. One laboratory study using partially purified fractions of odorless precursors of grape-varietal characters found that interspecific hybrid strains could release more grape aromatic character than non-hybrid strains³². Thus, another important advantage of hybrid strains may be the enhancement of varietal character rather than the production of yeast-derived aroma compounds. Hybrids also offer a distinct advantage over the use of two strains individually in that

there is a single strain in a given fermentation, so there is no competition between two different strains. An additional benefit may be the unique profile of compounds produced by hybrids as compared with those produced by their two parents. Hybrids may produce higher or lower concentrations of important aromatic compounds than either of their parents, and this property might be important to the overall wine aroma profile. A study of 212 strains of commercial and native isolates of *Saccharomyces* in Australia found that there is strong genetic similarity among non-hybrid strains, with roughly one-third of all isolates tested being related *Prise de Mousse* strains³³. In this collection, they discovered only 17 (8%) hybrid strains, 10 of which were hybrids with *S. kudriavzevii*. The authors speculate that novel characters for wines may come from the use of interspecific hybrids that can bring more metabolic diversity to the production of wine.

Interspecies hybrids can thus offer novel metabolic options in winemaking. However, there are always issues to consider before a winery adopts any new strain (Figure 3). Wineries can become colonized by robust fermenters, which can make it difficult for an introduced strain to actually implant in the fermentation. Thus, it is important that the hybrid strain be fully dominant against the native flora of the vineyard and winery. If conditions prevent implantation of the added strain, then it will of course have limited impact on the aroma profile of the wine. Since hybrids perform better at lower temperatures, high-temperature fermentations may be more readily dominated by non-hybrid strains of *S. cerevisiae*.

Second, yeast strains vary in their tolerance of wine stresses. These stresses can be caused by inhibitors in the fermentation, lack of ethanol tolerance, or nutrient limitation or excess. Many of our existing fermentation management strategies are tailored to *S. cerevisiae*.

Concerns with Use of New Wine Yeast Strains

- Dominance of fermentation
- Tolerances to stress factors
- Genetic stability
- Aroma profile and stability
- Impact of different juice chemistries
- Impact on and tolerance to wild microorganisms

Figure 3 Potential concerns in the adoption of a novel wine yeast strain.

Since *S. kudriavzevii* does not have the same ability to tolerate the inhibitory conditions found in winemaking²², hybrids may have inherited the reduced tolerance traits from this parent.

Genetic stability is also an important factor for hybrid strains. Non-hybrid strains undergo genetic modification, and strains can change over time, but the chromosomal constitution of hybrids can accelerate adaptive responses²³. Thus, these strains may continue to change at a more rapid pace than a non-hybrid strain.

Fourth, an important question to consider with any strain that has the capacity to dramatically alter the aroma profile is whether you want those specific aromas. The hybrids produce higher levels of the positive fruity esters, and that level of aromatic intensity might not be appropriate for some wine styles. The hybrids can also produce higher levels of the more negatively perceived higher or fusel alcohols³¹.

Last, one attractive attribute of hybrid strains is their ability to enhance varietal aroma via the release or modification of specific grape odorless precursor compounds³². However, these precursors have to be present in the first place in the fruit at harvest. The chemical composition of the grapes may not enable higher levels of production of the desired aromatic compounds. The pH, acidity, and phenolic composition of the juice may affect the appearance of a character by either masking or modifying it in unexpected ways. Thus, the strain may not perform as expected if the chemistry of the matrix is not compatible with the appearance or expression of the desired characters.

Significance

As with any new tool or technique, it is important to conduct winery trials to evaluate the impact of a potential change in production. It is also important to understand the benefits and limitations of any strain in light of the specific production conditions. Strains that do not have the ethanol or temperature tolerance associated with the initial Brix reading should not be selected. Hybrid wine strains, whether natural or constructed via natural mating processes, are going to offer a host of novel winemaking traits and enhance existing and even add new types of aromas and flavors to the wine. Accurately determining how these strains perform under specific winemaking conditions will be essential for capitalizing on this new source of aroma profile modification.

Correction: On page 29, in the last paragraph, the word “intraspecific” was changed to “interspecific” in the following sentence: “Over one-third of the wasps developed interspecific hybrids of *S. paradoxus* and *S. cerevisiae*, indicating that illicit as well as licit mating occurs within the insect.”

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