The Genetics of Non-conventional Wine Yeasts: Current Knowledge and Future Challenges

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Saccharomyces cerevisiae is by far the most widely used yeast in oenology. However, during the last decade, several other yeasts species has been purposed for winemaking as they could positively impact wine quality. Some of these non-conventional yeasts (Torulaspora delbrueckii, Metschnikowia pulcherrima, Pichia kluyveri, Lachancea thermotolerans, etc.) are now proposed as starters culture for winemakers in mixed fermentation with S. cerevisiae, and several others are the subject of various studies (Hanseniaspora uvarum, Starmerella bacillaris, etc.). Along with their biotechnological use, the knowledge of these non-conventional yeasts greatly increased these last 10 years. The aim of this review is to describe the last updates and the current state-of-art of the genetics of non-conventional yeasts (including S. uvarum, T. delbrueckii, S. bacillaris, etc.). We describe how genomics and genetics tools provide new data into the population structure and biodiversity of non-conventional yeasts in winemaking environments. Future challenges will lie on the development of selection programs and/or genetic improvement of these non-conventional species. We discuss how genetics, genomics and the advances in next-generation sequencing will help the wine industry to develop the biotechnological use of non-conventional yeasts to improve the quality and differentiation of wines.

Keywords: non-conventional yeast, non-Saccharomyces, wine, enology, oenology, microsatellite

INTRODUCTION

In oenology, alcoholic fermentation is generally performed by Saccharomyces cerevisiae yeast, the “conventional” wine yeast. Currently, the winemakers have the choice between hundreds of S. cerevisiae starters that have been selected for various characteristics including their ability to complete alcoholic fermentation in oenological conditions, their low release of off-flavor compounds, their positive impact on wine aromas, etc., (Pretorius, 2000; Marullo and Dubourdieu, 2010). The growing demand for more diversified wines or for specific characteristics (low ethanol content, etc.) has led to the exploration of new species for winemaking. These non-conventional yeasts may contribute to the wine's flavor and taste by producing a broad range of secondary metabolites and extracellular enzymes (Hong and Park, 2013; Ciani et al., 2014; Wang et al., 2015). Some species could be interesting for alcohol level reduction in wine (Masneuf-Pomarede et al., 2010; Bely et al., 2013) or for greater fermentative ability in harsh conditions due to enhanced fructophily (Sutterlin, 2010; Magyar and Tóth, 2011). It has to be noted that, as only...
some *Saccharomyces* species (i.e., *S. cerevisiae*, *S. uvarum*, and some interspecific hybrids) are able to consume all the sugar contained in grape must, non-*Saccharomyces* yeasts must be used in co- or semifungal fermentation with a *Saccharomyces* spp. able to secure AF completion (Jolly et al., 2006; Bely et al., 2013).

The wine industry currently proposes starters of a few non-conventional yeasts (*Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, *Lachancea thermotolerans*, etc.), while several other species (*Hanseniaspora uvarum*, *Starmerella bacillaris*, etc.) are subject of various studies to assess both positive contribution (Table 1) and negative impact (if any) on wine quality (Bely et al., 2013; Maturano et al., 2015). These non-conventional yeasts are widely distributed amongst the *Saccharomyces* (Figure 1). In order to evaluate theoenological potential of a given species, several strains are usually compared for phenotypes of interest like fermentation ability (Renaut et al., 2009) or glycerol production (Magyar and Tóth, 2011). However, in most cases, neither the relationships between the tested strains are described, nor the genetic structuration of the species is known. This lack of genetic knowledge is clearly detrimental, since we are not able to determine whether the phenotypic diversity described is representative of the species or not. The recent advances in next-generation sequencing (NGS) have triggered the development of genomic and genetic tools for some of these non-conventional yeasts, but the field is still in its infancy. The objective of this paper is thus to review the current state-of-art of the genetics of non-conventional wine yeasts and to discuss the future prospects and challenges from an enological viewpoint.

**BASIC GENETIC KNOWLEDGE OF WINE YEASTS**

As a model organism, the genomic outline of *S. cerevisiae* is well-known: its genome size is around 12 Mb organized in 16 chromosomes, with a mitochondrial genome of 85 Kb (Table 1). The genome sequences of several hundreds of strains of various origins are available, and much more sequences are produced easily using NGS technology and subsequently assembled even by lab with moderate bioinformatics skills. The population genomics of *S. uvarum* has been improved recently with the sequencing of more than 50 strains of various origins (Almeida et al., 2014). The type strain CBS7001 has a genome size of 11.5 Mb and 16 chromosomes (Clifton et al., 2003). By contrast, such basic knowledge (genome size, chromosome number, etc.) is available only for a small number of non-conventional wine species: *T. delbrueckii* has a genome of 9–11 Mb distributed on eight chromosomes; *L. thermotolerans* has a 10.4 Mb genome with eight chromosomes. Other wine yeast species usually have genome size ranging from 8 to 12 Mb, with chromosomes number unknown yet (*P. kluyveri*, *M. pulcherrima*, etc.). Moreover, there is still a lack of reference genome sequence for several non-conventional wine yeasts of interest like *S. bacillaris*, *P. fermentans*, etc., (Table 1). Disparities exist also for the mitochondrial genome, with full sequences available for some species like *L. thermotolerans* or *H. uvarum*, and partial sequences for other species (*C. stellata*, *P. membranifaciens*, etc.). Thus, although the genomic data of non-conventional wine yeast greatly increased this last decade, there is still a lot of work to achieve in this field.

**THE LIFE-CYCLE OF WINE YEASTS**

The life cycle of *Saccharomyces* wine species is well-known: both *S. cerevisiae* and *S. uvarum* are diploid species that divide asexually by mitosis. They are able to enter meiosis and form asci containing generally four haploid spores (tetrads). While haploid cells can undergo mitosis, the haploid level is generally transient and crosses between haploid spores of opposite mating types are readily observed, leading to diploid zygote formation. Moreover, haploid cells are usually able to switch mating type at mitosis (homothallism). The physical proximity between mother and daughter haploid cells of opposite mating type usually results in high level of inbreeding (Ruderfer et al., 2006; Cubillos et al., 2009; Ruggieri et al., 2011). Variations in this breeding system were described for *S. cerevisiae* like near-diacy or higher level of outcrossing, but seemed quite rare and associated with environmental specificities (Knoop, 2006; Al Safadi et al., 2010; Murphy and Zeyl, 2010).

By comparison, the precise life-cycle of most non-*Saccharomyces* yeasts is unknown yet. Sporulation was observed for most non-conventional yeast, albeit forming non-tetrad asci in many cases (*T. delbrueckii*, *D. Hansenii*, *H. vinae*, etc., Table 1). No evidence of sporulation ability was recorded to date for *Starterella/Candida* species. Data regarding the occurrence of sexual reproduction is usually scarce for most non-*Saccharomyces* yeasts, so classical genetic manipulations are impossible to date. To circumvent this limitation, both intra and inter specific hybridizations by protoplast fusion can be achieved as demonstrated in the past (Ball, 1984; Pina et al., 1986).

The basic ploidy level is also usually unresolved (Table 1): *T. delbrueckii* has been considered as a haploid species for a long time, but the detection of several strains harvesting several loci with two alleles (26.4% of strains showing heterozygosity), its ability to sporulate and the presence of mating type genes is more congruent with a diploid status (Albertin et al., 2014a). Conversely, for *S. bacillaris*, the proportion of heterozygous strains was almost null (0.01%). This, combined with its inability to sporulate, is more consistent with an hypothesis of an haploid status (Masneuf-Pomarede et al., 2015) but has still to be formally demonstrated. Finally, despite its fully sequenced genome, the ploidy status of *L. thermotolerans* is controversial: haploid or diploid depending on the authors (Souciet et al., 2009; Frebel et al., 2014). In conclusion, the biological life-cycle of many non-*Saccharomyces* yeasts remains to be elucidated.

**ECOLOGY OF WINE YEAST**

Most wine yeasts can colonize several ecological niches, including wine-related environments like grape, must, winery equipment and premise (Table 1). Moreover, many of them can be isolated from other human-associated processes (brewery, bakery, dairy,
| Species/synonym (anamorph) | Features of interest in winemaking | Genome size | Full nuclear genome sequence | Basic ploidy level | Sporulation/zygote formation | Heterozygosity | Ecological niches | Genetic subgroups | Genetic diversity from winemaking environments |
|---------------------------|-----------------------------------|-------------|-----------------------------|-------------------|----------------------------|---------------|------------------|----------------|-----------------------------------------------|
| *Saccharomyces cerevisiae* | AF completion                      | Nucleus: 12.0 Mb, 16 chromosomes (Goffeau et al., 1996). Mitochondrion: 85 Kb (Foury et al., 1998). | Several hundred sequences: lab strain S288c (Goffeau et al., 1996), wine strains EC1118 (Novo et al., 2009) and AWRI1631 (Borneman et al., 2008); the 100-genomes strains (Strope et al., 2015), etc. | Diploid, occasional tetraploid associated with specific environments (Alberin et al., 2009; Al Safadi et al., 2010) | 4 spores per ascus. Zygotes readily observed. (Kurtzman et al., 2011) | 75.1–81.9% (308/410 clones, 136/166 clones) (Legras et al., 2007; Muller and McCusker, 2009) | Wild environments: fruit, plant, insect, soil. Anthropic environments: wine, other distilled and traditional fermented beverages, food fermentation, dairy product, bioethanol. Lab environments. Clinical environments. (Fay and Benavides, 2005; Legras et al., 2007; Almeida et al., 2015), multiple domestication events (Schacherer et al., 2009). | Wild and domestic populations associated with wine, beer, bread, etc. (Fay and Benavides, 2005; Legras et al., 2007; Almeida et al., 2015), multiple domestication events (Schacherer et al., 2009). | 0.39–0.65 (Alberin et al., 2014b); 0.00–1.00 (Schuller et al., 2012); 0.27–0.35 (Hall et al., 2011) |
| *Saccharomyces uvarum*     | AF completion                      | Nucleus: 11.5 Mb, 16 chromosomes (Almeida et al., 2014). | More than 50 genomes of which CBS7001 — Cliften et al., 2003; Almeida et al., 2014 | 4 spores per ascus. Zygotes readily observed. (Kurtzman et al., 2011) | 0% (0/40 strains) (Masneuf-Pomarede et al., 2007) | Wild environments: plant. Anthropic environments: wine and cider. (Almeida et al., 2014) | Wild and domestic populations associated with wine and cider (Almeida et al., 2014) | 0.00–0.62 (Masneuf-Pomarede et al., 2007) |

(Continued)
| Species/ synonym (anamorph) | Features of interest in winemaking | Genome size | Full nuclear genome sequence | Basic ploidy level | Sporulation/ zygote formation | Heterozygosity | Ecological niches | Genetic subgroups | Genetic diversity from winemaking environments |
|-----------------------------|---------------------------------|-------------|----------------------------|-------------------|----------------------------|---------------|-----------------|-----------------|----------------------------------|
| **Torulaspora delbrueckii** *(Candida collabcua)* | Volatile acidity reduction (Bely et al., 2008); Aroma and complexity (Ciani and Maccarelli, 1998; Renault et al., 2009; Azzolini et al., 2012) | Nucleus: 9.2–11.5 Mb, 8 chromosomes (Gordon et al., 2011; Gomez-Angulo et al., 2015); Mitochondrion: 28–45 Kb (Wu et al., 2015) | 2 genomes: CBS 1146 and NRRL Y-50541 (Gordon et al., 2011; Gomez-Angulo et al., 2015) | Unclear, could be diploid (Albertin et al., 2014a) | One spore per ascus, occasional 2–3 spores/ascus (Kurtzman et al., 2011; Albertin et al., 2014a). | 26.4% (29/110 strains) (Albertin et al., 2014a). | Wild environments: fruit, plant, insect, soil. Anthropic environments: wine, other distilled and traditional fermented beverages, food fermentations, dairy products. (Albertin et al., 2014a) | | 0.35–1.00 (Albertin et al., 2015). |
| **Hanseniaspora uvarum** *(Kloeckera apiculata)* | Aroma (Rojas et al., 2001) | Nucleus: 8.08–9.08 Mb, 8 to 9 chromosomes (Esteve-Zarzoso et al., 2001). Mitochondrion: 11 Kb (Pramateftaki et al., 2006). | 2 genomes: DSM 2768 and 34–9 (NCBI¹) | Unclear, could be diploid (Albertin et al., 2016) | One, seldom two spores per ascus (Kreger-van Rij, 1977). Zygotes described³. | 82.6% (95/115 strains) (Albertin et al., 2016). | Wild environments: fruit, plant, insect, bird, mollusc, shrimp, soil. Anthropic environments: wine, other distilled and traditional fermented beverages. (Grangeteau et al., 2015; Albertin et al., 2016) | | 1.00 (but low number of strains per sample) (Albertin et al., 2016). |
| **Hanseniaspora guillermontii** *(Kloeckera apiculata)* | Acetate ester production (Rojas et al., 2001; Moreira et al., 2008; Viana et al., 2008) | Nucleus: 8 to 9 chromosomes (Esteve-Zarzoso et al., 2001). | – | – | Four spores per ascus (Barnett et al., 2000). Zygotes described³. | – | Wild environments: fruit, soil. Anthropic environments: wine. | | – |
| **Hanseniaspora vinacea** *(Kloeckera africana)* | Acetate ester production (Viana et al., 2011) | Nucleus: 11.4 Mb, 5 chromosomes (Esteve-Zarzoso et al., 2001; Giorello et al., 2014). | 1 genome: T02/19AF (Giorello et al., 2014) | – | One, seldom two spores per ascus (Kreger-van Rij, 1977). | – | Anthropic environments: wine. | | – |

(Continued)
| Species/synonym (anamorph) | Features of interest in winemaking | Genome size | Full nuclear genome sequence | Basic ploidy level | Sporulation/zygote formation | Heterozygosity | Ecological niches | Genetic subgroups | Genetic diversity from winemaking environments |
|-----------------------------|----------------------------------|-------------|-----------------------------|-------------------|-----------------------------|----------------|------------------|-----------------|-----------------------------------------------|
| **Starmerella bacillaris** (Candida zemplinina) | Fructophily ([Magyar and Tóth, 2011; Tofalo et al., 2012; Englezos et al., 2015]; reduced ethanol production ([Mao et al., 2012; Bely et al., 2013; Giaramida et al., 2013]; glycerol production ([Mao et al., 2012; Giaramida et al., 2013; Zara et al., 2014]; aroma release ([Andorra et al., 2012]; other characteristics ([Mangani et al., 2011; Sadoudi et al., 2012; Tofalo et al., 2012; Dorrizo et al., 2014; Magyar et al., 2014]) | Nucleus: 3 chromosomes ([Spiczki, 2004]. Mitochondrion: 23 Kb ([Pramateftaki et al., 2008]. | – | Unclear, could be haploid (Masneuf-Pomarede et al., 2015) | No evidence of sporulation ability (Masneuf-Pomarede et al., 2015) | 0.01% (1/163) (Masneuf-Pomarede et al., 2015) | Rare in wild environments. Anthropic environments: grape and wine. (Masneuf-Pomarede et al., 2015) | No evidence of domestication event, geographical clustering. (Masneuf-Pomarede et al., 2015) | 0.80–0.97 (Masneuf-Pomarede et al., 2015) |
| **Candida stellata**/Torula polysaccharophila | Glycerol production ([Ciani and Maccarelli, 1998]; Fructophily ([Magyar and Tóth, 2011]) | Nucleus: 3 chromosomes ([Spiczki, 2004]. | – | – | No evidence of sporulation ability | – | Anthropic environments: wine ([Csoma and Sipiczki, 2008]) | – | – |
| **Lachancea thermotolerans**/Kluveromyces thermotolerans | Glycerol overproduction ([Comitini et al., 2011]; Acetate ester production ([Comitini et al., 2011]; reduction of volatile acidity ([Comitini et al., 2011] | Nucleus: 10.4 Mb, 8 chromosomes ([Malpertuy et al., 2000]. Mitochondrion: 21.9–25.1 Kb ([Tala et al., 2005; Freel et al., 2014]. | 1 genome: CBS 6340 ([Malpertuy et al., 2000]. | Controversial: haploid ([Freel et al., 2014] or diploid ([Souciet et al., 2009]. | One to four spores per ascus ([Barrett et al., 2000]. Zygoetes described1. | – | Wild environments: fruit, plant. Anthropic environments: wine and agave fermentations ([Freel et al., 2014]) | – | – |

1. (Continued)
| Species/synonym (anamorph) | Features of interest in winemaking | Genome size | Full nuclear genome sequence | Basic ploidy level | Sporulation/zygote formation | Heterozygosity<sup>a</sup> | Ecological niches | Genetic subgroups | Genetic diversity from winemaking environments<sup>b</sup> |
|---------------------------|-----------------------------------|-------------|-----------------------------|-------------------|-----------------------------|-------------------------|-------------------|-----------------|---------------------------------------------------------------|
| Lachancea kluyveri         | NA                                | Nucleus: 11.3 Mb, 8 chromosomes (<cite>Souciet et al., 2009</cite>) | 1 genome:NCYC 543<sup>1</sup> (<cite>Souciet et al., 2009</cite>) | Diploid, occasional triploid (<cite>Freel et al., 2014</cite>) | – | – | Wild environments: soil, insect, plant | Geographical clustering (<cite>Jung et al., 2012</cite>) | – |
| Debaryomyces hansenii/Pichia hansenii (Candida famata) | Enzymatic activities (<cite>Yanai and Sato, 1999</cite>) | Nucleus: 11–12.18 Mb, 7 chromosomes (<cite>Dujon et al., 2004</cite>) | 2 genomes: CBS 767 and MTCC 234 (<cite>Dujon et al., 2004</cite>; <cite>Kumar et al., 2012</cite>) | Haploid (<cite>Breuer and Harms, 2006</cite>) | One (occasionally two) spores per ascus (<cite>Barnett et al., 2000</cite>). Zygotes described (<cite>Breuer and Harms, 2006</cite>) | – | Wild environments: ocean. Anthropic environments: cheese, grape. | – | – |
| Pichia kluyveri/Hanseluna kluyveri | Aromas (<cite>Anfang et al., 2009</cite>) | Mitochondrion: 43.1 Kb (CBS 7907)<sup>1</sup> | – | Diploid (<cite>Starmer et al., 1992</cite>) | Four spores per ascus (<cite>Barnett et al., 2000</cite>). Zygotes described (<cite>Starmer et al., 1992</cite>) | – | Wild environments: fruit, insect. Anthropic environments: wine, cheese. (<cite>Starmer et al., 1992</cite>) | – | – |
| Pichia kudriavzevii/Issatchenka orientalis (Candida krusei) | Under assessment (<cite>Clemente-Jimenez et al., 2004; Wang and Liu, 2013; Steensels and Verstrepen, 2014</cite>) | Nucleus: 10.18–12.94 Mb (<cite>Chan et al., 2012</cite>) | 3 genomes:SD108, M12, NBRC 1279 (<cite>Chan et al., 2012</cite>) | Diploid | One or two spores per ascus (<cite>Barnett et al., 2000</cite>). Zygotes described<sup>1</sup>, | – | Wild environments: plant. Anthropic environments: wine, other traditional fermented beverages, food fermentation, dairy product. (<cite>Chan et al., 2012</cite>) | – | – |

<sup>a</sup>Genetic diversity from winemaking environments: soil, insect, plant. Genetic clustering (<cite>Jung et al., 2012</cite>).
| Species/synonym (anamorph) | Features of interest in winemaking | Genome size | Full nuclear genome sequence | Basic ploidy level | Sporulation/zygote formation | Heterozygosity$^a$ | Ecological niches | Genetic subgroups | Genetic diversity from winemaking environments$^b$ |
|-----------------------------|----------------------------------|-------------|----------------------------|-------------------|----------------------------|-----------------|-----------------|-----------------|-----------------------------------------------|
| *Pichia membranifaciens* (*Candida valida*) | Esters production *(Viana et al., 2008)* | Nucleus: 11.58 Mb, between 2 and 8 chromosomes *(Naumov and Naumova, 2009)* | 1 genome$^2$ | – | One to four spores per ascus *(Barnett et al., 2000)*. | – | Wild environments: plant. Anthropic environments: AF and food spoilage yeast. | – | – |
| *Pichia fermentans* (*Candida lambica*) | Aromas *(Clemente-Jimenez et al., 2009)* | Maybe 2 chromosomes *(Miler et al., 1989)* | – | – | Two to four spores per ascus *(Barnett et al., 2000)*. Zygotes described$^3$. | – | Wild environments: plant, water, soil. Anthropic environments: wine, brewery. Clinical environments. | – | – |
| *Pichia anomala/Hanseluna anamala* (*Candida pelliculosa*) | Aromas *(Rojas et al., 2001; Domizio et al., 2011a,b); killer against Dekkera/Brettanomyces *(Comini et al., 2004)* | Nucleus: 26.55 Mb, 6 chromosomes *(Friel et al., 2005)*. | 1 genome: NRRL Y-366$^1$ | Diploid | One to four spores per ascus *(Barnett et al., 2000)*. Zygotes described$^3$. | – | Wild environments: soil, water, plant, animal. Anthropic environments: wine, fermentation contaminant, ensilage *(Kurtzman et al., 2011)* | – | – |
| *Metschnikowia pulcherrima*/ *Torulopsis pulcherrima* (*Candida pulcherrima*) | Aromas and esters production *(Clemente-Jimenez et al., 2004; Parapouli et al., 2010; Zott et al., 2011; Sadoudi et al., 2012)* | – | – | Diploid | One to two spores *(Barnett et al., 2000)*. | – | Wild environments: plant. Anthropic environments: wine | – | – |
| *Zygosaccharomyces bailii* | Fructophily *(Sutterlin, 2010)* | Nucleus: 10.27–21.14 Mb, 5 to 13 chromosomes *(Mra et al., 2014)* | 2 genomes: CUB 213$^1$ and ISA 1307 *(NCBI)* | Haploid and diploid strains *(Rodrigues et al., 2003)* | One to four spores per ascus *(Barnett et al., 2000)*. | – | Wild environments: fruit, tree. Anthropic environment: food spoilage | – | – |

$^a$Proportion of strains with heterozygous microsatellite loci.

$^b$Genetic diversity (0 means fully clonal population and 1 means fully diversified population)

Web sites: NCBI$^1$, http://www.ncbi.nlm.nih.gov/genome/; JGI$^2$, http://genome.jgi.doe.gov/; UCDAVIS$^3$, http://wineserver.ucdavis.edu/industry/enology/winemicro/wineyeast/diversity.html.
FIGURE 1 | Phylogeny of 41 species of Saccharomycetales on the basis of 18S ribosomal DNA sequence. Multiple sequence alignment (1951 bases) was performed by Clustal Omega (EMBL-EBI website). Genetic distance was computed using the K80 Kimura model (Kimura, 1980), phylogenetic tree was built using Neighbor joining clustering method and bootstrapping (1000 replicates) was used to assess the robustness of the nodes by means of R package ape (Paradis et al., 2004). Schizosaccharomyces pombe was used as outgroup species. The following sequences and strains (mostly type strains) were used: AB000642.1|Dipodascus albidus IFO 1984; AB013504.1|C. tanzawaensis JCM 1648; AB018175.1|C. stellata JCM 9476; AB023473.1|M. pulcherrima IFO 1678; AB040997.1|S. kudriavzevii IFO 1802; AB040998.1|S. mikatae IFO 1815; AB054561.1|C. silvicultrix JCM 9831; AB103529.1|C. sake JCM 2951; AF548094.1|S. cerevisiae CBS 1171; AJ271813.1|S. cariocanus UFRJ 50816; AY046254.1|H. valbyensis NRRL Y-1626; AY046256.1|H. guilliermondii NRRL Y-1625; AY046257.1|H. uvarum NRRL Y-1614; AY046258.1|H. vineae NRRL Y-17529; S. bacillaris CBS 4949; EF550365.1|P. membranifaciens NRRL Y-2026; EF550372.1|P. fermentans Y-1619; EF550389.1|P. kluyveri NRRL Y-11519; EF550396.1|D. anomalus NRRL Y-17522; EF550479.1|Wickerhamomyces anomalus NRRL Y-366; EU011714.1|C. ovalis NRRL Y-17862; EU011734.1|D. bruxellensis NRRL Y-12983; EU048783.1|C. albicans NRRL Y-12983; FJ153136.1|L. thermotolerans NRRL Y-6284; FJ153143.1|T. franciscae NRRL Y-6686; GU266277.1|S. arborcola AS 2.3317; GU597328.1|T. yegammaensis CBS 5839; H0651939.1|Scheffersomyces stipitis ATCC 58376; JQ698884.1|Saccharomycopsis capsularis NRRL Y-1769; JQ698890.1|Clavispora lucentiae NRRL Y-11827; JQ698891.0|Debaryomyces hansenii NRRL Y-7426; JQ698926.1|Timrowska lipolytica NRRL YB-423; JQ698936.1|Schizosaccharomyces pombe NRRL Y-12796; M55528.1|P. kudriavzevii MUCL 29849; S. eubayanus FM1318; S. uvarum CBS7001; X69846.1|M. bicuspidata MUCL 31145; X89623.1|L. marxianus CBS 712; X91083.1|T. yegammaensis CBS 5839; X97805.1|S. pastorianusNCYC 392; X97806.1|S. paradoxus CBS 432; X98120.1|T. delbrueckii CBS 1148; Z75580.1|L. kluyveriNCYC 543.
bioethanol, distillery, etc.) and also from wild substrates (soil, insect, plant, etc.). Isolation from clinical specimens is rarely described yet possible (yeasts being opportunistic microorganisms), and most wine yeasts are Generally Recognized As Safe (GRAS). Dissemination and transfer between the different ecological reservoirs could be performed through insects (Parle and Di Menna, 1966; Stefanini et al., 2012; Palanca et al., 2013), but also through human activities like material exchanges, etc., (Goddard et al., 2010). Indeed, although most wine yeasts are described as ubiquitous from an ecological viewpoint, some species have a restricted substrate range. This is the case of *H. guilliermondii* and *Starmerella* species for example, which are very rarely isolated from non-wine-related substrates (Masneuf-Pomarede et al., 2015). Thus, the study of most wine yeast should consider not only wine strains but also isolates from other technological processes and substrates in order to assess their biodiversity.

**ADAPTATION TO WINEMAKING ENVIRONMENTS AND EVOLUTIONARY MECHANISMS**

Wine environments are particularly harsh and inconstant: winemaking is a seasonal practice, so that yeasts present at the surface of grape berries at harvest suddenly have to survive in grape must containing high sugar concentrations, usually with sulfur dioxide content. Moreover, from an ecological viewpoint, the ensuing alcoholic fermentation is a rapidly fluctuating ecosystem: within a few days, grape must is depleted of nitrogen nutrients, while ethanol concentration and temperature increase steadily thanks to *Saccharomyces* spp. metabolism, thus conferring a fitness advantage for *Saccharomyces* spp. over the other wine yeasts (Goddard, 2008; Salvadó et al., 2011). In addition, the range of temperature can be quite high, with either short-term variations (daily variations) or long-term evolution (seasonal variations). As a result, within wine yeast species, some strains show specific wine-adaptation (Steenkens and Verstrepen, 2014) like sulphite resistance (Divol et al., 2012), ethanol tolerance (García-Rios et al., 2014), low pH adaptation (Pretorius, 2000), temperature adaptation (Naumov et al., 2000), etc. The underlying adaptive mechanisms vary greatly from one species to another: in *S. cerevisiae*, molecular approaches identified allelic variations as molecular causes of adaptation to the winemaking process (Aa et al., 2006; Marullo et al., 2007; Ambroset et al., 2011; Salinas et al., 2012; Jara et al., 2014). At the chromosome level, translocations were shown to be responsible for adaptation to sulphite (Zimmer et al., 2014). Polyploidy and hybridization are also major evolutionary processes that probably triggered adaptation to wine environments (Borneman et al., 2012; Erny et al., 2012) and are currently explored for biotechnological application (Timberlake et al., 2011; Plech et al., 2014; Blein-Nicolás et al., 2015; da Silva et al., 2015). Large genomic introgressions were evidenced in *S. uvarum* strains associated with human-driven fermentations, suggesting a link between introgressions and domestication (Almeida et al., 2014). Various horizontal gene transfers were also evidenced for wine *S. cerevisiae* strains (Novo et al., 2009), and were shown to favor adaptation to the nitrogen-limited wine fermentation environment (Marsit et al., 2015). Other evolutionary mechanisms were described (Dujon et al., 2004; Barrio et al., 2006; Scannell et al., 2007), and it is highly probable that further investigations will allow the identification of additional adaptation processes in wine yeasts. In particular, it could be interesting to focus on transposon families and their possible implication in environmental adaptation (Zeyl, 2004; Liti et al., 2005; Sarilar et al., 2015), to explore the impact of mitochondrial genome variation regarding adaptation to wine environments and practices (Picazo et al., 2015; Wu et al., 2015) or to describe the landscape of gene duplication and prion involvement in fitness issues (Landry et al., 2006; Jarosz et al., 2014). However, to date, most of these data were obtained from *Saccharomyces* species and could now be obtained from non-*Saccharomyces* of interest.

**POPULATION GENETICS OF YEAST SPECIES ASSOCIATED WITH WINEMAKING**

Within a given species, the colonization of different ecosystems can lead to the evolutionary differentiation of the subpopulations, in relationship with their adaptation to environmental specificities. This is the case of *S. cerevisiae* species that shows genetic subgroups of wild and domestic strains associated with human activities like wine, bread, beer, sake, etc., (Fay and Benavides, 2005; Liti et al., 2009; Sicard and Legras, 2011; Almeida et al., 2015), that probably originated through multiple domestication events (Schacherer et al., 2009). In a recent study, Almeida et al. (2014) showed that *S. uvarum* was also divided in genetic subgroups, one of domestic strains used in both winemaking and cidermaking and associated with the northern hemisphere, while others subgroups were composed of wild isolates from South America and Australasia. The current hypothesis is that a Patagonian “wild” sub-population gave rise to the domestic subpopulation through a recent bottleneck (Almeida et al., 2014). Another wine species was recently described as domesticated: *T. delbrueckii* is also divided in genetic subgroups of wild and domestic strains (Albertin et al., 2014a). Moreover, the wine/grape-related group showed an increase ability to ferment sugar in oenological condition, confirming the occurrence of phenotypic domestication (Albertin et al., 2015). By contrast, no hint of domestication was recorded to date for *S. bacillaris* and *H. uvarum* whose genetic diversity is shaped by geographical localization and/or time variation (Masneuf-Pomarede et al., 2015; Albertin et al., 2016).

**Biodiversity in winemaking conditions**

Several molecular methods were developed in order to perform intra-specific discrimination, like pulsed field electrophoresis, RAPD-PCR fingerprinting, tandem repeat-tRNA, Fourier
transform infrared spectroscopy, RFLP, etc., (Barquet et al.,
2012; Tofalo et al., 2013, 2014; Pfiegl er et al., 2014; Grangeteau
et al., 2015). However, these approaches do not allow the
establishment of the genetic relationships within a given species
and subs equent population genetics studies. An alternative is
the use of microsatellite genotyping. It has been successfully
applied to S. cerevisiae (Legras et al., 2005; Richards et al., 2009),
S. uvarum (Masneuf-Pomarede et al., 2009), T. delbrueckii
(Albertin et al., 2014a), S. bacillaris (Masneuf-Pomarede et
al., 2015), H. uvarum (Albertin et al., 2016) as well as to
the spoilage wine yeast Brettanomyces bruxellensis (Albertin
et al., 2014c), and is currently developed for additional wine
species like Meyerozyma guilliermondii (Wrent et al., 2015).
In addition to population genetic clustering, microsatellites
allow measuring the genetic diversity of a given species in
specific conditions. In S. cerevisiae, the genetic diversity varied
greatly, from 0 (fully clonal populations) to 1 (fully diversified
population, Table 1). The precise impact of S. cerevisiae
diversity (or absence of diversity) on wine quality is still
debated/studied (Egli et al., 1998; Howell et al., 2006; King et al.,
2008) and the direct link between microbial diversity and wine
complexity should be considered with caution. S. uvarum and
T. delbrueckii showed also a large range of diversity (0.35–1
and 0–0.62). By contrast, other species show systematic high
diversity (>0.9 for H. uvarum or S. bacillaris), suggesting
that they are not under selective pressure in winemaking
environments (Masneuf-Pomarede et al., 2015; Albertin et al.,
2016).

FUTURE CHALLENGES

Definite progresses in the genetics of non-conventional yeasts
were made in the last decade. However, there is still a great lack
of data compared to the conventional wine yeast S. cerevisiae.
Such knowledge is nowadays within reach thanks to the NGS
revolution (Solieri et al., 2013). NGS allows the development
of genome-assisted approaches like whole genome sequencing
and resequencing, transcriptome profiling, ChIP-sequencing
as ection DNA-structure, etc. (Solieri et al., 2013). De novo
sequencing is greatly needed as some wine species still lack
of nuclear and mitochondrial reference genomes (S. bacillaris,
P. fermentans, M. pulcherrima, etc.). However, de novo assembly
is sometimes difficult to conduct due to high heterozygosit y
level or sequence repeat, and led to draft genome with high
number of contigs or scaffolds. For example, H. uvarum DSM
2768 genome displays 335 contigs, P. kudriavzevii M12 has 621
scaffolds, and P. anomala NRRL Y-366 shows 1932 scaffolds.
Thus, the first aim of non-conventional wine yeast studies should
be the completion of robust genomic sequences. Then, additional
genome sequencing could be performed: genome re-sequencing
using NGS captures individual genotypes and allows population
genetics and ecologic studies within species. Such comparative
genomics approaches were successfully applied to S. cerevisiae
(Liti et al., 2009) and S. uvarum (Almeida et al., 2014), and could
now address non-Saccharomyces yeasts of technological interest.
In addition to intraspecific genomics, comparative genomics
between yeast species is particularly useful to understand
genome evolution (Liti and Louis, 2005). The identification
of specific metabolic pathways, gene duplications or functions
between species may increase our appreciation of adaptation’s
mechanisms and their biotechnological interest (Blein-Nicolas
et al., 2015). It has to be noted that several species genetically
close to wine yeasts show no peculiar affinity with winemaking
environment (Figure 1). This is the case of S. paradoxus:
despite being the most closely related species to S. cerevisiae,
S. paradoxus is essentially associated with wild environments
and particularly trees (Sniegowski et al., 2002; Johnson et al.,
2004). Comparative genomics of wine vs. non-wine yeast species
could thus increase our knowledge of the common genomic
requirement for grape/wine colonization, if any. Finally, NGS
technologies have greatly improved genome-assisted approaches
aiming at detecting genetic variants associated with phenotypes
in S. cerevisiae (Ehrenreich et al., 2010). In particular, QTL-
seq or genome-wide association studies (GWAS) could now
be applied to non-conventional yeasts depending on whether
classical breeding is possible (QTL-seq) or not (GWAS). These
fields are blank pages waiting to be filled in the next future of
ownology microbial research.

The use of mixed-cultures, combining both non-conventional
yeasts and one Saccharomyces species able to complete AF,
is increasing in winemaking. Thus, another challenge lies in
understanding yeast-yeast interactions and their underlying
mechanisms (Ciani et al., 2010; Ciani and Comitini, 2015).
Indeed, several types of yeast-yeast interactions have been
described in enological conditions: competition for nutriments,
release of toxic compounds (Fleet, 2003), and even “quorum-
sensing” like mechanisms (Nissen and Arneborg, 2003; Nissen
et al., 2003; Renault et al., 2013). Understanding these complex
interactions is of first importance as the combination of some
yeast strains seems condemned to failure: for example, cell-cell
contact was recently shown to be involved in the death of strains
of T. delbrueckii and L. thermotolerans during mixed-culture
alcoholic fermentation with S. cerevisiae (Renault et al., 2013;
Kemsawasd et al., 2015). In some cases, yeast death was
associated with the release of metabolites or killer toxin (Pérez-
Nevado et al., 2006; Albergaria et al., 2010; Branco et al., 2015;
Ramírez et al., 2015). The precise impact of such interactions regarding wine
quality and aromas is still unclear (Ciani et al., 2006), but will
have to be considered to control and optimize complex mixed
oenological fermentation.

Finally, in addition to NGS-assisted approaches and interactions
studies, another prospect in the field of non-conventional wine
yeast lies in classical genetic approaches: indeed, one of the limits of the previously detailed approaches
is their low ability in elucidating the basic life-cycle of wine
yeasts, particularly regarding the occurrence and control of
sexual reproduction. Still, classical breeding is one of the
key issues for genetic improvement of industrial strains of
S. cerevisiae (Pretorius, 2000; Giudici et al., 2005; Marullo et
al., 2006; Steensels et al., 2014) and represents a technological
barrier that must be overcome for actual improvement of
non-Saccharomyces wine yeasts. There is an important need for
traditional sporulation assays, spore microdissection attempts,
successive segregant analyses, breeding assays, etc. In addition,
genetic transformation of non-conventional wine yeasts would be
a welcomed tool for subsequent functional studies (Pacheco et al.,
2015).
2009; Roberts and Oliver, 2011). These classical approaches are time-consuming and necessitate traditional yeast-manipulation know-how, sometimes viewed as old-fashioned and therefore neglected. However, these old approaches are essential for our future understanding of the genetics of non-conventional wine yeast, and are complementary to the more en vogue NGS-assisted approaches.

**REFERENCES**

Aa, E., Townsend, J. P., Adams, R. L., Nielsen, K. M., and Taylor, J. W. (2006). Population structure and gene evolution in *Saccharomyces cerevisiae*. FEMS Yeast Res. 6, 702–715. doi: 10.1111/j.1567-1346.2006.00059.x

Albergaria, H., Francisco, D., Gori, K., Arneborg, N., and Girio, F. (2010). *Saccharomyces cerevisiae* CCMI 885 secretes peptides that inhibit the growth of some non-*Saccharomyces* wine-related strains. Appl. Microbiol. Biotechnol. 86, 965–972. doi: 10.1007/s00253-009-2409-6

Albertin, W., Chasseriaud, L., Comte, G., Panfili, A., Delcamp, A., Salin, F., et al. (2014a). Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast *Torulaspora delbrueckii*. *PloS ONE* 9:e94246. doi: 10.1371/journal.pone.0094246

Albertin, W., Marullo, P., Aigle, M., Bourgais, A., Bely, M., Dillmann, C., et al. (2014b). Oenological prefermentation practices strongly impact yeast populations and aroma profile. *Curr. Opin. Food Sci.* 4, 122, 312–320. doi: 10.1016/j.foodsci.2014.03.012

Albertin, W., Mirot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., et al. (2014b). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *Int. J. Food Microbiol.* 178, 87–97. doi: 10.1016/j.ijfoodmicro.2014.03.009

Albertin, W., Mirot-Sertier, C., Bely, M., Mostert, T. T., Colonna-Ceccaldi, B., Coulon, J., et al. (2016). Hanseniaspora uvarum from winemaking environments show spatial and temporal genetic clustering. *Front. Microbiol.* 6:1569. doi: 10.3389/fmicb.2015.01569

Almeida, P., Gonçalves, C., Teixeira, S., Libkind, D., Bontrager, M., Masneuf-Pomarède, I. (2015). Biodiversity of Wine Yeasts: New Insights from Population Genetics. Oeno2015.

Almeida, P., Mirot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., et al. (2014b). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *Int. J. Food Microbiol.* 178, 87–97. doi: 10.1016/j.ijfoodmicro.2014.03.009

Almeida, P., Mirot-Sertier, C., Bely, M., Mostert, T. T., Colonna-Ceccaldi, B., Coulon, J., et al. (2016). Hanseniaspora uvarum from winemaking environments show spatial and temporal genetic clustering. *Front. Microbiol.* 6:1569. doi: 10.3389/fmicb.2015.01569

Almeida, P., Mirot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., et al. (2014b). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *Int. J. Food Microbiol.* 178, 87–97. doi: 10.1016/j.ijfoodmicro.2014.03.009

Almeida, P., Mirot-Sertier, C., Bely, M., Mostert, T. T., Colonna-Ceccaldi, B., Coulon, J., et al. (2016). Hanseniaspora uvarum from winemaking environments show spatial and temporal genetic clustering. *Front. Microbiol.* 6:1569. doi: 10.3389/fmicb.2015.01569

Barnett, J. A., Payne, R. W., and Yarrow, D. (2000). Yeasts: Characteristics and Identification. Cambridge, UK: Cambridge University Press.

Barquet, M., Martin, V., Medina, K., Pérez, G., Carrau, F., and Gaggero, C. (2012). Tandem repeat-IRNA (TIRRNA) PCR method for the molecular typing of non-*Saccharomyces* subspecies. *Appl. Microbiol. Biotechnol.* 93, 807–814. doi: 10.1007/s00253-011-7314-4

Barrio, E., Gonzalez, S., Arias, A., Belloclo, C., and Querol, A. (2006). “Molecular mechanisms involved in the adaptive evolution of industrial yeasts,” in *Yeasts in Food and Beverages*, eds A. Querol and G. Fleet (Berlin; Heidelberg: Springer-Verlag), 153–174.

Bely, M., Renault, P., da Silva, T., Masneuf-Pomarède, I., Albertin, W., Moine, V., et al. (2013). “Non-conventional yeasts and alcohol level reduction,” in *Alcohol Level Reduction in Wine* (Vigne et Vin Publications Internationales), 33–37.

Bely, M., Stoeckle, P., Masneuf-Pomarède, I., and Dubourdieu, D. (2008). Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* 122, 312–320. doi: 10.1016/j.ijfoodmicro.2007.12.023

Blein-Nicolas, M., Albertin, W., da Silva, T., Valot, B., Ballau, T., Masneuf-Pomarède, I., et al. (2015). A systems approach to elucidate heterosis of protein abundances in yeast. *Mol. Cell. Proteomics* 14, 2056–2071. doi: 10.1074/mcp.M115.048058

Borneman, A. R., Desany, B. A., Riches, D., Affourtitt, J. P., Forgan, A. H., Pretorius, I. S., et al. (2012). The genome sequence of the wine yeast *V.IN3* reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. *FEMS Yeast Res.* 12, 88–96. doi: 10.1111/j.1567-1364.2011.00773.x

Borneman, A. R., Forgan, A. H., Pretorius, I. S., and Chambers, P. J. (2008). Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. *FEMS Yeast Res.* 8, 1185–1195. doi: 10.1111/j.1567-1364.2008.00434.x

Branco, P., Viana, T., Albergaria, H., and Arneborg, N. (2015). Antimicrobial peptides (AMPs) produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora guilliermondii* cells. *Int. J. Food Microbiol.* 205, 112–118. doi: 10.1016/j.ijfoodmicro.2015.04.015

Breuer, U., and Harms, H. (2006). *Debryomyces Hansenii*—an extremophilic yeast with biotechnological potential. *Yeast* 23, 415–437. doi: 10.1002/yea.1374

Chan, G. F., Gan, H. M., Ling, H. L., and Rashid, N. A. (2012). Genome sequence of *Pichia kudriavzevii* M12, a potential producer of bioethanol and phytase. *Eukaryot. Cell* 11, 1300–1301. doi: 10.1128/EC.00229-12

Ciani, M., Beco, L., and Comitini, F. (2006). Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. *Int. J. Food Microbiol.* 108, 239–245. doi: 10.1016/j.ijfoodmicro.2005.11.012

Ciani, M., Canonico, L., Oro, L., and Comitini, F. (2014). Sequential fermentation using non- *Saccharomyces* yeasts for the reduction of alcohol content in wine. *BIO Web Conf.* 9:02015. doi: 10.1051/bioweb/20140302015

Ciani, M., and Comitini, F. (2015). Yeast interactions in multi-starter wine fermentation. *Curr. Opin. Food Sci.* 1, 1–6. doi: 10.1016/j.cofs.2014.07.001

Ciani, M., Comitini, F., Mannazzu, I., and Domizio, P. (2010). Controlled mixed culture fermentation: a new perspective on the use of non- *Saccharomyces* yeasts in winemaking. *FEMS Yeast Res.* 10, 123–133. doi: 10.1111/j.1567-1364.2009.00579.x

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Ciani, M., and Maccarelli, F. (1998). Oenological properties of non-
Saccharomyces yeasts associated with wine-making. *World J. Microbiol. Biotechnol.* 14, 199–203. doi: 10.1023/A:10086825928354

Clemente-Jiménez, J. M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Heras-Vázquez, F. J. L., and Rodríguez-Vico, F. (2004). Molecular characteristics and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. *Food Microbiol.* 21, 149–155. doi: 10.1016/S0740-0020(03)00063-7

Clemente-Jiménez, J. M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Las Heras-Vázquez, F. J., and Rodríguez-Vico, F. (2005). Influence of sequential yeast mixtures on wine fermentation. *Int. J. Food Microbiol.* 98, 301–308. doi: 10.1016/j.ijfoodmicro.2004.06.007

Cliftén, P., Sudarsanam, P., Desikan, A., Fulton, L., Fulton, B., Majors, J., et al. (2003). Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. *Science* 301, 71–76. doi: 10.1126/science.1084337

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, L., et al. (2011). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiol.* 28, 873–882. doi: 10.1016/j.fm.2010.12.001

Comitini, F., De Ingenis, J., Pepe, L., Mannazzu, L., and Ciani, M. (2004). *Pichia anomala* and *Kluyveromyces wickerhamii* killer toxins as new tools against *Debora/Brettanomyces* spoilage yeasts. *FEMS Microbiol. Lett.* 238, 235–240. doi: 10.1111/j.1574-6968.2004.tb09761.x

Cosma, H., and Sipiczki, M. (2008). Taxonomic reclassification of *Candida* (synonym *Candida zemplinina*). *Presence of* [genus Results in Homoeostasis](http://www.frontiersin.org) *Saccharomyces cerevisiae/Saccharomyces kudriavzevii* hybrids in the Northern European wine making environment. *Appl. Environ. Microbiol.* 8, 3256–3265. doi: 10.1128/AEM.06752-11

Esteve-Zarzoso, B., Peris-Torán, M., Ramón, D., and Querol, A. (2001). Molecular characterisation of *Hanseniaspora* species. *Antonio von Leeuwenhoek* 80, 85–92. doi: 10.1023/A:1012268913569

Fay, J. C., and Benavides, J. A. (2005). Evidence for domesticated and wild populations of *Saccharomyces cerevisiae*. *PLoS Genet.* 1, 66–71. doi: 10.1371/journal.pgen.0010005

Fleet, G. H. (2003). Yeast interactions and wine flavour. *Int. J. Food Microbiol.* 86, 11–22. doi: 10.1016/S0168-1605(03)00245-9

Fourny, F., Roganti, T., Legrenier, N., and Purnelle, B. (1998). The complete sequence of the mitochondrial genome of *Saccharomyces cerevisiae*. *FEBS Lett.* 440, 325–331. doi: 10.1016/S0014-5793(98)01467-7

Freed, K. C., Friedrich, A., Hou, J., and Schacherer, J. (2014). Population genomic analysis reveals highly conserved mitochondrial genomes in the yeast species *Lachancea thermotolerans*. *Genome Biol. Evol.* 6, 2586–2594. doi: 10.1093/gbe/evu203

Friel, D., Vandenbol, M., and Jijakli, M. H. (2005). Genetic characterization of the yeast *Pichia anomala* (strain K), an antagonist of postharvest diseases of apple. *J. Appl. Microbiol.* 98, 783–788. doi: 10.1111/j.1365-2672.2004.02520.x

García-Rios, E., Gutiérrez, A., Salvadó, Z., Arroyo-López, F. N., and Guillamon, J. M. (2014). The fitness advantage of commercial wine yeasts in relation to the nitrogen concentration, temperature, and ethanol content under microvinification conditions. *Appl. Environ. Microbiol.* 80, 704–713. doi: 10.1128/AEM.03405-13

Giaramida, P., Ponticello, G., Di Maio, S., Squadrito, M., Genna, G., Barone, E., et al. (2013). *Candida zemplinina* for production of wines with less alcohol and more glycerol. *S. Afr. J. Enol. Viticult.* 34, 204–211. doi: 10.1128/AEM.00768-11

Giordano, F. M., Berna, L., Greif, G., Camesasca, L., Salzman, V., Medina, K., et al. (2014). Genome sequence of the native apiculate wine yeast *Hanseniaspora vineae* TO219AE. *Genome Announc.* 2, e00530–e00614. doi: 10.1128/genomeA.00530-14

Giudici, P., Solieri, L., Pulvirenti, A. M., and Cassanelli, S. (2005). Strategies and perspectives for genetic improvement of wine yeasts. *Appl. Microbiol. Biotechnol.* 66, 622–628. doi: 10.1007/s00253-004-1784-2

Goddard, M. R. (2008). Quantifying the complexities of *Saccharomyces cerevisiae*’s ecosystem engineering via fermentation. *Ecology* 89, 2077–2082. doi: 10.1890/07-2060.1

Goddard, M. R., Anfang, N., Tang, R., Gardner, R. C., and Jun, C. (2010). A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-sired global dispersal in oak barrels. *Environ. Microbiol.* 12, 63–73. doi: 10.1111/j.1462-2920.2009.02035.x

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., et al. (1996). Life with 6000 genes. *Science* 274, 546; 543–547. doi: 10.1126/science.274.5287.546

Gomez-Angulo, J., Vega-Alvarado, L., Escalante-Garcia, Z., Grande, R., Giscaher-Mathis, A., Amaya-Delgado, L., et al. (2015). Genome sequence of *Torulaspora delbrueckii* NRRL Y-30541, isolated from mezcal fermentation. *Genome Announc.* 3, e00438–e00515. doi: 10.1128/genomeA.00438-15

Gordon, J. L., Ariméndez, D., Proux-Wéra, E., ÓhÉigeartaigh, S. S., Byrne, K. P., and Wolfe, K. H. (2011). Evolutionary erosion of yeast sex chromosomes by mating-type switching accidents. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20024–20029. doi: 10.1073/pnas.1122808108

Grangeau, T., Gerhardt, D., Rousseaux, S., van Wallbrunn, C., Alexandre, H., and Guilloux-Benatier, M. (2015). Diversity of yeast strains of the genus *Hanseniaspora* in the winery environment: what is their involvement in grape must fermentation? *Food Microbiol.* 50, 70–77. doi: 10.1016/j.fm.2015.03.009

Hall, B., Durall, D. M., and Stanley, G. (2011). Population dynamics of *Saccharomyces cerevisiae* during spontaneous fermentation at a British Columbia Winery. *Appl. J. Enol. Viticult.* 62, 66–72. doi: 10.5344/ajev.2010.11054

Hong, Y.-A., and Park, H.-D. (2013). Role of non- *Saccharomyces* yeasts in Korean wines produced from campbell early grapes: potential use of...
Hanseniaspora uvarum as a starter culture. Food Microbiol. 34, 207–214. doi: 10.1016/j.fm.2012.12.011
Howell, K. S., Cozzolino, D., Bartowsky, E. J., Fleet, G. H., and Henschke, P. A. (2006). Metabolic profiling as a tool for revealing Saccharomyces interactions during wine fermentation. FEMS Yeast Res. 6, 91–101. doi: 10.1111/j.1567-1364.2005.00010.x
Jara, M., Cubillos, F. A., García, V., Salinas, F., Aguilara, O., Liti, G., et al. (2014). Mapping genetic variants underlying differences in the central nitrogen metabolism in fermenter yeasts. PLoS ONE 9:e86533. doi: 10.1371/journal.pone.0086533
Jarosz, D. F., Brown, J. C., Walker, G. A., Datta, M. S., Ung, W. L., Lancaster, A. K., et al. (2014). Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism. Cell 158, 1083–1093. doi: 10.1016/j.cell.2014.07.025
Johnson, L. J., Koufopanou, V., Goddard, M. R., Hetherington, R., Schafer, S. M., and Burt, A. (2004). Population genetics of the wild yeast Saccharomyces paradoxus. Genetics 166, 43–52. doi: 10.1534/genetics.166.1.43
Jolly, N. P., Augustyn, O. P. H., and Pretorius, I. S. (2006). The role and use of non-Saccharomyces yeasts in wine production. S Afr. J. Enol. Vitic. 27, 15–39.
Jung, P. P., Friedrich, A., Reisser, C., Hou, J., and Schacherer, J. (2012). Genetic contribution of bottlenecked lineages to the diversity of the yeast population of a temperate vineyard. Mol. Phylogenet. Evol. 62, 773–783. doi: 10.1016/j.ympev.2012.04.008
Kvitek, D. J., Will, J. L., and Gasch, A. P. (2008). Variations in Stress Sensitivity and Survival of the Yeast Hanseniaspora uvarum in a Cold Environment. Microbiology 154, 1763–1775. doi: 10.1099/mic.0.2007/007571-0
Legras, J. L., Merdinoglu, D., Cornuet, J. M., and Harmer, J. (2007). Selection of hypervariable microsatellite loci for the characterization of Saccharomyces cerevisiae strains. Int. J. Food Microbiol. 102, 73–83. doi: 10.1016/j.ijfoodmicro.2004.12.007
Liti, G., Carter, D. M., Moses, A. M., Warringer, J., Parts, L., James, S. A., et al. (2009). Population genomics of domestic and wild yeasts. Nature 458, 337–341. doi: 10.1038/nature07743
Liti, G., and Louis, E. J. (2005). Yeast evolution and comparative genomics. Annu. Rev. Microbiol. 59, 135–153. doi: 10.1146/annurev.micro.59.030804.121100
Liti, G., Peruffo, A., James, S. A., Roberts, I. N., and Louis, E. J. (2005). Inferences of evolutionary relationships from a population survey of LTR-retrotransposons and telomeric-associated sequences in the Saccharomyces sensu stricto complex. Yeast 22, 177–192. doi: 10.1002/yea.1200
Magyar, L., Nyitrai-Sárdy, D., Léskó, A., Pomázi, A., and Kállay, M. (2014). Anaerobic organic acid metabolism of Candida zemplinina in comparison with Saccharomyces wine yeasts. Int. J. Food Microbiol. 178, 1–6. doi: 10.1016/j.ijfoodmicro.2014.03.002
Malpertuy, A., Llorente, B., Blandin, G., Artiguenave, F., Wincker, P., and Dubon, B. (2000). Genomic exploration of the hemiascomycetous yeasts: 10. Kluyveromyces thermotolerans. FEBS Lett. 487, 61–65. doi: 10.1016/S0014-5793(00)02281-X
Mangani, S., Buscioni, G., Collina, L., Bocci, E., and Vencenzini, M. (2011). Effects of microbial populations on anthocyanin profile of sangiovese wines produced in Tuscany, Italy. Am. J. Enol. Vitic. 62, 487–494. doi: 10.5344/ajev.2011.11047
Maris, S., Mena, A., Bigey, F., Sauvage, F. X., Couloux, A., Guy, J., et al. (2015). Evolutionary advantage conferred by an euakaryote-to-eukaryote gene transfer event in wine yeasts. Mol. Biol. Evol. 32, 1695–1707. doi: 10.1093/molbev/msv057
Marullo, P., Aigle, M., Bely, M., Masneuf-Pomarède, I., Durrens, P., Dubourdieu, D., et al. (2007). Single QTL mapping and nucleotide-level resolution of a physiological trait in wine Saccharomyces cerevisiae strains. FEMS Yeast Res. 7, 941–952. doi: 10.1111/j.1567-1364.2007.00252.x
Marullo, P., Bely, M., Masneuf-Pomarède, I., Pons, M., Aigle, M., and Dubourdieu, D. (2009). Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res. 6, 268–279. doi: 10.1111/j.1567-1364.2006.00034.x
Marullo, P., and Dubourdieu, D. (2010). “Yeast selection for wine flavour modulation,” in Managing Wine Quality, Vol. 2, ed A. G. Reynolds (Cambridge: Woodhead Publishing), 293–345.
Masneuf-Pomarède, I., Bely, M., Marullo, P., Loonv-Funel, A., and Dubourdieu, D. (2010). Reassessment of phenotypic traits for Saccharomyces bayanus var. uvarum wine yeast strains. Int. J. Food Microbiol. 139, 79–86. doi: 10.1016/j.ijfoodmicro.2010.01.038
Masneuf-Pomarède, I., Juquin, E., Miot-Sertier, C., Renault, P., Laizet, Y. H., Salin, F., et al. (2015). The yeast Starmereella bacillaris (syronym Candida zemplinina) shows high genetic diversity in winemaking environments. 15:fov045. FEMS Yeast Res. doi: 10.1093/femsyr/fov045
Masneuf-Pomarède, I., Le Jeune, C., Durrens, P., Lolière, M., Aigle, M., and Dubourdieu, D. (2007). Molecular typing of wine yeast strains Saccharomyces bayanus var. uvarum using microsatellite markers. Syst. Appl. Microbiol. 30, 75–82. doi: 10.1016/j.syam.2006.02.006
Miller, M., Kock, J. L. F., Pretorius, G. H. J., and Coetzee, D. J. (1989). The value of Hanseniaspora uvarum grown as a starter culture. FEMS Yeast Res. 1364.2005.00010.x
Mora, N. P., Münsterkötter, M., Dias-Valada, F., Santos, J., Palma, M., Roque, F. C., et al. (2014). The genome sequence of the highly acetic acid-tolerant Zygosaccharomyces bailii-derived interspecies hybrid strain ISA1307, isolated from a sparkling wine plant. DNA Res. 21, 299–313. doi: 10.1093/dnares/dst050
Moreira, N., Mendes, F., Guedes de Pinho, P., Hogg, T., and Vasconcelos, I. (2008). Heavy sulphur compounds, higher alcohols and esters production profile of Hanseniaspora uvarum and Hanseniaspora guilliermondii grown as pure and mixed cultures in grape must. Int. J. Food Microbiol. 124, 231–238. doi: 10.1016/j.ijfoodmicro.2008.03.025
Muller, L. A. H., and McCusker, J. H. (2009). Microsatellite analysis of genetic diversity among clinical and nonclinical Saccharomyces cerevisiae isolates suggests heterozygote advantage in clinical environments. Mol. Ecol. 18, 2779–2786. doi: 10.1111/j.1365-294X.2009.04234.x
Souciet, J. L., Dujon, B., Gaillardin, C., Johnston, M., Baret, P. V., Cliften, P., et al. (2009). Comparative genomics of protoploid Saccharomyces cerevisiae. Genome Res. 19, 1696–1709. doi: 10.1101/gr.091546.109

Starrmer, W. T., Ganter, P. F., and Aberdeen, V. (1992). Geographic distribution and genetics of killer phenotypes for the yeast Pichia kluyveri across the United States. Appl. Environ. Microbiol. 58, 990–997.

Steensels, J., Snoek, T., Meersmans, E., Picca Nicolino, M., Voordekers, K., and Verstrepen, K. J. (2014). Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbiol. Rev. 38, 947–995. doi: 10.1111/1574-6976.12073

Steensels, J., and Verstrepen, K. J. (2014). Taming wild yeast: potential of conventional and nonconventional yeasts in industrial fermentations. Annu. Rev. Microbiol. 68, 61–80. doi: 10.1146/annurev-micro-091213-113025

Stefanini, I., Dapporto, L., Legras, J. L., Calabretta, A., Di Paola, M., De Filippo, C., et al. (2012). Role of social wasps in Saccharomyces cerevisiae ecology and evolution. Proc. Natl. Acad. Sci. U.S.A. 109, 13398–13403. doi: 10.1073/pnas.1208362109

Strope, P. K., Skelly, D. A., Kozmin, S. G., Mahadevan, G., Stone, E. A., Magwene, P. M., et al. (2015). The 100-genomes strain, an S. cerevisiae resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. Genome Res. 25, 762–774. doi: 10.1101/gr.185538.114

Sutterlin, K. A. (2010). Fructophilic Yeasts to Cure Stick Fermentations in Alcoholic Beverages. Ph.D. thesis, University of Stellenbosch.

Talla, E., Anthouard, V., Bouchier, C., Frangeul, L., and Dujon, B. (2005). The complete mitochondrial genome of the yeast Klyuyveromyces thermotolerans. FEMS Lett. 579, 30–40. doi: 10.1016/j.femsle.2004.10.106

Timberlake, W. E., Frizzell, M. A., Richards, K. D., and Gardner, R. C. (2011). A new yeast genetic resource for analysis and breeding. Yeast 28, 63–80. doi: 10.1002/yea.1821

Tofalo, R., Perpetuini, G., Gasoli, G., Schirone, M., Corsetti, A., and Suzuki, G. (2014). Biodiversity study of wine yeasts belonging to the “terroir” of Montepulciano d’Abruzzo “Colline Teramane” revealed Saccharomyces cerevisiae strains exhibiting atypical and unique 5.8S-ITS restriction patterns. Food Microbiol. 39, 7–12. doi: 10.1016/j.fm.2013.10.001

Tofalo, R., Perpetuini, G., Schirone, M., Gasoli, G., Aguzzi, L., Corsetti, A., et al. (2013). Biogeographical characterization of Saccharomyces cerevisiae wine yeast by molecular methods. Front. Microbiol. 4:166. doi: 10.3389/fmicb.2013.00166

Tofalo, R., Schirone, M., Torriani, S., Rantsiou, K., Cocolin, L., Perpetuini, G., et al. (2012). Diversity of Candida zemplinina strains from grapes and Italian wines. Food Microbiol. 29, 18–26. doi: 10.1016/j.fm.2011.08.014

Viana, F., Belloch, C., Vallés, S., and Manzanares, P. (2011). Monitoring a mixed starter of Hanseniaspora uvarum–Saccharomyces cerevisiae in natural must: impact on 2-phenylethyl acetate production. Int. J. Food Microbiol. 151, 235–240. doi: 10.1016/j.ijfoodmicro.2011.09.005

Viana, F., Gil, J. V., Genovés, S., Vallés, S., and Manzanares, P. (2008). Rational selection of non-Saccharomyces wine yeasts for mixed starters based on oster formation and enological traits. Food Microbiol. 25, 778–785. doi: 10.1016/j.fm.2008.04.015

Wang, C., and Liu, Y. (2013). Dynamic study of yeast species and Saccharomyces cerevisiae strains during the spontaneous fermentations of Muscat blanc in Jingyang, China. Food Microbiol. 33, 172–177. doi: 10.1016/j.fm.2012.09.014

Wang, C., Mas, A., and Esteve-Zarzoso, B. (2015). Interaction between Hanseniaspora uvarum and Saccharomyces cerevisiae during alcoholic fermentation. Int. J. Food Microbiol. 206, 67–74. doi: 10.1016/j.ijfoodmicro.2015.04.022

Wang, Q.-M., Liu, W.-Q., Liti, G., Wang, S.-A., and Bai, F.-Y. (2012). Surprisingly diverged populations of Saccharomyces cerevisiae in natural environments remote from human activity. Mol. Ecol. 21, 5404–5417. doi: 10.1111/j.1365-294X.2012.05732.x

Warringer, J., Zörögö, É., Cubillos, F. A., Zia, A., Gjøsland, A., Simpson, J. T., et al. (2011). Trait variation in yeast is defined by population history. PLoS Genet. 7:e1002111. doi: 10.1371/journal.pgen.1002111

West, P., Rivas, E. M., Peinado, J. M., and de Silóniz, M. I. (2015). Development of an affordable typing method for Meyerozyma guilliermondii using microsatellite markers. Int. J. Food Microbiol. 217, 1–6. doi: 10.1016/j.ijfoodmicro.2015.10.008

Wu, B., Bulić, A., and Hao, W. (2015). Extensive horizontal transfer and homologous recombination generate highly chimeric mitochondrial genomes in yeast. Mol. Biol. Evol. 32, 2559–2570. doi: 10.1093/molbev/msv127

Yanai, T., and Sato, M. (1999). Isolation and properties of ß-glucosidase produced by Debaryomyces hansenii and its application in winemaking. Am. J. Enol. Vitic. 50, 231–235.

Zara, G., Mannazzu, I., Del Caro, A., Budroni, M., Pinna, M. B., Murru, M., et al. (2014). Wine quality improvement through the combined utilisation of yeast hulls and Candida zemplinina/Saccharomyces cerevisiae mixed starter cultures. Aust. J. Grape Wine Res. 20, 199–207. doi: 10.1111/ajgw.12078

Zeyl, C. (2004). Capturing the adaptive mutation in yeast. Res. Microbiol. 155, 217–223. doi: 10.1016/j.resmic.2003.12.006

Zimmer, A., Durand, C., Loira, N., Durrens, P., Sherman, D. J., and Marullo, P. (2014). QTL dissection of lag phase in wine fermentation reveals a new transcriptional responsibility for Saccharomyces cerevisiae adaptation to sulfite. PLoS ONE 9:e86298. doi: 10.1371/journal.pone.0086298

Zott, K., Thibon, C., Bely, M., Lonvaud-Funel, A., Dubourdieu, D., and Masneuf-Pomarede, I. (2011). The grape must non-Saccharomyces microbial community: impact on volatile thiol release. Int. J. Food Microbiol. 151, 210–215. doi: 10.1016/j.ijfoodmicro.2011.08.026

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