FEMS Yeast Research, 2023, 23, 1–18
DOI: 10.1093/femsyr/foad010
Advance access publication date: 9 February 2023
Minireview

Biology and physiology of *Hanseniaspora vineae*: metabolic diversity and increase flavour complexity for food fermentation

Francisco Carrau[1,2,*], Eduardo Dellacassa[3], Eduardo Boido[1], Karina Medina[1], Maria Jose Valera[1], Laura Fariña[1,3], Gabriel Perez[1], Valentina Martin[1], Fernando Alvarez-Valin[4], Lucia Balestrazzi[4]

[1]Universidad de la República, Facultad de Quimica Av. Gral. Flores 2124, Área Enología y Biotecnología de Fermentaciones, 11800 Montevideo, Uruguay
[2]Universidad de la República, Facultad de Medicina Av. Gral. Flores 2125, CEINBIO, 11800 Montevideo, Uruguay
[3]Universidad de la República, Facultad de Quimica Av. Gral. Flores 2124, Laboratorio de Biotecnología de Aromas, 11800 Montevideo, Uruguay
[4]Universidad de la República, Facultad de Ciencias Igua 4225, Instituto de Biología, Laboratorio de Genomica Evolutiva/Sección Biomatemática, 11400 Montevideo, Uruguay
*Corresponding author. Director of Enology and Fermentation Biotechnology Area, Facultad de Quimica, Av. Gral. Flores 2124, 11800 Montevideo, Uruguay. E-mail: fcarrau@fq.edu.uy
Editor: [Isak Pretorius]

Abstract

Apiculate yeasts belonging to the genus *Hanseniaspora* are predominant on grapes and other fruits. While some species, such as *Hanseniaspora uvarum*, are well known for their abundant presence in fruits, they are generally characterized by their detrimental effect on fermentation quality because the excessive production of acetic acid. However, the species *Hanseniaspora vineae* is adapted to fermentation and currently is considered as an enhancer of positive flavour and sensory complexity in foods. Since 2002, we have been isolating strains from this species and conducting winemaking processes with them. In parallel, we also characterized this species from genes to metabolites. In 2013, we sequenced the genomes of two *H. vineae* strains, being these the first apiculate yeast genomes determined. In the last 10 years, it has become possible to understand its biology, discovering very peculiar features compared to the conventional *Saccharomyces* yeasts, such as a natural and unique G2 cell cycle arrest or the elucidation of the mandelate pathway for benzenoids synthesis. All these characteristics contribute to phenotypes with proved interest from the biotechnological point of view for winemaking and the production of other foods.

Keywords: *Hanseniaspora vineae*, flavour diversity, benzenoids metabolism, wine yeast, fermentation adaptations, cell apoptosis

Introduction

Searching for flavour diversity represents the main target in today's wine industry, and in fact in the entire food fermentation industry. In a massive market where quality has improved through standardization of production, in the case of wine the accessibility of consistent strains of *Saccharomyces cerevisiae* yeast provides similar flavour and characteristics to different grape varieties all around the world. Although this strategy avoids the appearance of many wine defects, it may pose some limitations. In fact after many years of its application, the human palate evolves, and consumers 'learn' to enjoy diversity of colour and flavours to discover what is defined as the 'terroir identity'. Following this concept, many winemakers have started to search for nonconventional yeast species focusing on the natural microbial flora of the grapes (Suárez-Lepe and Morata 2012). This situation brought some ideas of returning to the ancestral roots of working with the spontaneous community, which gives increased flavour complexity to some industrially fermented foods. However, this style of work is much more complicated for a master producer, requiring a certain yeast management, i.e. not very easy to control in a small fermentation facility. This complication is exacerbated when these producers start to grow in larger volume as has been happening lately with many craft wineries. Spontaneous or mother sourdough or a 'pied de cuve' as it was called by the traditional winemakers of only 50 years ago, has come to be seen as a romantic way of producing some wine batches. Regrettably, only some to these batches are of good quality, whereas others are of poor quality and even some, which might be discarded. This situation convinced researchers and winemakers in the 2000s to systematically work on the selection of diverse yeasts of non-*Saccharomyces* strains to increase complexity (Fleet 2008).

Some of the studies of non-*Saccharomyces* species began with species such as *Schizosaccharomyces pombe*, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Pichia kluyveri*, or *Metschnikowia pulcherrima*.

However, the first commercial non-*Saccharomyces* starter was launched in 2004 and was a combination of *T. delbrueckii* and *Kluyveromyces thermotolerans*, with *Saccharomyces*. It was not until 2011, that other species were launched on the market, such as *M. pulcherrima* and *M. fructicola* (Roudil et al. 2019).

In 2002, we focused on the selection of apiculate yeasts that usually were considered not very attractive in terms of flavour, but on the other hand this group is the most abundant in grapes and other fruits (genus *Hanseniaspora/Kloeckera*) (see Fig. 1).

There are some reviews about these species of the *Hanseniaspora* yeast group that are found in the literature (Capece et al. 2007,

Received: October 6, 2022. **Revised:** January 2, 2023. **Accepted:** February 8, 2023
© The Author(s) 2023. Published by Oxford University Press on behalf of FEMS. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

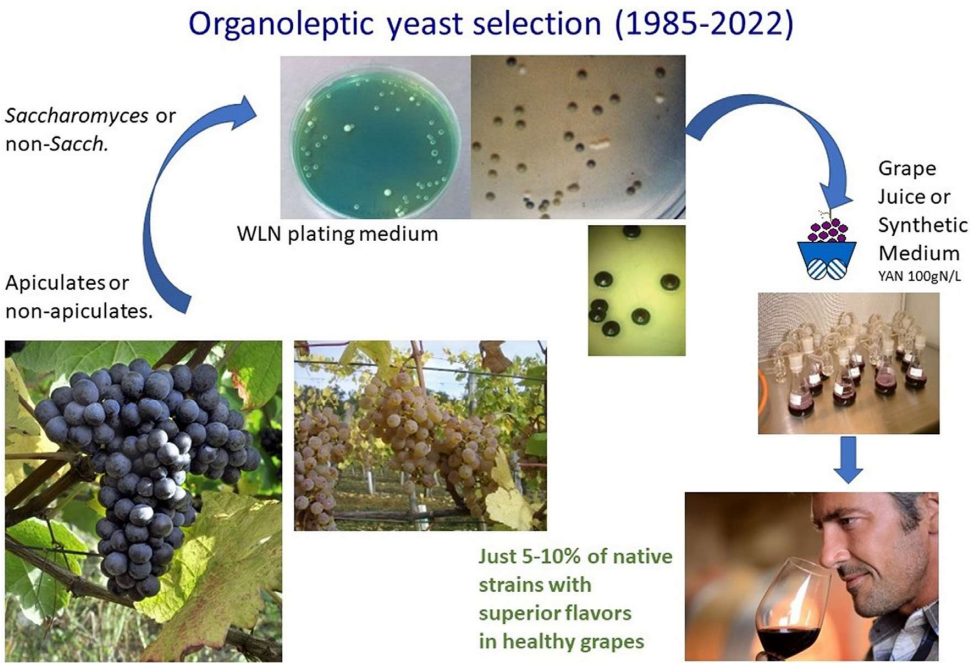

Figure 1. We discovered *H. vineae* from the whole apiculate yeast group by the nose. Less than 5% of the apiculate genus *Hanseniaspora* were producers of superior flavours, and from this selection about 90% were *H. vineae* strains using a limited yeast assimilable nitrogen (YAN) concentration of 100 mgN/l (Carrau et al. 2015). Interestingly, *H. vineae* shows the best flavour characteristics of the genus and secondly *H. osmophila*, the closest species to *H. vineae* of the fermentation group (see Fig. 4). WLN, WL nutrient medium where apiculates grow with dark green colonies.

Zott et al. 2008, Díaz-Montaño and de Jesús Ramírez Córdova 2009, Čadež et al. 2014, 2021, Martin et al. 2018, Steenwyk et al. 2019, Carrau et al. 2020, Valera et al. 2022). Interestingly, reviews before 2014 contain almost no mention of the species *H. vineae*.

In this article, we review the main results obtained in the last decade on the biology and applied characteristics of *H. vineae*. A wide variety of technological approaches have been applied to study this species, such as genome sequencing, transcriptomics, flow cytometry, and metabolomics studies using mainly GCMS/HPLC and sensory analysis. Recent results about its biology are discussed with particular emphasis on phenomena such as a cell cycle arrest at the G2 phase, a very unstable genome in terms of DNA repair, very variable mitochondrial genomes that involves the very fast gain and loss of mobile Group I self-splice introns in genes *COB* and *COXI*, and the development of the pathways related to the synthesis of aromatic amino acid derived compounds such as their acetate esters.

In reference to food applications, many of these characteristics have been shown to affect the flavour phenotype. Examples that have been studied are a fast cell lysis process increasing palate; an overproduction of key flavours such as acetates of 2-phenylethanol, tyrosol and tryptophol, benzenoids, or acetoin-related compounds; and an active protease activity that increases free amino acids and protein stability.

In conclusion, *H. vineae* could help to identify and understand genetic adaptations that emerged during yeast domestication for fermentation, being at a intermediary stage between the wild fruit yeast *Hanseniaspora uvarum* and the efficient fermentor *S. cerevisiae*. We propose here that *H. vineae* is attractive for evolutionary analysis as an interesting eukaryotic model to study ancestral fermentor genomes and a greater diversity of secondary metabolic pathways of alcoholic fermentation.

Origins, genomics, and the cell cycle of *H. vineae*

The development of molecular techniques allowed the detailed analysis and comparison of genomes from different *Hanseniaspora* species with *Saccharomyces* and other yeast species.

The genome of *H. vineae* was the first of the genus *Hanseniaspora* to be sequenced in 2013. A total of 4733 gene models were predicted from the assembly (Giorello et al. 2014). The number of genes shared with *S. cerevisiae* was on average 83% (see Fig. 2). This is more than other species of the genus, such as *Hanseniaspora guilliermondii*, *Hanseniaspora opuntiae*, and *H. uvarum*, which shared 70% of genes with *Saccharomyces* (Seixas et al. 2019).

Interestingly, compared to other budding yeasts from the Saccharomycotina, including *Saccharomyces*, the genus *Hanseniaspora* exhibited very high evolutionary rates ((Riley et al. 2016, Zhang et al. 2018). The study performed by Steenwyk et al. (2019), based on the analysis of 25 genomes, demonstrated that species in the genus *Hanseniaspora* lost many genes involved in diverse processes and identified two lineages within the genus: a faster-evolving lineage (FEL), which comprises *H. guilliermondii*, *H. opuntiae*, *H. uvarum*, and other fruit species, and a slower-evolving lineage (SEL) corresponding to *Hanseniaspora osmophila*, *Hanseniaspora occidentalis*, and *H. vineae*. The FEL lineage began diversifying approximately 87 million years ago (mya), and the SEL lineage began diversifying approximately 54 mya. Remarkably, both lineages lost genes associated with the cell cycle and genome integrity, but these losses were greater in the FEL group (Steenwyk et al. 2019).

In a recent publication, Schwarz et al. (2022) evaluated the ability to growth in an aerobic and discontinuous system of four *Hanseniaspora* species to analyse their viability and cell cycle progression. *Hanseniaspora uvarum* and *H. opuntiae* (representing the FEL group), and *H. osmophila* (SEL group) exhibited a typical arrest

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

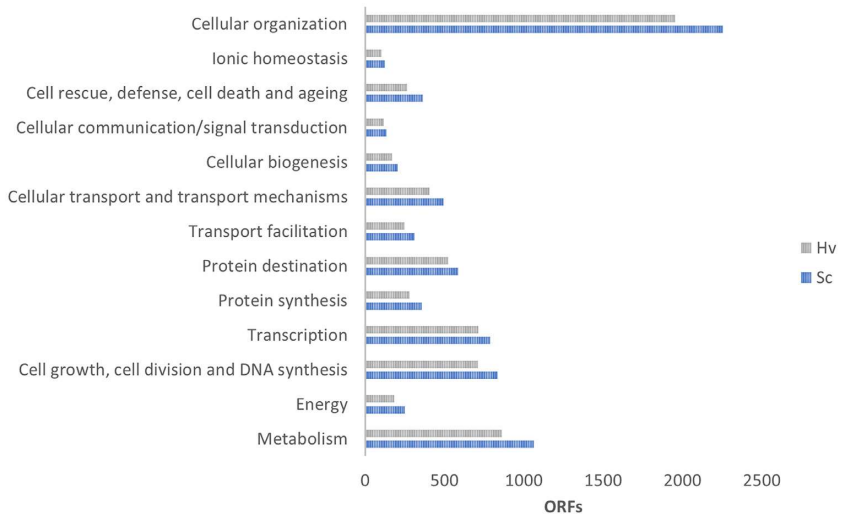

Figure 2. Functional clustering of predicted proteins from the *H. vineae* (Hv) genome. Clustering was performed using MIPS [Munich Information Center for Protein Sequences (MIPS-GSF, Neuherberg, Germany)] functional catalogue and *S. cerevisiae* S288c (Sc) genome database was used for comparison.

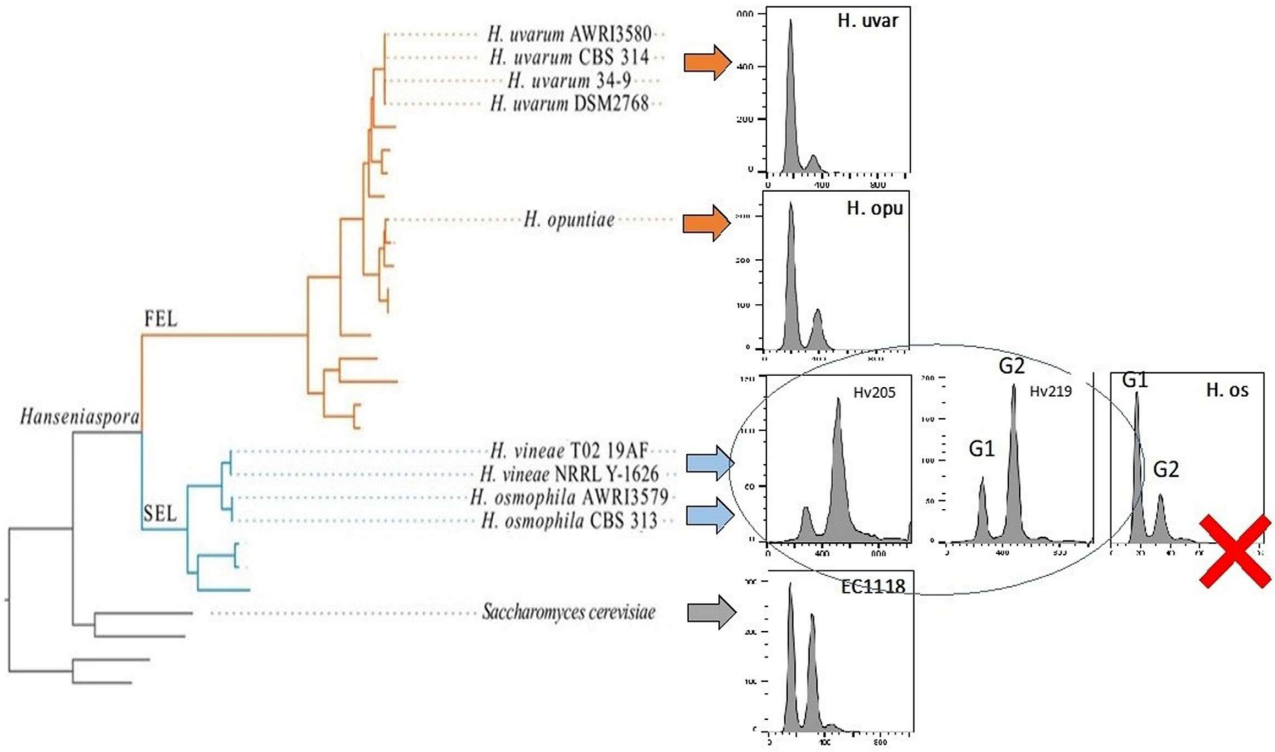

Figure 3. Proportions of cells with one copy of DNA content (G1), or with two copies of DNA content (G2), and the representative flow cytometry profiles of *S. cerevisiae*, *H. opuntiae*, *H. uvarum*, *H. osmophila*, *H. vineae* (Hv205), and *H. vineae* (Hv219) during aerobic growth. Adapted from Schwarz et al. (2022).

in G0/G1 during the stationary phase (Fig. 3), as is also observed in *S. cerevisiae*. Conversely, three different strains of *H. vineae* (SEL group) presented G2/M arrest and lost viability rapidly when entering the stationary phase compared with other *Hanseniaspora* and *Saccharomyces* yeasts. Studies are now underway to determine whether G2 arrest is modulated by the carbon/nitrogen ratio.

From the oenological point of view, *H. vineae* presents outstanding characteristics. The phenotypic differences found in fermentative environments (Viana et al. 2009, 2011, Medina et al. 2013, Lleixà et al. 2016b, Martin et al. 2016b, Del Fresno et al. 2020) are consistent with the fact that the genes of the species present higher identities with *S. cerevisiae* compared to all the other species of *Hanseniaspora*. Predicted protein sequences of three key enzymes involved in glycolysis and fermentation, hexokinase (HXK2), phosphofructokinase (PFK1 and PFK2), and pyruvate kinase (CDC19) from *H. vineae* exhibit higher amino acid identities with *S. cerevisiae* than with *H. uvarum*, *H. guilliermondii*, *H. valbyensis*, *H. opuntiae* and *H. osmophila*. Also, a significantly higher

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

Table 1. Genes involved in sugar transport, glycolysis, alcoholic fermentation, and aroma biosynthesis from *S. cerevisiae* and *H. vineae*. Gene CNs are indicated in brackets.

Biological function	*S. cerevisiae*	*H. vineae*
Sugar transport and sensors	*HXT* (x17); *SNF3*; *RGT2*;*FPS1*; *GPR1*; *GUP1*; *GUP2*; *STL1*; *JEN1*; *ASC1*; *ASC2*; *GPA2*	*HXT* (x2); *SNF3*; *GPR1*; *GUP1*; *STL1* (x2); *JEN1*; *ASC1*; *GPA2*
Glycolysis	*HXK1*; *HXK2*; *PGI1*; *PFK1*; *PFK2*; *FPBA1*; *TPI1*; *TDH1*; *TDH2*; *TDH3*; *PGK1*; *GPM1*; *ENO1*; *ENO2*; *CDC19*; *PYK2*	*HXK2*; *PGI1*; *PFK1*; *PFK2*; *FPBA1*; *TPI1*; *TDH2*; *TDH3*; *PGK1*; *GPM1*; *ENO1*; *ENO2*; *CDC19*
Alcoholic fermentation	*PDC1*; *PDC2*; *PDC5*; *PDC6*; *ADH* (x8)	*PDC1*; *ADH* (x8)
Key genes of wine yeasts adaptations	*SSU1*; *CUP1* (x2); *SUC2*; *THI5*; *THI11*; *THI12*; *THI13*; *THI14*; *THI16*; *THI20*; *THI21*; *THI72*; *THI73*; *THI80*; *TPC1*	*SSU1*; *SUC2*; *THI7*; *THI72*; *THI80*; *TPC1*
Aroma biosynthesis (higher alcohols, esters, and volatile organic acids)	*ARO8*; *ARO9*; *BAT1*; *BAT2*; *ARO10*; *PDC1*; *PDC5*; *PDC6*; *THI3*; *SFA1*; *GRE2*; *YPR1*; *PAD1*; *SPE1*; *OYE2*; *HOM2*; *AAD3*; *AAD4*; *AAD6*; *AAD10*; *AAD14*; *AAD15*; *AAD16*; *ARO80*; *GAT2*; *GLN3*; *GZF3*; *DAL80*; *ATF1*; *ATF2*; *EEB1*; *EHT1*; *MGL2*; *AAD*; *IAH1*; *ALD2*; *ALD3*; *ALD4*; *ALD5*; *ALD6*	*ARO8* (x3); *ARO9* (x4); *BAT1*; *ARO10* (x2); *PDC1* (x2); *ADH2* (x2); *ADH3* (x2); *ADH6* (x4); *SFA1*; *GRE2* (x4); *OYE2* (x3); *HOM2*; *ARO80*; *ATF2*; ATF-like (x4); *EHT1*; *MGL2*; *IAH1*; *ALD2* (x2); *ALD5*; *ALD6*

copy number (CN) of alcohol dehydrogenase genes (ADH) present in the genome of *H. vineae* might account for the increased alcohol resistance of *H. vineae* compared to other *Hanseniaspora* species. The molecular basis of aroma production has been thoroughly described (Giorello et al. 2019, Valera et al. 2021) showing marked differences to other *Hanseniaspora* species and *Saccharomyces*. The high number of predicted acetyl transferase genes in *H. vineae* and *H. osmophila* is remarkable, presenting six genes with alcohol acetyl transferase (AATase) domains, superior to *S. cerevisiae* S288c that presents just three or *H. uvarum* with only two genes (Valera et al. 2021). These activities are involved in the esterification of higher alcohols with acetate. This fact would explain the enhanced production of acetate esters by *H. vineae* contributing to wine aroma (Viana et al. 2009, 2011, Medina et al. 2013, Giorello et al. 2019), mainly from the aromatic higher alcohols tryptophol, tyrosol, and phenylethanol (Valera et al. 2021). See Table 1.

Hanseniaspora and *Saccharomyces* diverged before the whole genome duplication event that took place in the latter species (Langenberg et al. 2017, Giorello et al. 2019), however, some genes involved in oenological traits exhibited by *H. vineae* are present in high CN, similarly to *S. cerevisiae* (Table 1), supporting the idea of a convergent evolutionary adaptation to fermentative environments as discussed below.

A similar, but partial, adaptation to fermentative environments is also observed in *H. osmophila* and *H. occidentalis*. However, this latter species has been isolated from grape wine fermentations and might be confined to orange juice fermentations (Martin et al. 2018).

The new species *H. gamundiae* was recently described and as shown in Fig. 4, belongs to the same clade of *H. vineae*. This species was isolated from a rare fermented beverage of Patagonia (Čadež et al. 2019), which might explain its close relationship with other species of the fermentation group, contrasting with other *Hanseniaspora* species of fruits such as *H. uvarum* (Martin et al. 2018, Valera et al. 2020a). The genetic and oenological differences found in diverse studies (Viana et al. 2009, 2011, Medina et al. 2013, Martín 2016, Lleixà et al. 2016b, Giorello et al. 2019, Valera et al. 2020a), allow us to categorize the *Hanseniaspora* genus into two clusters that have a techological: the fermentation group and the fruit group (Fig. 4) (Martin et al. 2018). Interestingly, these two groups coincide with the FEL and SEL reported by Steenwyk et al. (2019). This fact might explain that the fermentation niche demands more stable genomic events compared to the increased variability that appears in the fruit group of species, as the fruit niche might be highly diverse compared to grape juice.

In Table 1, a group of key genes that are related to wine fermentation are shown; their presence in *H. vineae* is considered to be a signal of wine domestication. One of the most interesting genes is *SSU1*, which encodes a sulphur transport protein that increases sulphite resistance of the yeast cell. This gene is present only in the fermentation clade of *Hanseniaspora*. Notably, within eukaryotes this gene has only been identified in some fungi within eukaryotes. Figure 5 presents a phylogenetic tree built with the group of yeasts that contain *SSU1*.

Hanseniaspora vineae mitochondrial genome

Studies of mitochondrial genomes within the genus *Hanseniaspora* are limited (Pramateftaki et al. 2006). We assembled the mitochondrial genomes from two *H. vineae* strains. Figure 6 shows some important features of these genomes. A worth mentioning aspect is that there is remarkably different intron content between strains HV219 and HV205 of *H. vineae*. In particular, the genes encoding *COXI* and cytochrome B exhibit high variability within species that involves the very fast gain and loss of mobile Group I self-splice introns as observed in *S. cerevisiae*. Specifically in *COX I* gene, only the HV219 strain contains an intron located between exons 1 and 2 that encodes endonuclease aI5 alpha. Moreover in the same gene, in the region located between exons 3 and 4 there is another Type I intron but this one is very different between the two *H. vineae* strains described here. Finally, in cytochrome B gene only HV219 contains an intron that also contains an ORF encoding a maturase. Overall, these results illustrate the existence of a highly dynamic composition of introns in these two mitochondrial genes, which appears to be more fluid than that observed in other yeast groups.

Evolution and a domestication model within the genus *Hanseniaspora*

Overall, there is a clear trajectory of adaptations evidencing signs of yeast domestication from the fruit ecosystem (Fig. 7). The main

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

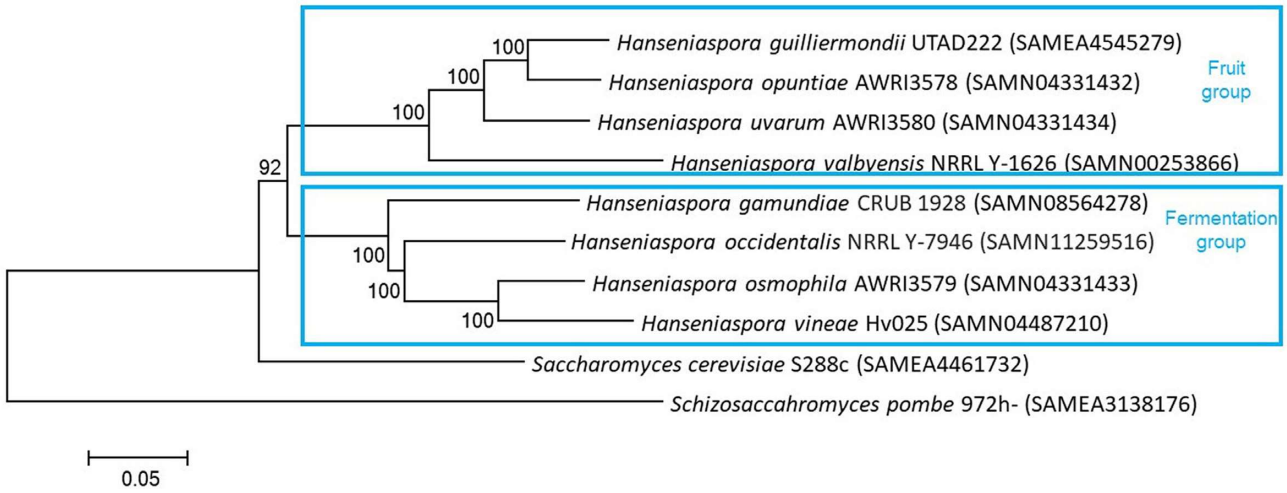

Figure 4. Phylogenetic tree obtained using the concatenated DNA sequences of nine genes of the alcohol fermentation pathway (*CDC19*, *FBA1*, *PGI1*, *PFK1*, *PFK2*, *HXK2*, *ENO1*, *PGK1*, and *PDC1*). The tree was built using the neighbour-joining method. The robustness of nodes is indicated by bootstrap values (%) calculated using 1000 pseudoreplicates. The entries in brackets correspond to NCBI BioSample identifiers. Interestingly, *H. osmophila* and *H. vineae* diverged more recently from each other than to the other species of the fermenter group, and might be more efficient in fermentation as will be discussed.

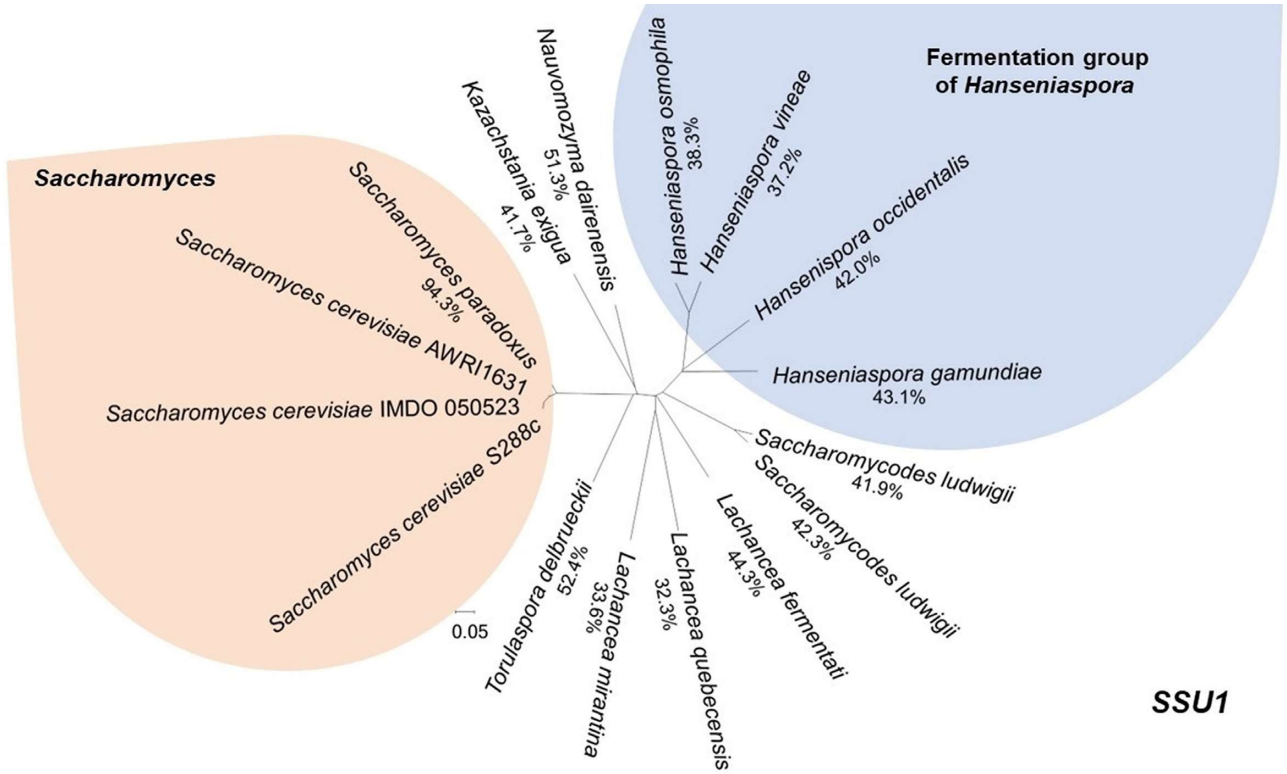

Figure 5. Phylogenetic tree depicting the relationships and genetic distances among predicted amino acid sequences of *SSU1* from different yeasts. Amino acid identity (%) was calculated for each species against *S. cerevisiae* sequences. This gene is involved in one of the key functions related to domestication for wine fermentation. It is a very peculiar gene, not found in eukaryotic cells other than fungi and is related to the sulphite resistance during fermentation. Something similar is also observed with the gene encoding *SUC2* (beta-fructofuranosidase), another enzyme, i.e. key in wine adaptations of yeast. It is found in *S. cerevisiae*, other fungi, but not in other eukaryotes.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

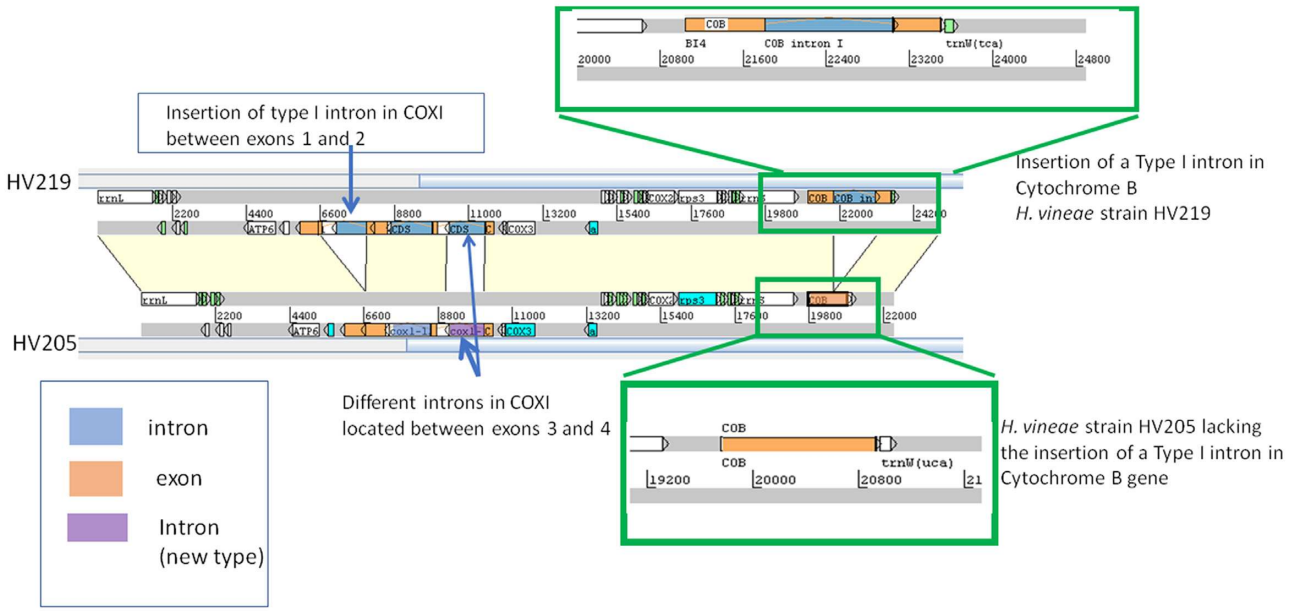

Figure 6. Mitochondrial genomes of two strains of *H. vineae* were assembled. Differences in Type I introns containing homing endonucleases and maturases are highlighted. Introns and exons of the genes encoding cytochrome oxidase I (COX I) and cytochrome B (Cyt B) are shown.

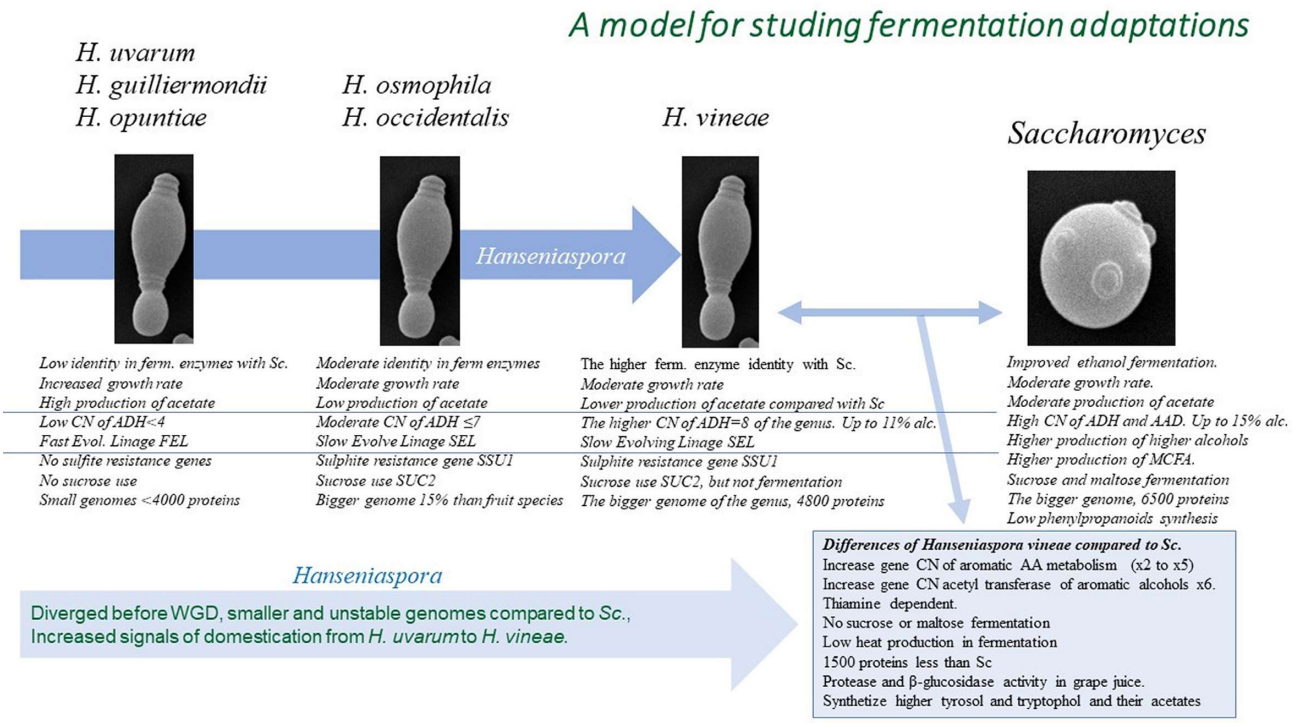

Figure 7. Four-stage model of genetic adaptations that might explain the process of domestication within the genus *Hanseniaspora*, and how *H. vineae* shows an approximation to the characteristics of the most adapted juice fermenting yeast: *S. cerevisiae*. The highly adapted species for ethanol production lost some flavour pathways that reduced the formation of desirable aroma compounds, such as the benzenoid pathway and aromatic higher alcohols production. This model might explain how the extreme specialization for ethanol fermentation reduced the activity of the secondary metabolism, mainly flavour compounds. In each stage of the figure, domestication signals or genetic adaptations are described. Sc: *S. cerevisiae*; CN: copy numbers; WGD: whole genome duplication event in Sc; and MCFA: medium-chain fatty acids.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

representative species in fruit is *H. uvarum*, which is closely related to other species such as *H. guilliermondii* and *H. opuntiae*, that are not successful for fermentations above 6% of alcohol by volume. Throughout the process of adaptation from fruits to the fermentative ecosystem, clearly *H. vineae* is the most adapted species.

In the last decade, many similarities of *H. vineae* with *Saccharomyces* were characterized, and this species of the genus was found to be unique in having the capacity to ferment up to 10%–11% of alcohol by volume (Martin et al. 2022b). The fact that it has a higher percentage of glycolytic enzymes similarities with *Saccharomyces*, and the presence of a significantly higher CN of ADH genes compared to the rest of the genus, might explain its increased ethanol resistance. Although the concept of domestication is still under discussion (Pontes et al. 2020), clear signs of domestication appear in *H. vineae* but not in the other species of this genus (Liti et al. 2009, Borneman et al. 2011, Libkind et al. 2011, Almeida et al. 2014). These data suggest the existence in this species of a clear advanced stage of domestication for fermentation within the genus *Hanseniaspora*. *Hanseniaspora vineae* characteristics might help understanding adaptation processes from fruit to juice fermentation niches. Interestingly, adaptations found in *H. vineae* compared to *Saccharomyces* show that some flavour pathways are still present in *H. vineae* and explain the increased flavour synthesis, i.e. detected in this species.

Future advances in the genetic and transcriptomic mechanism utilized by *H. vineae* will be developed with new molecular tools. Genetic manipulation of *Hanseniaspora* species is still very limited, but recently some tools were developed for successful transformation of *H. uvarum* (Badura et al. 2021). These studies might be useful for *H. vineae* genetic modification, e.g. to improve our knowledge of the mandelate pathway recently discovered for the synthesis of benzenoids (Valera et al. 2020b, c), or to develop the overproduction of the intense flavours of acetate esters of higher aromatic alcohols.

Flavour and particular metabolism in *H. vineae*

Wine aroma must be visualized not just as the sum of individual components, but the result of complex interactions between many chemical compounds. Volatile components can interact with each other, in a synergistic or antagonistic manner (Ferreira et al. 2016). In this sense, from an organoleptic perspective the use of *H. vineae* could provide distinctive features in comparison to the use of conventional *S. cerevisiae* strains as it confers a moderate fermentation process, but with a new balance of volatile metabolites, with an increased formation of aromatic flavour compounds. The use of *H. vineae* has been reported to be beneficial for many types of wines. The contribution of this yeast to the aroma of white and rosé wines has been highlighted at the pilot and winery levels (Medina et al. 2007, 2013, Lleixà et al. 2016b, Del Fresno et al. 2020, Del Fresno et al. 2021b). The production of 2-phenylethyl acetate represents the main contribution of *H. vineae* from the point of view of wine volatile composition (Martin et al. 2022b). This compound is characterized by its fruity, honey, and floral notes with an olfactory perception threshold of 0.250 mg/l. As shown in Table 2 in different fermentations using this yeast either in pure form or in a sequential culture (with *S. cerevisiae*), using red or white grapes of different varieties, the perception threshold is exceeded in all cases, in a range of 2–60 times depending on the type of vinification used (Medina et al. 2013, Martin et al. 2018, Del Fresno et al. 2021b). Although many of the data shown in Table 1 are at the winery scale, where there might not be perfect yeast implantation controls, a comparison of the metabolic capacity of β-2-phenylethyl acetate production of *H. vineae* with *S. cerevisiae* shows that it is on average 18 times greater (ranging from 1.1 to 90 times) (Table 2). This capacity can be explained by the increased CN of genes that are expressed and have alcohol acetyltransferase (AATase) domains present in the *H. vineae* genome (x6), compared to industrial *S. cerevisiae* strains (x2) (Giorello et al. 2019).

In wine, the presence of ethyl acetate and acetic acid at levels above the perception threshold is undesirable being a clear indication of the presence of microbial spoilage. But it has been reported that at low levels the presence of these components adds complexity and enhances the fruity aroma of the wine (Cliff and Pickering 2006, Jackson 2017). *Hanseniaspora vineae* produces similar or lower levels of acetic acid and a smaller increase in ethyl acetate than *S. cerevisiae* (R average 1.2 and 1.8, respectively) as shown in Table 2. However, the wines obtained in these fermentations, instead of being associated with the presence of aromatic defects, have been assigned to fruity descriptors and increased palate volume (Medina et al. 2013, Lleixà et al. 2016b, Del Fresno et al. 2021b). These two key components of the sensory perception of the wine are in equilibrium in wines produced with *H. vineae*.

Phenylpropanoids synthesis pathways in *H. vineae*. A model eukaryote

It was well-determined that at least in a synthetic medium *H. vineae* produced high concentrations of monoterpenes and sesquiterpenes compared to *Saccharomyces* (Martin et al. 2018) and natural white wine fermentations (Del Fresno et al. 2020). Interestingly, it was recently detected the formation of safranal in Albillo Mayor white wine by *H. vineae* above the threshold level, and this aroma compound was not found in the same trials with *H. opuntiae* or *Saccharomyces* control fermentations (Del Fresno et al. 2022). Studies are now underway to determine whether safranal was synthetized *de novo* or by a particular biotransformation pathway of *H. vineae* from grape precursors.

Benzyl alcohol, benzaldehyde, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzyl alcohol are synthesized *de novo* in the absence of grape-derived precursors by *H. vineae* (Martin et al. 2016b). The levels of benzyl alcohol produced by different strains of *H. vineae* were 20–200 times higher than those measured in fermentations with *S. cerevisiae*. Table 2 shows the results of different vinifications with *H. vineae*, including fermentations in mixed culture, obtaining an average benzyl alcohol production 14 times higher than in vinifications with *S. cerevisiae* solely. The absence of the phenylalanine lyase pathway (*PAL*) in *H. vineae* suggests that benzenoids synthesis necessarily depends on *de novo* synthesis from chorismate (Martín 2016, Martin et al. 2016b). Recently, this feature has allowed the elucidation of the biosynthetic pathway of these aromatic compounds in ascomycetous yeasts using *H. vineae* as a model microorganism (Valera et al. 2020b). Benzyl alcohol is synthesized from phenylalanine through the so-called mandelate pathway, which has been validated in *S. cerevisiae* using deletion mutants of key genes such as *ARO10* encoding not only phenylpyruvate decarboxylase but also a benzoylformate decarboxylase function (Valera et al. 2020c). Using ^{13}C precursors, the authors showed that a simultaneous parallel biosynthetic pathway in *H. vineae* synthetizes 4-hydroxybenzyl alcohol from

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

Table 2. Main flavour compounds produced under real winemaking conditions by *H. vineae* and *S. cerevisiae* with different grapes.

	Acetic acid (g/l)	Ethyl acetate (mg/l)	2-Phenylethyl acetate (mg/l)	2-Phenyl ethyl alcohol (mg/l)	Benzyl alcohol (μg/l)	Acetoin (μg/l)	2,3-Butanediol (μg/l)	Isobutanol (mg/l)	2 and 3-methyl-1-butanol (mg/l)[a]	Hexanoic acid (μg/l)	Octanoic acid (μg/l)	Decanoic acid (μg/l)	Grape and wine characteristics	Fermentation characteristics	Fermentation vessel	*S. cereviseae*	*H. vineae*	Reference
Odour threshold Descriptor	0.7 g/l Vinegar	12.3 mg/l Fruity to solvent	0.25 mg/l Fruity , honey, floral	14 mg/l Honey, rose, spicy	200 000 μg/l Floral, rose, phenolic, balsamic	150 000 μg/l Creamy, butter, fat	600 000 μg/l Butter, fat	40 (mg/l) Fusel oil, chemical	30 (mg/l)[b] Alcoholic, fruity at low conc.	420 μg/l Sweet, acid rancid	500 μg/l Sweet, cheese	1000 μg/l Rancid, fatty						
H. vineae	0.42 ± 0.01	58 ± 5	15.4 ± 1.2	15.2 ± 0.3	n.r.[c]	6.5 ± 1.3	382 ± 42	21.1 ± 0.95	144.4 ± 0.7	n.r.	n.r.	n.r.	50% Tempranillo and 50% Albillo. rose wine	Pure[e]	150 l stainless steel	Fermivin 3C	T02/5A (Universidad de la República)	Del Fresno et al. (2021b)
S. cerevisiae	0.44 ± 0.11	71 ± 4	9.3 ± 1.6	16.5 ± 0.9	n.r.	5.5 ± 0.6	536 ± 81	17.7 ± 0.40	112.6 ± 4.7	n.r.	n.r.	n.r.						
R[d]	1.0	0.8	1.7	0.9	–	0.9	0.7	1.2	1.3	–	–	–						
H. vineae	0.40 ± 0.01	63 ± 3	7.4 ± 0.4	16.2 ± 1.5	n.r.	5.7 ± 0.4	395 ± 89	26.2 ± 1.0	150.9 ± 3.0	n.r.	n.r.	n.r.		Pure	225 l oak barrels	Fermivin 3C	T02/5A (Universidad de la República)	
S. cerevisiae	0.52 ± 0.02	85 ± 3	6.7 ± 0.6	17.1 ± 1.6	n.r.	5.7 ± 1.7	360 ± 14	18.2 ± 0.4	131.1 ± 1.3	n.r.	n.r.	n.r.						
R	0.8	0.7	1.1	0.9	–	1.0	1.1	1.4	1.2	–	–	–						
H. vineae	0.34 ± 0.02	n.r.	10.5 ± 3.2	14.1 ± 0.9	25 ± 5	20 419.0 ± 6535.0	268 ± 384	n.r.	63.1 ± 21.2	211 ± 109	128 ± 29	473 ± 276	Chardonnay	Pure	0 125 l Erlenmeyer cotton plugs	ALG 804	HV205 (T02/5A) (Universidad de la República)	Valera et al. (2021)
S. cerevisiae	0.45 ± 0.05	n.r.	0.12 ± 0.05	25.7 ± 5.4	7 ± 1	1764.0 ± 2628.0	1594 ± 351	n.r.	113.5 ± 35.4	1365 ± 133	2143 ± 370	472 ± 51						
Relation	0.8	–	90.7	0.5	3.6	11.6	0.2	–	0.6	0.2	0.1	1.0						
H. vineae	0.40 ± 0.01	n.r.	2.32 ± 0.05	8.1 ± 0.2	37 ± 7	15.0 ± 13.0	n.r.	2.4 ± 0.3	36.4 ± 4.1	330 ± 35	734 ± 12	979 ± 31	Macabeo	Pure	100 l tank	QA23	T02/5A (Universidad de la República)	Lleixà et al. (2016b)
S. cerevisiae	0.30 ± 0.04	n.r.	0.05 ± 0.00	16.8 ± 0.9	0.0 ± 0.0	56.0 ± 59.0	n.r.	1.9 ± 0.2	61.4 ± 5.1	777 ± 70	1757 ± 335	389 ± 212						
R	1.3	–	49.4	0.5	37.0	0.3	–	1.3	0.6	0.4	0.4	2.5						
H. vineae	0.53 ± 0.01	24.8 ± 0.6	1.53 ± 0.03	28.5 ± 1.9	n.r.	n.r	n.r	18.0 ± 0.1	94.8 ± 1.3	3140 ± 100	3030 ± 50	710 ± 70	Petit Manseng	Sequential[f]	4.5 l	Lalvin® D25 4™	CVE-HV6 (China Agricultural University)	Zhang et al. (2022b)
S. cerevisiae	0.28 ± 0.03	16.4 ± 0.2	0.20 ± 0.01	18.0 ± 0.4	n.r.	n.r	n.r	14.7 ± 0.2	84.8 ± 1.3	3760 ± 140	3520 ± 110	690 ± 80						
R	1.9	1.5	7.5	1.6	–	–	–	1.2	1.1	0.8	0.9	1.0						
H. vineae	0.36 ± 0.02	79 ± 3	7.9 ± 0.3	9.5 ± 0.3	n.r.	5.5 ± 0.1	644 ± 29	n.r.	n.r.	n.r.	n.r.	n.r.	Albillo Mayor	Sequential	120 l stainless steel barrel	Fermivin 3C	T02/5A (Universidad de la República)	Del Fresno et al. (2021c)
S. cerevisiae	0.45 ± 0.07	46 ± 3	6.3 ± 0.4	9.7 ± 0.3	n.r.	5.7 ± 0.2	892 ± 97	n.r.	n.r.	n.r.	n.r.	n.r.						
R	0.8	1.7	1.3	1.0	–	1.0	0.7	–	–	–	–	–						
H. vineae	0.30 ± 0.00	81 ± 1	6.2 ± 1.9	25.6 ± 1.5	n.r.	7.3 ± 1.4	470 ± 54	23.4 ± 1.7	141.6 ± 13.1	n.r.	n.r.	n.r.	Tempranillo (late harvest)	Sequential	0.1 l glass flask with Muller valves	7VA (Technical University of Madrid)	T02/5A (Universidad de la República)	Escott et al. (2021)
S. cerevisiae	0.20 ± 0.00	20 ± 2	5.2 ± 0.4	76.6 ± 6.5	n.r.	5.8 ± 0.3	446 ± 24	27.0 ± 0.5	257.2 ± 37.5	n.r.	n.r.	n.r.						
R	1.5	4.1	1.2	0.3	–	1.3	1.1	0.9	0.6	–	–	–						
H. vineae	0.35	14.6	4.34	13.6	n.r	126.0	n.r.	n.r.	54.3	1902	4184	4607	Chardonnay	Sequential	225 l French oak barrels	ALG 804	T02/5A (Universidad de la República)	Medina et al. (2013)
S. cerevisiae	0.40	8.0	0.45	32.9	n.r	17.0	n.r.	n.r.	63.2	3834	8478	3796						
R	0.9	1.8	10.2	0.4	–	7.4	–	–	0.9	0.5	0.5	1.2						
H. vineae	0.38	7.4 ± 0.2	0.43 ± 0.00	23.84 ± 0.08	203 ± 5	98.7 ± 4.6	206 ± 3	3.6 ± 0.1	83 231 ± 0.1	840 ± 3	988 ± 21	174 ± 13	Tannat	Sequential	5000 l oak vats	ALG 111	T02/5A (Universidad de la República)	Martin et al. (2018), Medina et al. (2021)
S. cerevisiae	0.30	4.42 ± 0.03	0.19 ± 0.00	23.3 ± 1.3	164 ± 5	182.9 ± 3.5	250 ± 2	5.2 ± 0.2	78.3 ± 5.7	956 ± 4	1086 ± 64	148 ± 13						
R	1.3	1.7	2.2	1.0	1.2	0.5	0.8	0.7	1.1	0.9	0.9	1.2						
R minimum	0.8	0.7	1.1	0.3	1.2	0.3	0.2	0.7	0.6	0.2	0.1	1.0						
R maximum	1.9	4.1	90.7	1.6	37	11.6	1.1	1.4	1.3	0.9	0.9	2.5						
R average	1.1	1.8	18.4	0.8	14.0	3.0	0.8	1.1	0.9	0.6	0.5	1.4						

[a]Sum of 2- and 3-methyl butanol;
[b]odour threshold of 3-methyl-butanol;
[c]n.r. not reported;
[d]R: relation between concentration of compound produced by *H. vineae*/concentration of compound produced by *S. cerevisiae*;
[e]pure fermentation with *H. vineae*;
[f]sequential fermentation with *H. vineae* followed by *S. cerevisiae*.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

tyrosine via the 4-hydroxymandelate pathway, which was confirmed in *S. cerevisiae* by decreasing yeast assimilable nitrogen (YAN) levels in the synthetic mediums (Martín 2016). Interestingly, the discovery of this pathway was key to understanding the synthesis of a compound such as the coenzyme Q6 head, the 4-hydroxy benzoic acid in ascomycetes fungi (Fernández-del-Río and Clarke 2021). More recently, 4-hydroxymandelate was detected in human cells (Banh et al. 2021), and the authors speculate that these cells might be using the 4-hydroxymandelate pathway proposed for yeast. Tyrosol is found in significantly higher concentrations in *S. cerevisiae* fermentations, but its acetate ester, which was shown to increase its sensory impact, is highly produced in coinoculations with *H. vineae* and not in *S. cerevisiae* fermentations (Martin et al. 2018). Similar results were obtained with tryptophol in fermentations with *H. vineae*, where practically all this aromatic alcohol was acetylated to another little-known aroma compound, tryptophol acetate. It is worth noting that this compound was not found in *Saccharomyces*, or in *H. osmophila* and *H. uvarum* (Valera et al. 2021). These results indicate that some of the acetyl transferase (*ATF*) genes that were identified in *H. vineae* are specialized in aromatic alcohols acetylation. Phenylpropanoid synthesis is clearly dependent on the availability of nitrogenous nutrients. Tests with different levels of assimilable nitrogen in fermentations with *H. vineae* have shown that the reduction of DAP salts leads to an increase of these aromatic compounds (Martin et al. 2016a). When we decreased YAN levels in *S. cerevisiae* below 100 mgN/l, we could detect some small concentrations of benzenoids formed by this yeast.

Increase of acetoin in *H. vineae*

Acetoin affects the wine bouquet; it is of particular interest as a precursor for the biosynthesis of 2,3-butanediol and diacetyl. Three possible metabolic pathways have been proposed for acetoin biosynthesis in *S. cerevisiae* based directly on the decarboxylation of pyruvate to form acetaldehyde (Cheynier et al. 2010). One of the main factors affecting acetoin formation is the intracellular redox state (NAD+/NADH ratio) and the intracellular concentration of pyruvic acid (Cheynier et al. 2010). In the case of *H. vineae*, a higher production of acetoin is observed compared to conventional fermentations with *S. cerevisiae* for the production of Chardonnay and Tempranillo wines (Table 2). This is in agreement with what has been reported previously for apiculate yeasts (Cheynier et al. 2010). In the case of 2,3 butanediol, its production by *H. vineae* is on average a little lower than that obtained by fermentation with *S. cerevisiae* (Table 2).

Decrease of higher alcohols, ethyl esters, and medium-chain fatty acids in *H. vineae*

Vinifications with *H. vineae* in different grape varieties show that in general a lower concentration of higher alcohols is produced compared to *Saccharomyces* (Table 2). The mean *R* (relation between compound concentration produced by *H. vineae*/compound concentration produced by *S. cerevisiae*) is greater than 1, although in some vinifications the opposite result is obtained. This behaviour observed for some vinifications can be explained by the ability of *H. vineae* to form acetates from higher alcohols (Giorello et al. 2019), which reduces the alcohol concentration compared to vinifications with *S. cerevisiae*.

Medium-chain fatty acids (MCFA) are synthesized from acetyl Co-A and their high concentration is associated with toxic effects on yeast, but they play a fundamental role as biosynthetic intermediates of long-chain fatty acids, which are essential constituents in the cell membrane (Restrepo et al. 2019). The presence of MCFA is associated with descriptors that are not often desirable in wine aroma (acid, rancid, fatty, and cheese), but are part of the pool of compounds conforming the common aroma base (Ferreira et al. 2016). Among the MCFA, hexanoic acid and octanoic acid have similar perception thresholds and when analyzing wines fermented by *S. cerevisiae*, their concentration far exceeds this threshold. As shown in Table 2, this has been reported previously (Ferreira et al. 2000, Fariña et al. 2015). For decanoic acid, its perception threshold is twice that of hexanoic and octanoic acids and in conventional fermentations it does not usually reach this threshold. *Hanseniaspora vineae* produces a lower concentration of hexanoic and octanoic acid ($R = 0.6$ and 0.5, respectively) compared to *S. cerevisiae* and produces higher concentrations of decanoic acid that rarely exceeds its threshold (1 in 4 times reported). This MCFA balance achieved by *H. vineae* from a sensory point of view could enhance the fruity aromas due to other components originating from the yeast.

Application and quality impact on winemaking

Hanseniaspora vineae presents different outstanding characteristics that make its industrial application possible and interesting, namely resistance to sulphur dioxide ($SO_{2)}$ and ethanol, exocellular protease and β-glucosidase enzymatic activity, fast cell lysis with early release of polysaccharides, DNA and proteins, ideal for aging on lees and other highly produced flavours that are not found in *S. cerevisiae* fermented wines. All these characteristics make it a very interesting yeast for cellar application, since it allows the production of wines with characteristics that differentiate them from the rest without the sensory defect risks. It was successfully used with different grape varieties, to produce increased complexity of white wines with neutral grapes such as Trebbiano, Ugni blanc, Airen, Albillo, or Macabeo, or to increase floral flavour notes in Chardonnay, Viognier, or Petit Manseng, or rosé wines produced with red varieties such as Tempranillo, Cabernet, and Tannat.

One of the outstanding characteristics that gives it good applicability at the industrial level is its resistance to SO_2. This compound is the main additive for winemaking when used moderately at harvest time (2–4 g/Hl) and depending on varieties protects grapes from oxidation in conventional or low input winemaking processes (Tavares et al. 2021). Therefore, it is important that the yeast to be used resists the presence of this compound in the must. In a study carried out with 11 strains of *H. vineae* (Martín 2016), even though all the yeasts analyzed reduced their growth as the concentration of SO_2 increased in the medium (Fig. 8, all *H. vineae* showed a good performance under 75 mg/l SO_2, a considerably high level at the industrial scale, where 50 mg/l is considered enough to protect grape juice processes before fermentation. Except yeast strain C12 213F, all *H. vineae* showed similar SO_2 resistance levels compared to the *S. cerevisiae* control. Indicating that for most of the strains of *H. vineae* analyzed, the concentration of SO_2 in the medium of 50 mg/l was acceptable. Some contrasting reports described for other species of *Hanseniaspora* (Fleet and Heard 1993), suggest that species such as *H. uvarum* have a very

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

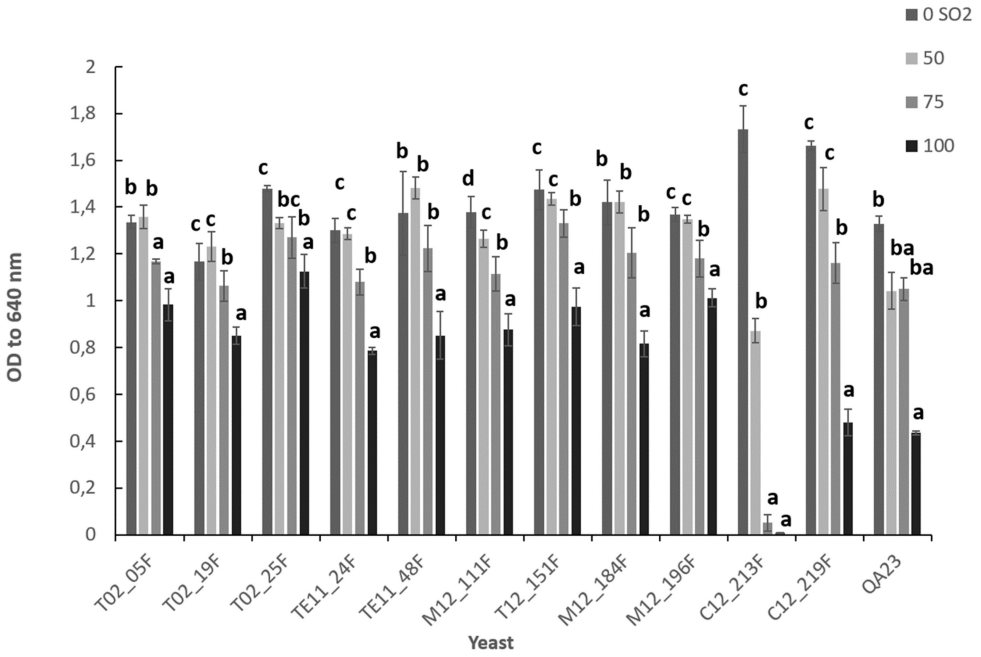

Figure 8. Growth measured as optical density (OD at 640 nm) recorded for each *H. vineae* strain at four SO_2 concentrations levels (mg/l). Data with the same letter do not differ from each other (LSD test, 95% confidence level). QA23 was the *S. cerevisiae* control strain.

weak resistant capacity to SO_2. This is confirmed by the findings of Valera et al. (2020a), where both *H. vineae* and *H. osmophila* present the key gene *SSU1* that confers resistance to sulphites as well as *S. cerevisiae* and that is not found in the rest of the species of the genus *Hanseniaspora*.

Another important characteristic to consider when selecting a yeast for fermentation is its ability to tolerate the conditions of the fermentation medium. Most non-*Saccharomyces* yeasts have been described as poorly tolerant to the presence of high concentrations of sugars or ethanol and responsible for fermentation defects and/or stuck fermentations, which is why they have not been selected for winemaking (Toit and Pretorius 2000, Padilla et al. 2016). With the passage of time, these concepts about nonconventional yeasts have changed. In the work carried out by Viana et al. (2011) on Tempranillo musts coinoculated with *S. cerevisiae* in a sequential manner, they were able to demonstrate the presence of *H. vineae* in the final stages of fermentation. More recently this species was again identified at the end of fermentation (Lleixà et al. 2016a), showing the ability of *H. vineae* to resist high ethanol concentrations. This was also seen in ice-wine production where *H. vineae* in sequential inoculation with *S. cerevisiae* had a better growth rate and higher cell number during fermentation compared to another fermentation coinoculated with *M. pulcherrima* (Zhang et al. 2018). In agreement with these works are the results obtained by Valera et al. (2020a), where *H. vineae* was able to grow in conditions of higher alcohol content, 10% ethanol, compared to other species of the genus *Hanseniaspora sp.*, such as the species of the fruit clade shown in Fig. 4, that were not able to grow when alcohol was above 5%–10% by volume. These works exemplify the ability of *H. vineae* yeasts to adapt to fermentation conditions compared to other non-*Saccharomyces* species. Interestingly, Giorello et al. (2019) reported that *H. vineae*, unlike the rest of the species of the genus *Hanseniaspora*, had a higher CN of ADH genes (see Fig. 5), which may be associated with its high tolerance to ethanol and other adaptations to fermentation conditions (Fig. 8).

Extracellular enzymes of *H. vineae* in winemaking

Another desirable characteristic present in *H. vineae* is the extracellular enzymatic activity like β-glucosidase and protease, as yeasts with enzymatic activity are able to transform grape compounds and in so doing, modify the sensory profile of the wines.

Some of the compounds that affect the organoleptic quality of wines are found in the must in conjugated form, bound to a glucose moiety in the form of β-glucosides. This implies, in the case of volatile aroma compounds, that they are not yet in their volatile form. As for the precursors of aromatic compounds, their hydrolysis determines the typicity of certain varieties such as Muscats and other aromatic varieties such as Chardonnay, Riesling, or Cabernet. The glycosidic β-(1–4) bonds are characteristic of the aroma precursors present in grape musts and are the most frequently cleaved by yeasts. Therefore, the presence of β-glucosidase activity in yeasts is of great interest as it allows breaking these bonds and releasing these aromatic precursors.

For *H. vineae*, β-glucosidase activity has been reported in several papers. In a study with 11 strains of the species, all showed activity *in vitro* with plating methods at pH 6, without significant differences between strains (Martín 2016). This agrees with results previously reported by us (Pérez et al. 2011), but in this case differences among strains could be observed. In another work (López et al. 2015), the presence of β-glucosidase activity in *H. vineae* was also determined at an optimum pH of 6 in contrast to another report where activity could be observed at pH 3.5 (Mostert 2013). There were other reports (López et al. 2014) about *H. vineae*

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

glucosidase activity in different strains with plating methods. Interestingly, in our experience many plating methods to detect enzymatic activity at the wine pH (3–4), are sometimes not seen in agar plates (glucosidase, protease, or killer toxins), however, they are functionally at real winemaking conditions in grape juice (Carrau et al. 2020).

From an oenological point of view, proteolytic activity is relevant because it is responsible for the hydrolysis of proteins and peptides present in musts and wines and, therefore, for obtaining protein stability specially in white wines (Waters et al. 1994, 2005). This provides a possible solution to address problems associated with clarification, stabilization, and filtration of wines. It contributes to reduce the haze in finished and bottled white wines, which causes great economic losses for the wine industry because it is perceived as a defect by consumers (Cosme et al. 2020, Saracino et al. 2021). In addition, the hydrolytic process releases small peptides and amino acids to the medium making them available to yeasts as a nitrogen source and, in certain circumstances, avoiding sluggish fermentations caused by nitrogen deficiency in the must.

For *H. vineae*, protease activity has been determined on several occasions.

During the characterization of the extracellular protease activity of 23 strains of *H. vineae* on skim milk medium plates, all presented protease activity at pH 6, three standing out for their higher activity, as indicated by the presence of a clear halo around the colony (Martin et al. 2022a). These results confirmed those reported by other authors (López et al. 2014).

Recently, by means of heat stability tests, the ability to reduce the appearance of haze in Sauvignon Blanc wines fermented with two strains (Martin et al. 2022b) and with twenty strains (Martin et al. 2022a) of *H. vineae* were evaluated. A total of four of the strains significantly reduced the haze of the wine compared a control fermented only with *S. cerevisiae*. These studies show that the presence of protease activity might reduce the needed amount of bentonite by 50% to achieve protein stability, reducing the risk of losing flavours. Protease activity is strain-dependent and not species-dependent.

Usually many wine yeasts degrade a certain percentage of malic acid of grape juices. Reduction of this acid sometimes might be attractive for some unripe grapes in cool regions (Benito et al. 2012, Loira et al. 2018) or for red wines. Recently, malic acid degradation by *H. occidentalis* was reported with a higher percentage than any *Saccharomyces* strain (van Wyk et al. 2022). *Hanseniaspora vineae* strains have some levels of degradation of malic acid that are currently under study mainly for white base sparkling wines due to the interest in avoiding the malolactic fermentation by LAB (Roman Villegas et al. 2021). Although the majority of *Hanseniaspora* species have the gene for the malate dehydrogenase synthesis, they do not have the malate cell transporter as happens with all *Saccharomyces* species. The lack of this transporter significantly limit malic acid degradation, as opposite to what happens in *Schizosaccharomyces* species. However, further studies are needed to understand how some of the species of *Hanseniaspora* degrade an increased level of malic acid compared to *Saccharomyces*. It is known in *Pichia kudriavzevii*, that the transporters of fumarate and succinic acid of the *JEN* family genes (Xi et al. 2021), play a role in malate import capacity into the cell. Interestingly, we have found these genes in *H. vineae*, and with highly protein similarity with *Pichia JEN* proteins.

Curiously, it was reported in *H. uvarum* that secrets active killer proteins similar to those of *Saccharomyces* produced by virus like particles of double stranded RNA (Radler et al. 1985, 1990, Schmitt and Neuhausen 1994). This killer activity was demonstrated recently against many pathogenic yeasts and other fungi in vitro (Hameed et al. 2019). However, we have screened 23 different strains of *H. vineae* for killer activity against other *Hanseniaspora*- and *Saccharomyces*-sensitive strains and all behaved as neutral. They did not kill and were not killed by *Saccharomyces* killer strains (data not shown).

Decrease of biogenic amines, and fast lysis

Another interesting feature of *H. vineae*, is the ability to influence the formation of biogenic amines. Cofermentations of *S. cerevisiae* with *H. vineae* show a decrease in the concentration of amines in comparison to fermentations with *S. cerevisiae* alone (Medina et al. 2013, Zhang et al. 2022a). The mechanism that *H. uvarum* uses to degrade some biogenic amines was described recently (Han et al. 2022), and may be the same as that of *H. vineae*.

Another aspect to consider when using *H. vineae* during fermentation is its contribution to the mouthfeel of the wine, as it has been shown that during fermentation there is an early release of polysaccharides, DNA, and proteins through the cell wall. In turn, these compounds are of higher molecular weight than those released by *S. cerevisiae*, thus allowing a better mouthfeel sensation ideal to produce wines aged on lees (Del Fresno et al. 2020). Lysis of *H. vineae* cells takes place as soon as sugars are exhausted from the grape must, reducing the need for lees contact from months to weeks compared to *Saccharomyces* (Carrau et al. 2020).

Hanseniaspora vineae and wine colour

Few studies have looked at the influence of yeast on wine colour although it is known that they synthetize some key compounds that promote anthocyanin reactions, such as the synthesis of compounds as pyruvic acid or acetaldehyde (Medina et al. 2018). A synthetic red grape juice medium (RGJM) supplemented with an anthocyanin extract obtained from grape skins of *Vitis vinifera* cv. Tannat, prepared according to a previous work (Medina et al. 2005), showed the formation of vitisin A, vitisin B, malvidin-3-glucoside-4-vinylphenol, and malvidin-3-glucoside-4-vinylguaiacol by *H. vineae*. In that study, *H. vineae* Hv205 showed the highest chemical age value with no correlation found between colour intensity and total anthocyanin content.

In addition, the cofermentation of *H. vineae* with *S. cerevisiae* resulted in a significantly higher concentration of acetaldehyde when compared with the pure culture of *S. cerevisiae*. The HPLC-DAD-MS analysis confirmed an increase of vitisin B, demonstrating the positive effect of mixed cultures of *H. vineae* with *S. cerevisiae* that increase acetaldehyde formation, a key compound for the formation of vitisins (Medina et al. 2016).

Interesting results were obtained recently in the production of rosé wines with Tempranillo and Albillo grapes of Ribera del Duero. A better colour intensity resulted from the application of

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

H. vineae in sequential inoculation with *Saccharomyces* (Del Fresno et al. 2021b). Winemaking was carried out in oak and stainless steel barrels, and *H. vineae* wines resulted in up to 44% more anthocyanin concentration in the final wines.

Practical application at winery scale and *H. vineae* implantation

White wines using *H. vineae* have been produced on a commercial scale since 2007 (Medina et al. 2007) and could be sensorially differentiated from wines produced by conventional *Saccharomyces* fermentations (Medina et al. 2013, Lleixà et al. 2016a, Martin et al. 2018, Del Fresno et al. 2020, Del Fresno et al. 2021a). It is clear from a practical point of view that to produce increased and intense flavour compounds of higher aromatic alcohols and their esters during winemaking, *H. vineae* likes low temperatures (15–22°C), grape must of low YAN (around 100 mgN/l), and moderate microaerobic conditions such as open tanks and barrel fermentations (Yan et al. 2020). The species requires thiamine addition of about 0.5 mg/l as it cannot synthetize this vitamin, but diammonium phosphate (DAP) addition should be avoided so as not to inhibit phenylpropanoid synthesis. *Hanseniaspora vineae* ferments glucose and fructose well, but not sucrose, so chaptalization with the disaccharide of sugarcane should be also avoided (Carrau et al. 2020).

Although red wines made with these treatments can be differentiated by chemical techniques, the sensory differentiation or qualitative evaluations of these processes at the winery scale were less clear. Our observation is that young red wines can be more easily differentiated than full-bodied and powerfully structured red wines, and even less so after barrel maturation. However, chemically, red wines made from *H. vineae* showed the presence of increased concentrations of benzenoids, acetoin, 2,3-butanediol, and acetate esters derived from aromatic alcohols such as tryptophol, tyrosol, and phenyl ethanol compared to conventionally vinified wines (Valera et al. 2021). Metabolic fingerprinting by gas chromatographic analysis (Howell et al. 2006) allowed us to demonstrate that *H. vineae* had contributed to the aromatic chemical composition on an industrial scale of wine fermentation (Martin et al. 2022b). Confirmation of a good implantation is easily effected by microscopy due to the distinctive cellular shape of apiculate yeast cells (see Fig. 7), although the analysis of the metabolic chemical fingerprint can determined its real contribution.

Nutrients and metabolites under mixed cultures. The concept of friendly yeasts

Saccharomyces starters are highly competitive with the natural community of grapes and grape-musts of a certain terroir, defined as a microbial terroir (Gilbert et al. 2014, Carrau et al. 2020). High alcohol excludes other yeast species from the grape juice at the winery. *Saccharomyces* has developed several mechanisms to control this environment, such as rapid removal of nitrogen and vitamins, high production of ethanol and CO_2, increasing temperature, and the production of certain metabolites that are considered toxic to many yeast species cells such as higher alcohols, short- and medium-chain fatty acids, and isoacids (Carrau and Henschke 2021). In the last two decades, mixed cultures fermentations have been studied in order to increase flavour diversity and sensory complexity of wines. The facts that defined *Saccharomyces* strains as 'selfish yeasts' have promoted work with sequential inoculation for mixed cultures conditions to give 24 or 48 hours to the non-*Saccharomyces* species to act in the medium. Sequential inoculation methods allowed non-*Saccharomyces* strains to influence the fermentation with diverse flavours for 1 or 2 days before introducing the *Saccharomyces* strain to the must to ensure the completion of fermentation (Domizio et al. 2011, Jolly et al. 2014, Loira et al. 2015, Borren and Tian 2021). The definition of *H. vineae* as a 'friendly yeast' is supported not by a humane sympathy, but by its metabolic characteristics. Unlike *Saccharomyces*, *H. vineae* produces very low levels of compounds considered toxic to cells, such as higher alcohols and MCFA (an average of ten times less), and instead has a high capacity to acetylate alcohols, which is considered a form of medium detoxification, decreasing the free acids and alcohols concentrations (Peddie 1990). All these aspects together with its capacity to produce moderate temperature and CO_2 levels during fermentation makes *H. vineae* an interesting model of friendly yeast that allowed growth of other yeast strains compared to the efficient *Saccharomyces*' single strain fermentations (Carrau and Henschke 2021).

Application of *H. vineae* in beer

More recently, *H. vineae* has begun to be studied for its use in the brewing industry, particularly for craft beer production. In this sense, *H. vineae* has been characterized for sugar utilization, alcohol production, aromas, phenolic off-flavours (POF), sensory trials, hops sensitivity, and organic acids production in beers.

Regarding sugar utilization, *H. vineae* is only able to ferment the wort sugars glucose and fructose (no sucrose, maltose, or maltotriose utilization), resulting in increased residual maltose sugar concentrations and contributing to enhanced sweetness in beers (Bellut et al. 2018, Bellut and Arendt 2019, Larroque et al. 2021, Postigo et al. 2022). The inability to ferment maltose and maltotriose introduces the possibility of brewing alcohol-free beer (AFB) (≤ 0.5% v/v) (Bellut et al. 2018). Bellut et al. (2018) conducted a study with five strains isolated from kombucha, one of them being *H. vineae*, which were compared to a commercially applied AFB strain *Saccharomycodes ludwigii* and a *S. cerevisiae* brewer's yeast. An experienced sensory panel could not discriminate between the *H. vineae* AFB and the one produced with the commercial AFB strain.

Additionally, Bellut and Arendt (2019) reported nonalcoholic beer reached final ethanol contents of 0.34% v/v. Again, in a sensory analysis with an expert sensory panel, the AFB produced with *Hanseniaspora* could not be distinguished from the AFB produced with the commercially employed *S. ludwigii* strain. *Hanseniaspora vineae* was given the attributes of 'black tea', 'honey', and 'caramel-like'.

The results mentioned above indicated its suitability in AFB brewing, opening the possibility to produce AFB product category, which offers economic benefits in the form of a growing market, a lower tax burden, enables brewers to expand their variety of products, and promote healthier and more responsible alcohol consumption.

In a recent study, Larroque et al. (2021) reported a cofermentation beer treatment using a native yeast strain of *H. vineae* (Hv205), because of its reported capacity to produce derived aromatic amino acids acetates, such as 2-phenylethyl acetate and benzyl alcohol in wines as is shown above. The results demonstrated that even though Hv T02/05F was maltose negative, it was a promising yeast for the production of fruity beers in mixed cul-

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

ture for its contribution to the aromatic profile by a high ester production, which adds fruitiness nuances to beer. In this study, mixed cultures of native non-*Saccharomyces* yeast strains were tested (Hv205, *Zygoascus meyerae*, Hv T12_135F, and *Pichia anomala* BCMO15_2), resulting that the mixed culture of *S. cerevisiae* Sc 00/35 + Hv205 had the highest fermentation capacity and the highest population of viable cells at the end of fermentation. Monitoring population dynamics and *H. vineae* implantation during fermentation, on the fourth day of fermentation, *S. cerevisiae* 00/35 dominated the culture, while the population of *H. vineae* was 32%, and 13% at the end of fermentation.

Postigo et al. (2022) confirmed previous reports for cofermentation studies (Larroque et al. 2021), and under single fermentation for low alcohol beer production (Bellut et al. 2018, 2019).

Regarding aromas in beer, Budroni et al. (2017), and Callejo et al. (2019) reported that higher alcohols are the most abundant organoleptic compounds generally regarded as desirable. These compounds impart refreshing, floral and pleasant notes in concentrations below 300 mg/l, and add complexity to the beer. The two most important higher alcohols in beers are isobutanol and isoamyl alcohol. In this sense, the results reported by Postigo et al. (2022) suggested that *H. vineae* (22.51–43.43 m/l) was a poor/medium producer of higher alcohols compared to *S. cerevisiae* (119.55 mg/l), as shown in wines. *Hanseniaspora vineae* was below the threshold levels for aldehyde and ketone production. For acetoin, *H. vineae* showed higher production than the commercial *Saccharomyces* strains, which is consistent with the reported data in wines. *Hanseniaspora vineae* produced low concentrations of γ-butyrolactone, which was contrary to results obtained in wine studies (Giorello et al. 2019). This compound imparts a sweet and worty flavour to beer. The absence of phenolic aromas in the sensory analysis confirmed that this species is POF negative (the phenolic aroma is associated mainly with a clove-like aroma) (Postigo et al. 2022), which is a desirable selection criterion for some brewing yeasts (Burini et al. 2021).

Additionally, Bellut et al. (2019) confirmed the absence of POF production and showed no signs of sensitivity toward iso-α-acids (hops component) concentrations of up to 100 mg/l.

Finally, another possible brewing application with *H. vineae* has been its use to produce sour beer. Osburn et al. (2018) reported for *H. vineae* an excellent attenuation, lactic acid production, and desirable sensory characteristics, positioning it as a viable alternative to lactic acid bacteria (LAB) for the production of sour beers. They suggested a new LAB-free paradigm to produce sour beer in a 'primary souring' because the lactic acid production and resultant pH decrease occurs during primary alcoholic fermentation. Similar results have been reported by de Souza Varize et al. (2019). In this study, *H. vineae* has been used to produce sour beers in a single fermentation step, without the need of LAB for souring. The resulting beers showed both lactic tartness and fruity aromatic and flavour notes. In our experience with wines, we never found production of lactic acid by *H. vineae*.

Application of *H. vineae* in other fermented foods

Cider

Apple juice to produce cider is also an interesting source of *Hanseniaspora* species (Valles et al. 2007, De Arruda Moura Pietrowski et al. 2012, Al Daccache et al. 2020a, b, 2021). *Hanseniaspora vineae* has the potential to produce cider in single yeast fermentation processes up to 10% by volume (Tournas et al. 2006, Wei et al. 2019, Hou et al. 2022). This is an interesting application for the species as complete fermentation might be done with single or mixed cultures of strains of other *Hanseniaspora* species independently of *Saccharomyces* strains. Aromatic acetate esters were produced at significantly higher concentrations compared to conventional fermentation processes with *Saccharomyces* at optimal temperatures of about 20°C (Hou et al. 2022). Some nutrient differences between apple juice and grape juice very probably have some effects indicting the need for further studies to understand the best conditions to obtain high quality ciders (Gschaedler et al. 2021).

Tequila and kombucha

Hanseniaspora vineae is a component of the native community of yeasts responsible for the fermentation of agave juice in the production of Tequila (Lachance 1995). The species was used to ferment agave and some difficulties in completing the fermentation were solved by the addition of yeast extract and not ammonium phosphate, a more common practice (González-Robles et al. 2015). This may be due to the lack of *de novo* synthesis of thiamine, i.e. characteristic in *H. vineae* (Carrau and Henschke 2021) and in other *Hanseniaspora* species (Seixas et al. 2019).

The evaluation of the application of *H. vineae* in agave fermentation was that it increased fruity flavour in the distillates. We could not find any published reference on the use of *H. vineae* in kombucha fermentation; this beverage is based on tea infusions with some other fruit components depending on the producer. However, our first trials with the addition of *H. vineae* in the initial mixed culture resulted in a decrease of acetic acid production and with an increased fruity sensory character in the final beverage (Peyrot Andrada 2021). Further studies are required to evaluate the potential of this species for kombucha.

Bread

Baker's yeast has been utilized for centuries and *S. cerevisiae* has always played an important role in the inocula. Depending on the processes, LAB or other yeasts may play a role. The key factor is the production of CO_2 for increasing dough volume, but secondary metabolism also increases the flavour complexity of the bread. Although very traditional production includes many yeast species, in the last decade alternative nonconventional yeast species have been explored to increase diversity with the commercial *Saccharomyces* usually added (Zotta et al. 2022). It was not expected that apiculate yeast might function for this process, but a few reports showed promising results with *H. uvarum* and *H. vineae*. Recently, *H. vineae* was successfully applied with good and even superior results for leavened doughs than *Saccharomyces* in laboratory tests (Takaya et al. 2019, 2021). Sucrose was replaced by glucose sources (fruit juices, honey, or commercial glucose), as *H. vineae* does not have the capacity to hydrolyse the former fermentatively. These authors confirm the increased flavour complexity produced by *H. vineae* with the production of acetoin and phenyl ethyl acetate. Further studies will contribute to a better understanding the potential of *H. vineae* for bread commercial application.

The timeline of the discovery of *H. vineae* since 1957

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

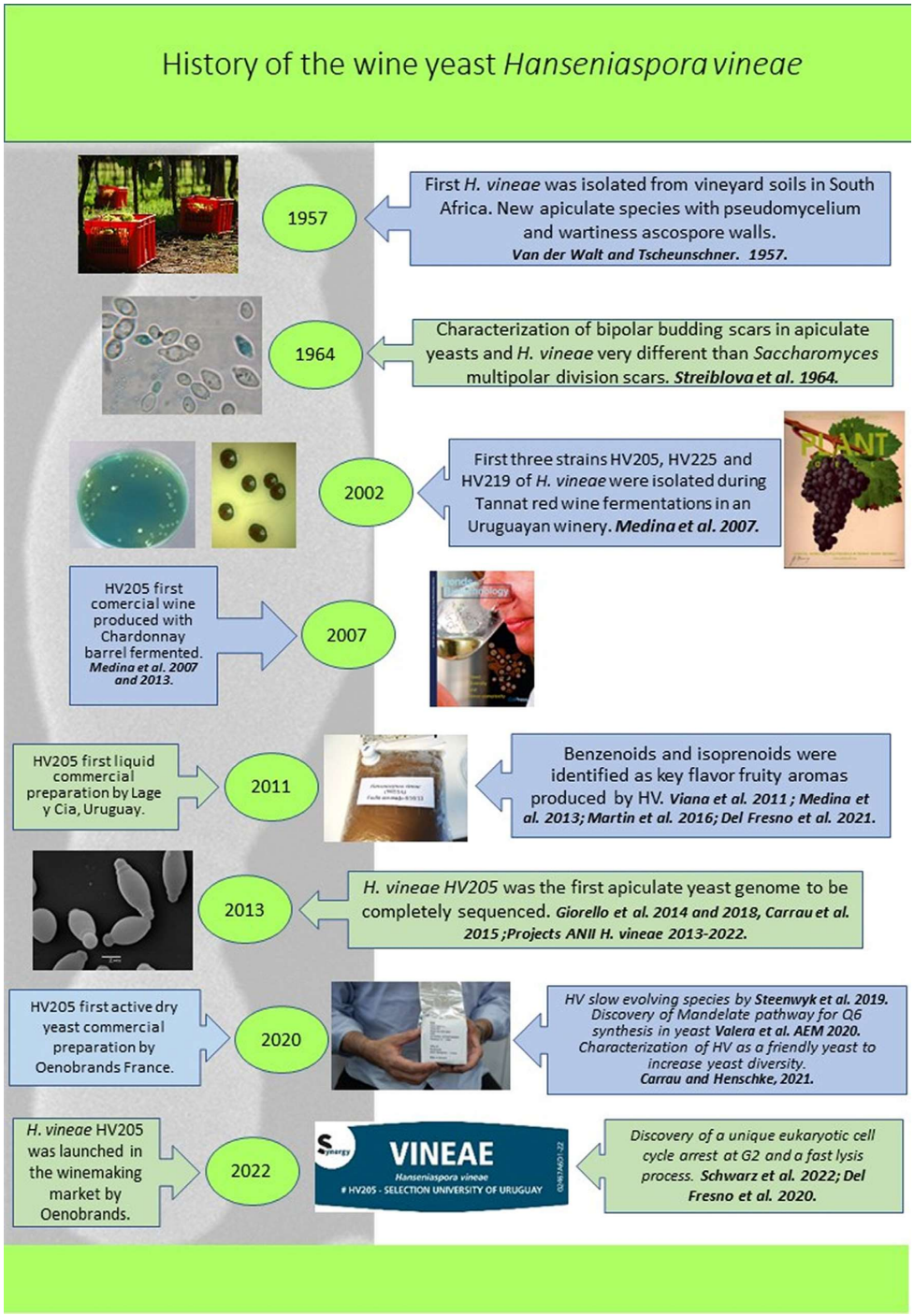

Figure 9. Timeline of relevant events in *H. vineae* history and its application for winemaking. After 20 years of research and development studies at our laboratory and winery level, a strain Hv205 was finally commercially launched this year by Oenobrands France.

Conclusions and future perspectives

In the last 20 years it has been shown that *H. vineae* posseses unusual characteristics within the genus *Hanseniaspora*. Species of this genus predominate in fruits, whereas *H. vineae* shows a special adaptation to fermentation. The species shares many similarities with *Saccharomyces* as opposed to congeners such as *H. uvarum*. The data presented in this review demonstrate that *H. vineae* is an ancestral fermenter that evolved with some key gene changes from fruit to fermentations niches. The increased similarity of glycolytic enzymes to *Saccharomyces*, the presence of genes such as *SSU1* and *SUC2*, and the increased CN of ADH genes, are good examples. We propose here that this species would be an excellent cell model to understand the concept of domestication for fermentation.

We have summarized the important contribution of *H. vineae* to the flavour phenotype in wines, and in other fermented products that are under study today in many research groups on food science, such as beer, bread, and cider. Today, *H. vineae* is the first apiculate yeast available commercially.

Finally, *H. vineae* can make a significant contribution as a model eukaryotic cell for understanding the phenolic aroma compounds

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

pathways that are very poor in the *Saccharomyces* genus. Many of the compounds in the phenolic group are very important not only for flavours in foods, but also as rich antioxidants or bioactive compounds for human nutrition. Examples of this are the precursors of the coenzyme Q in eukaryotes, or many other active compounds that are found uniquely in plants. Winemakers, masters of food fermentation, biotechnologists, and scientists will enjoy working with this particular yeast species of the genus *Hanseniaspora*.

Acknowledgements

This review about *H. vineae* contained results of 20 years of research with many collaboration projects and with colleagues that made possible the development of this yeast species for the industry. We would like to thank Albert Mas et al., URV and Antonio Morata et al., UPM from Spain, Tomas Roman et al., San Michele Institute, Italy, Remi Schneider et al., Oenobrands, France, Sergio Echeverrigaray et al., UCS, Brazil, Cathy Clark and Lucia Fernandez-Del Rio, UCLA, USA, Alberto Rosa, UNC, Argentina, Rafael Radi et al., UdelaR, Uruguay, and Eduardo Manta et al., UdelaR, Uruguay. In addition, we thank to all the winemakers and wineries that have tried Hv205 in Uruguay, Brazil, Italy, Spain, France, and the USA.

Conflict of interest. None declared.

Funding

We thank the following agencies for financial support: CSIC Group project numbers 802 and 983 of the Universidad de la República, Uruguay, the Agencia Nacional de Investigacion e Innovacion (ANII), *H. vineae* FMV 6956 project, postdoctoral fellowship (PD_NAC_2016_1_133945 to M.J.V.), and a grant of Espacio Interdisciplinario Proyecto Centros to CEINBIO and the Programa Alimentos y Salud Pays. We also thank the Instituto Pasteur of Montevideo for sequencing the first *H. vineae* strains, Oenobrands (France) and Lage y Cia-Lallemand (Uruguay) for supporting our cooperation project on *H. vineae* metabolic characterization ANII Alianza 2019–2022. (ALI_2_2019_1_155314).

References

Al Daccache M, Koubaa M, Maroun RG *et al.* Impact of the physico-chemical composition and microbial diversity in apple juice fermentation process: a review. *Molecules* 2020b;**25**;3698.

Al Daccache M, Koubaa M, Maroun RG *et al.* Suitability of the Lebanese "Ace Spur" apple variety for cider production using *Hanseniaspora* sp. yeast. *Fermentation* 2020a;**6**:32.

Al Daccache M, Salameh D, Chamy LEL *et al.* Evaluation of the fermentative capacity of an indigenous *Hanseniaspora* sp. strain isolated from Lebanese apples for cider production. *FEMS Microbiol Lett* 2021;**367**:fnaa093.

Almeida P, Gonçalves C, Teixeira S *et al.* A Gondwanan imprint on global diversity and domestication of wine and cider yeast *Saccharomyces uvarum*. *Nat Commun* 2014;**5**:4044.

Badura J, van Wyk N, Brezina S *et al.* Development of genetic modification tools for *Hanseniaspora uvarum*. *Int J Mol Sci* 2021;**22**:1943.

Banh RS, Kim ES, Spillier Q *et al.* The polar oxy-metabolome reveals the 4-hydroxymandelate CoQ10 synthesis pathway. *Nature* 2021;**597**:420–5.

Bellut K, Arendt E. Chance and challenge: non-*Saccharomyces* yeasts in nonalcoholic and low alcohol beer brewing—a review. *J Am Soc Brew Chem* 2019;**77**:77–91.

Bellut K, Michel M, Zarnkow M *et al.* Application of non-*Saccharomyces* yeasts isolated from kombucha in the production of alcohol-free beer. *Fermentation* 2018;**4**:66.

Bellut K. Arendt EK Chance and challenge: Non-saccharomyces yeasts in nonalcoholic and low alcohol beer brewing—A review. *J Am Soc Brew Chem* 2019;**77**:77–91.

Benito S, Palomero F, Morata A *et al.* New applications for *Schizosaccharomyces pombe* in the alcoholic fermentation of red wines. *Int J Food Sci Technol* 2012;**47**:2101–8.

Borneman AR, Desany BA, Riches D *et al.* Whole-genome comparison reveals novel genetic elements that characterize the genome of industrial strains of *Saccharomyces cerevisiae*. *PLos Genet* 2011;**7**:e1001287.

Borren E, Tian B. The important contribution of non-*Saccharomyces* yeasts to the aroma complexity of wine: a review. *Foods* 2021;**10**:13.

Budroni M, Zara G, Ciani M *et al.* Saccharomyces and non-Saccharomyces starter yeasts. In Brewing Technology. IntechOpen, .

Burini JA, Eizaguirre JI, Loviso C *et al.* Levaduras no convencionales como herramientas de innovación y diferenciación en la producción de cerveza. *Rev Argent Microbiol* 2021;**53**:359–77.

Čadež N, Bellora N, Ulloa R *et al.* Genomic content of a novel yeast species *Hanseniaspora gamundiae* sp. nov. from fungal stromata (Cyttaria) associated with a unique fermented beverage in Andean Patagonia, Argentina. *PLoS ONE* 2019;**14**:e0210792.

Čadež N, Bellora N, Ulloa R *et al.* Hanseniaspora smithiae sp. nov., a novel apiculate yeast species from Patagonian forests that lacks the typical genomic domestication signatures for fermentative environments. *Frontiers in microbiology* 2021;**12**:679894.

Čadež N, Pagnocca FC, Raspor P *et al.* *Hanseniaspora nectarophila* sp. nov., a yeast species isolated from ephemeral flowers. *Int J Syst Evol Microbiol* 2014;**64**:2364–9.

Callejo MJ, García Navas JJ, Alba R *et al.* Wort fermentation and beer conditioning with selected non-Saccharomyces yeasts in craft beers. *Eur Food Res Technol* 2019;**245**:1229–38.

Capece A, Fiore C, Romano P. Molecular and technological biodiversity in apiculate yeasts of wine origin. *Acta Hortic* 2007;**754**:139–46.

Carrau F, Boido E, Ramey D. Yeasts for low input winemaking: microbial terroir and flavor differentiation. *Adv Appl Microbiol* 2020;**111**:89–121.

Carrau F, Gaggero C, Aguilar PS. Yeast diversity and native vigor for flavor phenotypes. *Trends Biotechnol* 2015;**33**:148–54.

Carrau F, Henschke PA. *Hanseniaspora vineae* and the concept of friendly yeasts to increase autochthonous wine flavor diversity. *Front Microbiol* 2021;**12**:1–8.

Cheynier V, Schneider R, Salmon JM *et al.* Chemistry of wine. *Compr Nat Prod II Chem Biol* 2010;**3**:1119–72.

Cliff MA, Pickering GJ. Determination of odour detection thresholds for acetic acid and ethyl acetate in ice wine. *J Wine Res* 2006;**17**:45–52.

Cosme F, Fernandes C, Ribeiro T *et al.* White wine protein instability: mechanism, quality control and technological alternatives for wine stabilisation—an overview. *Beverages* 2020;**6**:19.

De Arruda Moura Pietrowski G, Dos Santos CME, Sauer E *et al.* Influence of fermentation with *Hanseniaspora* sp. yeast on the volatile profile of fermented apple. *J Agric Food Chem* 2012;**60**:9815–21.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

de Souza Varize C, Christofoleti-Furlan RM, Muynarsk E *et al.* Biotechnological applications of nonconventional yeasts. *Yeasts Biotechnol* 2019;**46**:107674.

Del Fresno JM, Escott C, Carrau F *et al.* Improving Aroma Complexity with Hanseniaspora spp.: Terpenes, Acetate Esters, and Safranal. *Fermentation* 2022;**8**:654.

Del Fresno JM, Escott C, Loira I *et al.* Impact of *Hanseniaspora vineae* in alcoholic fermentation and ageing on lees of high-quality whitewine. *Fermentation* 2020;**6**:66.

Del Fresno JM, Escott C, Loira I *et al.* The impact of *Hanseniaspora vineae* fermentation and ageing on lees on the terpenic aromatic profile of white wines of the Albillo variety. *Int J Mol Sci* 2021a;**22**:2195.

Del Fresno JM, Escott C, Loira I *et al.* The impact of *Hanseniaspora vineae* fermentation and ageing on lees on the terpenic aromatic profile of white wines of the Albillo variety. *Int J Mol Sci* 2021c;**22**:1–14.

Del Fresno JM, Loira I, Escott C *et al.* Application of *Hanseniaspora vineae* yeast in the production of rosé wines from a blend of Tempranillo and Albillo grapes. *Fermentation* 2021b;**7**:1–15.

Díaz-Montaño DM, de Jesús Ramírez Córdova J. The fermentative and aromatic ability of *Kloeckera* and *Hanseniaspora* yeasts. In: Satyanarayana T, Kunze G (eds), *Yeast Biotechnology: Diversity and Applications*. Dordrecht: Springer, 2009, 281–305.

Domizio P, Romani C, Comitini F *et al.* Potential spoilage non-*Saccharomyces* yeasts in mixed cultures with *Saccharomyces cerevisiae*. *Ann Microbiol* 2011;**61**:137–44.

Escott C, López C, Loira I *et al.* Improvement of must fermentation from late harvest cv. Tempranillo grapes treated with pulsed light. *Foods* 2021;**10**:1–14.

Fariña L, Villar V, Ares G *et al.* Volatile composition and aroma profile of Uruguayan Tannat wines. *Food Res Int* 2015;**69**:244–55.

Fernández-del-Río L, Clarke CF. Coenzyme Q biosynthesis: an update on the origins of the benzenoid ring and discovery of new ring precursors. *Metabolites* 2021;**11**:385.

Ferreira V, López R, Cacho JF. Quantitative determination of the odorants of young red wines from different grape varieties. *J Sci Food Agric* 2000;**80**:1659–67.

Ferreira V, Sáenz-Navajas M-P, Campo E *et al.* Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. *Food Chem* 2016;**199**:447–56.

Fleet GH, Heard GM. Yeasts-growth during fermentation. In: Fleet GH (ed.), *Wine Microbiology and Biotechnology*. Chur: Harwood Academic Publishers, 1993, 27–54.

Fleet GH. Wine yeasts for the future. *FEMS Yeast Res* 2008;**8**:979–95.

Gilbert JA, van der Lelie D, Zarraonaindia I. Microbial terroir for wine grapes. *Proc Natl Acad Sci* 2014;**111**:5–6.

Giorello F, Valera MJ, Martin V *et al.* Genomic and transcriptomic basis of *Hanseniaspora vineae*'s impact on flavor diversity and wine quality. *Appl Environ Microbiol* 2019;**85**:e01959–18.

Giorello FM, Bernā L, Greif G *et al.* Genome sequence of the native apiculate wine yeast Hanseniaspora vineae T02/19AF. *Genome announcements* 2014;**2**:e00530–14.

González-Robles IW, Estarrón-Espinosa M, Díaz-Montaño DM. Fermentative capabilities and volatile compounds produced by *Kloeckera/Hanseniaspora* and *Saccharomyces* yeast strains in pure and mixed cultures during *Agave tequilana* juice fermentation. *Antonie Van Leeuwenhoek* 2015;**108**:525–36.

Gschaedler A, Iñiguez-Muñoz LE, Flores-Flores NY *et al.* Use of non-*Saccharomyces* yeasts in cider fermentation: importance of the nutrients addition to obtain an efficient fermentation. *Int J Food Microbiol* 2021;**347**:109169.

Hameed AR, Al-Qaysi SA, Hameed ST. Killer activity of *Hanseniaspora uvarum* isolated from dates vinegar: partially purification and characterization of killer toxin. *Baghdad Sci J* 2019;**16**:140–50.

Han B, Gao J, Han X *et al.* *Hanseniaspora uvarum* FS35 degrade putrescine in wine through the direct oxidative deamination pathway of copper amine oxidase 1. *Food Res Int* 2022;**162**:111923.

Hou CY, Huang PH, Lai YT *et al.* Screening and identification of yeasts from fruits and their coculture for cider production. *Fermentation* 2022;**8**:1.

Howell KS, Cozzolino D, Bartowsky EJ *et al.* Metabolic profiling as a tool for revealing *Saccharomyces* interactions during wine fermentation. *FEMS Yeast Res* 2006;**6**:91–101.

Jackson RS. Chapter 3—olfactory sensations. In: Jackson RSBT-WT (ed.), *Wine Tasting*. 3rd edn. Cambridge: Academic Press, 2017, 41–101.

Jolly NP, Varela C, Pretorius IS. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res* 2014;**14**:215–37.

Lachance MA. Yeast communities in a natural tequila fermentation. *Antonie Van Leeuwenhoek* 1995;**68**:151–60.

Langenberg A-K, Bink FJ, Wolff L *et al.* Glycolytic functions are conserved in the genome of the wine yeast *Hanseniaspora uvarum*, and pyruvate kinase limits its capacity for alcoholic fermentation. *Appl Environ Microbiol* 2017;**83**:e01580–17.

Larroque MN, Carrau F, Fariña L *et al.* Effect of *Saccharomyces* and non-*Saccharomyces* native yeasts on beer aroma compounds. *Int J Food Microbiol* 2021;**337**:108953.

Libkind D, Hittinger CT, Valério E *et al.* Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proc Natl Acad Sci* 2011;**108**:14539–44.

Liti G, Carter DM, Moses AM *et al.* Population genomics of domestic and wild yeasts. *Nature* 2009;**458**:337–41.

Lleixà J, Manzano M, Mas A *et al.* *Saccharomyces* and non-*Saccharomyces* competition during microvinification under different sugar and nitrogen conditions. *Front Microbiol* 2016a;**7**:1959.

Lleixà J, Martín V, Portillo M del C *et al.* Comparison of fermentation and wines produced by inoculation of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. *Front Microbiol* 2016b;**7**:338.

Loira I, Morata A, Comuzzo P *et al.* Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Res Int* 2015;**76**:325–33.

Loira I, Morata A, Palomero F *et al.* *Schizosaccharomyces pombe*: a promising biotechnology for modulating wine composition. *Fermentation* 2018;**4**:70.

López MC, Mateo JJ, Maicas S. Screening of β-glucosidase and β-xylosidase activities in four non- *Saccharomyces* yeast isolates. *J Food Sci* 2015;**80**:C1696–704.

López S, Mateo JJ, Maicas S. Characterisation of *Hanseniaspora* isolates with potential aromaenhancing properties in muscat wines. *South Afr J Enol Vitic* 2014;**35**:292–303.

Martin V, Boido E, Giorello F *et al.* Effect of yeast assimilable nitrogen on the synthesis of phenolic aroma compounds by *Hanseniaspora vineae* strains. *Yeast* 2016a;**33**:323–8.

Martin V, Giorello F, Fariña L *et al.* De novo synthesis of benzenoid compounds by the yeast *Hanseniaspora vineae* increases the flavor diversity of wines. *J Agric Food Chem* 2016b;**64**:4574–83.

Martin V, Jose Valera M, Medina K *et al.* Oenological impact of the *Hanseniaspora/Kloeckera* yeast genus on wines—a review. *Fermentation* 2018;**4**:76.

Martin V, Risso C, Listur B *et al.* Proteolytic activity under white wine fermentation by *Hanseniaspora vineae* yeast strains. In: *Proceedings*

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

of the OIV Web Conference. Ensenada: International Organisation of Vine and Wine, 2022a.

Martin V, Valera MJ, Medina K *et al.* Application of *Hanseniaspora vineae* to improve white wine quality. In: Morata A (ed.), *White Wine Technology*. Cambridge: Academic Press, 2022b, 99–115.

Martín V. *Hanseniaspora vineae* : caracterización y su uso en la vinificación. Ph.D. Thesis, Universidad de la Republica, 2016.

Medina K, Boido E, Dellacassa E *et al.* Effects of non-*Saccharomyces* yeasts on color, anthocyanin, and anthocyanin-derived pigments of Tannat grapes during fermentation. *Am J Enol Viticul* 2018;**69**:148–56.

Medina K, Boido E, Dellacassa E *et al.* Yeast interactions with anthocyanins during red wine fermentation. *Am J Enol Viticul* 2005;**56**:104–9.

Medina K, Boido E, Fariña L *et al.* Impacto en el aroma de vinos Tannat producidos por diferentes levaduras en tres sistemas de vinificación. *South Florida J Dev* 2021;**2**:1565–71.

Medina K, Boido E, Fariña L *et al.* Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. *Food Chem* 2013;**141**:2513–21.

Medina K, Boido E, Fariña L *et al.* Non-*Saccharomyces* and *Saccharomyces* strains co-fermentation increases acetaldehyde accumulation: effect on anthocyanin-derived pigments in Tannat red wines. *Yeast* 2016;**33**:339–43.

Medina K, Ferreri L, Fariña L *et al.* Aplicación de la levadura *Hanseniaspora vineae* en cultivos mixtos con *Saccharomyces cerevisiae* en la vinificación. *Rev Enol* 2007;**4**:1–6.

Mostert TT. Investigating the secretome of non-*Saccharomyces* yeast in model wine. M.Sc. Thesis, Stellenbosch Institute for Wine Biotechnology, Faculty of AgriSciences, Stellenbosch University. 2013. Unpublished.

Osburn K, Amaral J, Metcalf SR *et al.* Primary souring: a novel bacteria-free method for sour beer production. *Food Microbiol* 2018;**70**:76–84.

Padilla B, Gil J V, Manzanares P. Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front Microbiol* 2016;**7**:411.

Peddie HAB. Ester formation in brewery fermentations. *J Inst Brew* 1990;**96**:327–31.

Pérez G, Fariña L, Barquet M *et al.* A quick screening method to identify β-glucosidase activity in native wine yeast strains: application of esculin glycerol agar (EGA) medium. *World J Microbiol Biotechnol* 2011;**27**:47–55.

Peyrot Andrada D. Caracterizacion fisicoquimica de bebidas fermentadas a base de te, Kombucha. Seleccion de levaduras para su utilizacion. Licenciatura en Quimica, National Autonomous University of Nicaragua, 2021. http/enologia.fq.edu.uy.

Pontes A, Hutzler M, Brito PH *et al.* Revisiting the taxonomic synonyms and populations of *Saccharomyces cerevisiae*—phylogeny, phenotypes, ecology and domestication. *Microorganisms* 2020;**8**:903.

Postigo V, Sánchez A, Cabellos JM *et al.* New approaches for the fermentation of beer: non-*Saccharomyces* yeasts from wine. *Fermentation* 2022;**8**:280.

Pramateftaki PV, Kouvelis VN, Lanaridis P *et al.* The mitochondrial genome of the wine yeast *Hanseniaspora uvarum*: a unique genome organization among yeast/fungal counterparts. *FEMS Yeast Res* 2006;**6**:77–90.

Radler F, Pfeiffer P, Dennert M. Killer toxins in new isolates of the yeasts *Hanseniaspora uvarum* and *Pichia kluyveri*. *FEMS Microbiol Lett* 1985;**29**:269–72.

Radler F, Schmitt MJ, Meyer B. Killer toxin of *Hanseniaspora uvarum*. *Arch Microbiol* 1990;**154**:175–8.

Restrepo S, Espinoza L, Ceballos A *et al.* Production of fatty acids during alcoholic wine fermentation under selected temperature and aeration conditions. *Am J Enol Viticul* 2019;**70**:169–76.

Riley R, Haridas S, Wolfe KH *et al.* Comparative genomics of biotechnologically important yeasts. *Proc Natl Acad Sci* 2016;**113**:9882–7.

Roman Villegas T, Cappello N, Gallo A *et al.* The use of Hanseniaspora vineae on the production of base sparkling wine. 2021. In: Macrowine 2021, online, June 23-30, 2021. url: https://ives-openscience.eu/8367/ handle: http://hdl.handle.net/10449/70642.

Roudil L, Russo P, Berbegal C *et al.* Non-*Saccharomyces* commercial starter cultures: scientific trends, recent patents and innovation in the wine sector. *Recent Patents Food Nutr Agric* 2019;**11**:27–39.

Saracino F, Brinco J, Gago D *et al.* DCMC as a promising alternative to bentonite in white wine stabilization. Impact on protein stability and wine aromatic fraction. *Molecules* 2021;**26**:6188.

Schmitt MJ, Neuhausen F. Killer toxin-secreting double-stranded RNA mycoviruses in the yeasts *Hanseniaspora uvarum* and *Zygosaccharomyces bailii*. *J Virol* 1994;**68**:1765–72.

Schwarz LV, Valera MJ, Delamare APL *et al.* A peculiar cell cycle arrest at g2/m stage during the stationary phase of growth in the wine yeast *Hanseniaspora vineae*. *Curr Res Microb Sci* 2022;**3**:100129.

Seixas I, Barbosa C, Mendes-Faia A *et al.* Genome sequence of the non-conventional wine yeast *Hanseniaspora guilliermondii* UTAD222 unveils relevant traits of this species and of the *Hanseniaspora* genus in the context of wine fermentation. *DNA Res* 2019;**26**:67–83.

Steenwyk JL, Opulente DA, Kominek J *et al.* Extensive loss of cell-cycle and DNA repair genes in an ancient lineage of bipolar budding yeasts. *PLoS Biol* 2019;**17**:e3000255.

Suárez-Lepe JA, Morata A. New trends in yeast selection for winemaking. *Trends Food Sci Technol* 2012;**23**:39–50.

Takaya M, Ohwada T, Oda Y. Characterization of the yeast *Hanseniaspora vineae* isolated from the wine grape "Yamasachi" and its use for bread making. *Food Sci Technol Res* 2019;**25**:835–42.

Takaya M, Ohwada T, Oda Y. Conventional bread making with a mixture of the yeast *Hanseniaspora vineae* and the baker's yeast *Saccharomyces cerevisiae* for improved quality. *Food Sci Technol Res* 2021;**27**:483–9.

Tavares MJ, Güldener U, Mendes-Ferreira A *et al.* Genome sequencing, annotation and exploration of the SO2-tolerant non-conventional yeast *Saccharomycodes ludwigii*. *BMC Genomics* 2021;**22**:1–15.

Toit M, Pretorius IS. Microbial spoilage and preservation of wine : using weapons from nature's own arsenal—a review. *South Afr J Enol Vitic* 2000;**21**:74–96.

Tournas VH, Heeres J, Burgess L. Moulds and yeasts in fruit salads and fruit juices. *Food Microbiol* 2006;**23**:684–8.

Valera MJ, Boido E, Dellacassa E *et al.* Comparison of the glycolytic and alcoholic fermentation pathways of *Hanseniaspora vineae* with *Saccharomyces cerevisiae* wine yeasts. *Fermentation* 2020a;**6**:78.

Valera MJ, Boido E, Ramos JC *et al.* The mandelate pathway, an alternative to the phenylalanine ammonia lyase pathway for the synthesis of benzenoids in ascomycete yeasts. *Appl Environ Microbiol* 2020b;**86**:e00701–20.

Valera MJ, Medina K, Martin V *et al.* Effect of non-conventional yeasts on the flavor profiles of fermented beverages. In: *Flavors and Fragrances in Food Processing: Preparation and Characterization Methods*. Washington: American Chemical Society, 2022, 225–44.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025

Valera MJ, Olivera V, Boido E *et al.* Wine aroma characterization of the two main fermentation yeast species of the apiculate genus *Hanseniaspora*. *Fermentation* 2021;**7**:162.

Valera MJ, Zeida A, Boido E *et al.* Genetic and transcriptomic evidences suggest ARO10 genes are involved in benzenoid biosynthesis by yeast. *Yeast* 2020c;**37**:427–35.

Valles BS, Bedriñana RP, Tascón NF *et al.* Yeast species associated with the spontaneous fermentation of cider. *Food Microbiol* 2007;**24**:25–31.

van Wyk N, Scansani S, Beisert B *et al.* The use of *Hanseniaspora occidentalis* in a sequential must inoculation to reduce the malic acid content of wine. *Appl Sci* 2022;**12**:6919.

Viana F, Belloch C, Vallés S *et al.* Monitoring a mixed starter of *Hanseniaspora vineae–Saccharomyces cerevisiae* in natural must: impact on 2-phenylethyl acetate production. *Int J Food Microbiol* 2011;**151**:235–40.

Viana F, Gil JV, Vallés S *et al.* Increasing the levels of 2-phenylethyl acetate in wine through the use of a mixed culture of *Hanseniaspora osmophila* and *Saccharomyces cerevisiae*. *Int J Food Microbiol* 2009;**135**:68–74.

Waters EJ, Alexander G, Muhlack R *et al.* Preventing protein haze in bottled white wine. *Proteins* 2005;**11**:215–25.

Waters EJ, Pellerin P, Brillouet JM. A *Saccharomyces* mannoprotein that protects wine from protein haze. *Carbohydr Polym* 1994;**23**:185–91.

Wei J, Wang S, Zhang Y *et al.* Characterization and screening of non-*Saccharomyces* yeasts used to produce fragrant cider. *LWT* 2019;**107**:191–8.

Xi Y, Zhan T, Xu H *et al.* Characterization of JEN family carboxylate transporters from the acid-tolerant yeast *Pichia kudriavzevii* and their applications in succinic acid production. *Microb Biotechnol* 2021;**14**:1130–47.

Yan G, Zhang B, Joseph L *et al.* Effects of initial oxygenation on chemical and aromatic composition of wine in mixed starters of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. *Food Microbiol* 2020;**90**:103460

Zhang B, Liu H, Xue J *et al.* Use of *Torulaspora delbrueckii* and *Hanseniaspora vineae* co-fermentation with *Saccharomyces cerevisiae* to improve aroma profiles and safety quality of Petit Manseng wines. *LWT* 2022a;**161**:113360.

Zhang B, Tang C, Yang D *et al.* Effects of three indigenous non-*Saccharomyces* yeasts and their pairwise combinations in co-fermentation with *Saccharomyces cerevisiae* on volatile compounds of Petit Manseng wines. *Food Chem* 2022b;**368**:130807.

Zhang BQ, Shen JY, Duan CQ *et al.* Use of indigenous *Hanseniaspora vineae* and *Metschnikowia pulcherrima* co-fermentation with *Saccharomyces cerevisiae* to improve the aroma diversity of Vidal blanc icewine. *Front Microbiol* 2018;**9**:2303.

Zott K, Miot-Sertier C, Claisse O *et al.* Dynamics and diversity of non-*Saccharomyces* yeasts during the early stages in winemaking. *Int J Food Microbiol* 2008;**125**:197–203.

Zotta T, Di Renzo T, Sorrentino A *et al.* Selection of non-*Saccharomyces* wine yeasts for the production of leavened doughs. *Microorganisms* 2022;**10**:1849.

Downloaded from https://academic.oup.com/femsyr/article/doi/10.1093/femsyr/foad010/7033425 by UREP user on 21 October 2025