



SPECIAL ARTICLE

Yeast diversity in *Vitis non-vinifera* ecosystems

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Abstract The surface of grapes lodges a complex community of yeast species responsible for spontaneous alcoholic fermentation. The study of indigenous *Saccharomyces* and “non-*Saccharomyces*” yeasts during grape must fermentation constitutes a major research area in microbial enology. Although there are detailed studies on the microbiota of *Vitis vinifera* L. grapes, little is known about the diversity of yeast communities present in non-*vinifera* *Vitis* ecosystems (*i.e.*, grapes and spontaneously fermenting grape musts). Potentially scientific and/or enological valuable yeast strains from these non-*vinifera* *Vitis* ecosystems might never be isolated from *V. vinifera* L. In this updated review, we summarize relevant aspects of the microbial studies conducted on *V. non-vinifera* grapes and spontaneously fermenting grape musts.

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PALABRAS CLAVE

Levaduras;
Saccharomyces;
Uvas;
Mostos;
V. vinifera L.;
V. labrusca L.

Diversidad de levaduras en ecosistemas de *Vitis no-vinifera*

Resumen La superficie de las uvas aloja una comunidad compleja de especies de levaduras responsables de la fermentación alcohólica espontánea. El estudio de estas levaduras *Saccharomyces* y «no-*Saccharomyces*» durante la fermentación del mosto de uvas constituye un área relevante de investigación microbiológica en enología. Si bien existen estudios detallados de la microbiota de uvas de *Vitis vinifera* L., poco se sabe sobre la diversidad de comunidades de levaduras presentes en ecosistemas de *Vitis no-vinifera* (*i.e.*, uvas y mostos en fermentación espontánea). Cepas de levaduras presentes en ecosistemas de *Vitis no-vinifera*, con valor

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potencial científico y/o enológico, podrían no estar presentes en *V. vinifera* L. En esta revisión actualizada, resumimos los aspectos relevantes de los estudios microbiológicos efectuados en mostos en fermentación espontánea de uvas de *V. non-vinifera*.

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Introduction

Microbial communities present during grape must fermentation largely contribute to the sensory and organoleptic characteristics of wines^{4,16}. Even in regular sulfur dioxide-treated must (*i.e.*, used to limit and/or kill the endogenous microbiota and as a protective antioxidant agent), the inoculated yeast starters coexist during fermentation with surviving indigenous non-*Saccharomyces* and *Saccharomyces* yeast, fungi and bacteria species¹⁹, shaping the final sensory and organoleptic profile of the produced beverages. In the case of spontaneous fermentation (*i.e.*, non-sulfited or mild sulfited musts), a challenging and risky winemaking process with potentially unpredictable outputs, the entire indigenous microbial community present in the must conducts the alcoholic fermentation^{11,19,29}. Due to its scientific and industrial importance, the study of indigenous microbial communities in grapes and spontaneously fermenting musts is a major research area in enology^{19,29,42,43}. Thus, different culture-dependent and/or metagenomic approaches, as well as DNA-based strategies, have been used to isolate and identify the complexity and population dynamics of microorganisms in enological ecosystems^{5,26,27}.

Indigenous yeast diversity in *V. non-vinifera* ecosystems

Most of the studies on the microbiota of grapes and fermenting grape musts involve *Vitis vinifera* ecosystems^{3,12,29,39}. Some of these studies suggest that the grape varieties themselves condition the microbial population structure during spontaneous fermentation^{8,13,24,36,39}. Supporting this idea, vineyards cultivating different grape varieties appear to harbor more diverse *Saccharomyces cerevisiae* and non-*Saccharomyces* strains than vineyards cultivating only one grape variety^{13,36}. In addition, it has been observed that particular yeast species show preferences for certain grape varieties (*e.g.*, red or reddish basidiomycetes predominate in white grapes, while equal amounts of ascomycetes and basidiomycetes were observed on red grapes)³². Thus, specific structural and/or general physicochemical grape varietal factors appear to influence the structure and fitness of certain yeast microbiota²⁴. Apparent specific associations between different *Vitis* and yeast species have recently been recognized³⁴ (see below).

Non-*Saccharomyces* are the predominant yeasts isolated at the early stages of the spontaneous fermentation of *V. vinifera* grape musts^{12,19,29}, with the most

important genera being *Hanseniaspora*, *Candida*, *Pichia* and *Metschnikowia*^{19,42}. By mid-fermentation, the population of non-*Saccharomyces* species decreases and the wine yeast *S. cerevisiae* completes the fermentation process¹. A similar pattern of non-*Saccharomyces* and *Saccharomyces* yeast species succession was evidenced during the fermentation of *V. non-vinifera* grapes^{4,6,34}. Baffi et al.⁴ identified *Hanseniaspora uvarum* as the most frequent non-*Saccharomyces* yeast species in the Isabel and Bordeaux varieties of *Vitis labrusca* grapes and must. Additionally, *Issatchenkia occidentalis* was the second and *Issatchenkia orientalis* the third most frequent yeast species isolated from Bordeaux grapes and Bordeaux/Isabel grapes and musts at all stages, respectively⁴. In a later study using Isabel and Bordeaux grapes from the same region⁶, *H. uvarum* was also a dominant yeast species, both on *V. non-vinifera* grape surfaces and at the initial stages of spontaneous fermentation. *Pichia kluyveri* was found at the beginning of fermentation while *I. orientalis* was isolated at the final stages of fermentation⁶. In both studies, *S. cerevisiae* was the most frequent yeast species during the middle and final phases of spontaneous fermentation^{4,6}.

Important differences have been identified in the diversity and identity of non-*Saccharomyces* species isolated in *V. vinifera* and *V. non-vinifera* ecosystems. For example, two independent studies on the *V. non-vinifera* grape varieties Isabel and Bordeaux (*V. labrusca*), found a higher yeast diversity in the Bordeaux grapes than the Isabel grapes, suggesting that yeast diversity might be characteristic of each grape variety^{4,6}. Similar yeast diversity was evidenced in the study of Danish grape varieties²². The hybrid variety 'Leon Millot' (*V. vinifera* and *V. riparia* × *Vitis rupestris*) reveals the same, or even higher, yeast diversity compared to the interspecific varieties (back crossings to *V. vinifera*) Rondo and Zalas Perle²².

Using a standard culture-dependent strategy, the population of non-*Saccharomyces* and *Saccharomyces* yeast species was recently studied on Isabella (*V. labrusca* L.) fermenting grape must in Argentina (*i.e.*, vintage of year 2015)³⁴. The dynamics of the yeast population during spontaneous fermentation of Isabella proved to be similar to that described for *V. vinifera*. *Starmerella bacillaris*, however, was the main yeast species at the early stages of spontaneous fermentation of Isabella must, dramatically decreasing its contribution in the middle and late stages of the process³⁴. This dominance of *S. bacillaris* in the same ecosystem, however, was not observed in fermenting Isabella grapes from vintage of year 2017³³. Additionally, rare non-*Saccharomyces* yeast species were also recognized in Isabella must at the

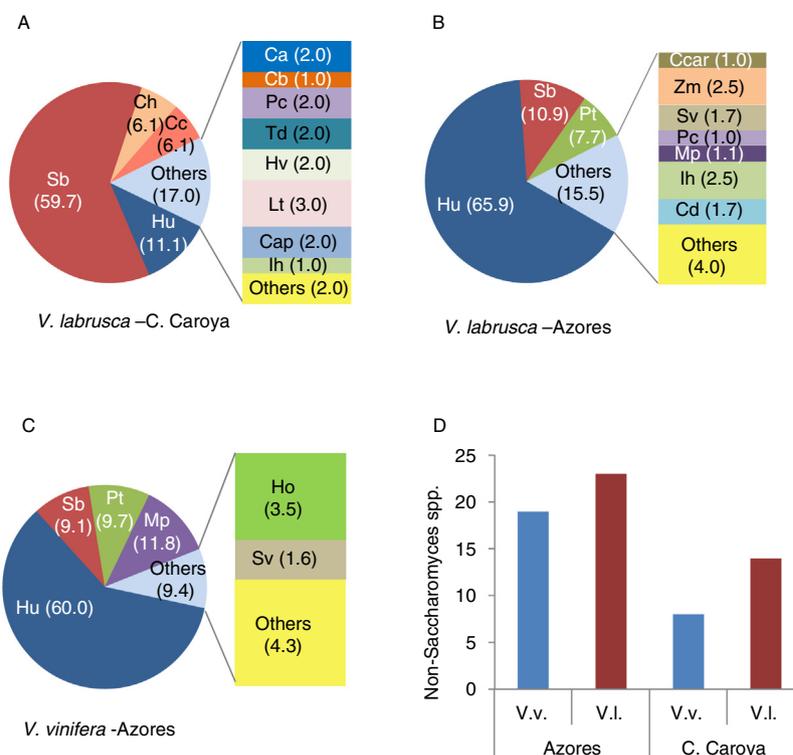


Figure 1 Diversity of non-*Saccharomyces* species isolated from grapes and spontaneously fermenting grape must from *V. vinifera* and *V. labrusca* vineyards from the Azores islands (Portugal) and C. Caroya (Córdoba, Argentina). (A, B, C) Relative contribution of non-*Saccharomyces* yeast species representing more than 1% of the isolates from spontaneously fermenting *V. labrusca* grape must from C. Caroya³⁴ (A) and grapes from *V. labrusca* (B) and *V. vinifera* (C) from the Azores islands^{14,15}. Numbers in parentheses indicate percentages. (D) Total number of non-*Saccharomyces* species identified on *V. vinifera* (blue bars) and *V. labrusca* (brown bars) grapes from the Azores islands (1710 and 3150 isolates, respectively) (data obtained from Refs. 14 and 15 and spontaneously fermenting grape musts from C. Caroya (40 and 100 isolates, respectively) (data obtained from Refs. 34 and 33). Non-*Saccharomyces* species are: *Ca* (*Candida azymoides*), *Cap* (*Candida apicola*), *Cb* (*Candida bentonensis*), *Cc* (*Candida californica*), *Ccar* (*Candida carpophila*), *Cd* (*Candida diversa*), *Ho* (*Hanseniaspora opuntiae*), *Hu* (*Hanseniaspora uvarum*), *Hv* (*Hanseniaspora vineae*), *Ih* (*Issatchenkia hanoiensis*), *Lt* (*Lachancea thermotolerans*), *Mp* (*Metschnikowia pulcherrima*), *Pc* (*Pichia cecembensis*), *Pk* (*Pichia kluyveri*), *Pt* (*Pichia terricola*), *Sb* (*Starmerella bacillaris*), *Sv* (*Saccharomycopsis vini*), *Td* (*Torulaspota delbrueckii*) and *Zm* (*Zygoascus meyeriae*).

initial stages of fermentation, including *Candida azymoides*, *Pichia cecembensis*, *Candida californica*, *Candida bentonensis*, *Issatchenkia hanoiensis* and *Candida apicola* (Fig. 1A). Interestingly, some yeast genera commonly isolated from *V. vinifera* L. grapes and musts¹⁹ (e.g., *Hanseniaspora*, *Torulaspota* and *Metschnikowia*) were rarely identified and almost never dominated the yeast flora in the *V. labrusca* L. must analyzed³⁴. These observations reinforce the research interest in biodiversity and extraordinary wine yeasts in ecological niches alternative to traditional *V. vinifera* ecosystems³⁴.

In the Azores Archipelago, different yeast microbiotas were identified on grapes harvested during vintages of years 2009 and 2010 from active versus abandoned *V. labrusca* vineyards¹⁴ (Fig. 1B) as well as on grapes from *V. vinifera* vineyards¹⁵ (Fig. 1C). In these studies, no apparent associations between grapevine and yeast species were found. Climatic conditions and geographic location seemed to be the underlying causes for the distribution of the predominant yeast species¹⁴. Interestingly, *P. cecembensis* and *C. azymoides* found on these *V. labrusca* grapes, two yeast species not previously recognized in either *V. vinifera* grapes or musts, were also found in the study on *V. labrusca*

L. grapes in Argentina³⁴ (Fig. 1A). These observations strongly suggest that at least these two yeast species are associated with *V. labrusca* L. grapes, regardless of their geographic origin and/or the associated human interventions. Moreover, in both locations *I. hanoiensis*, a yeast species rarely isolated in *V. vinifera* grapes, was also identified in *V. labrusca* grapes^{14,34}. These results suggest that *C. azymoides* and *P. cecembensis* are preferentially associated with *V. labrusca* L. grapes and that specific *Vitis*-microbial interactions may underlie the assembly of specific grapevine yeast communities³⁴. The great diversity of non-*Saccharomyces* species recognized in the *V. labrusca* and *V. vinifera* ecosystems studied by Drumonde-Neves et al.^{14,15} and Raymond Eder et al.³⁴ is illustrated in Fig. 1.

Microbial contributions to the sensory profiles of *V. non-vinifera* fermented beverages

V. non-vinifera species and their hybrids are popular in geographic areas where *V. vinifera* cannot develop properly^{10,18,23,45}. Among these, *V. aestivalis*, *V. labrusca*, *V. riparia* and *V. rotundifolia* are widely used to produce wine,

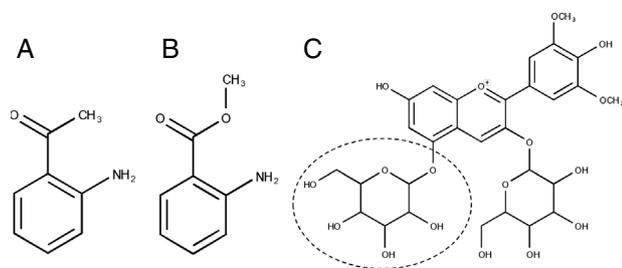


Figure 2 Chemical compounds in *Vitis* spp. 2-aminoacetophenone (A) and methyl anthranilate (B) are related to the perception of foxiness in *V. labrusca* L. wines. Malvidin 3,5-diglucoside (C) is exclusive to wines from *V. non-vinifera*. The glucoside in position 5 is indicated with a circle.

grape juice, table grapes and/or jam^{2,7,18,22,23,31}. Remarkable sensory differences have been observed between wines obtained using *V. vinifera* and *V. non-vinifera* grapes^{7,41}. Some *V. non-vinifera* fermented beverages are recognized as having disadvantages, including lower aroma complexity, high malic acid levels and/or increased amounts of some grape-derived vegetative odorants^{7,23}. Five grape-derived “vegetal, earthy, minty” families of compounds are uniquely linked to some *V. non-vinifera* (i.e., *V. riparia* and *V. cinerea*) wines: eugenol (“clove”-like aroma), 1,8-cineole (also known as eucalyptol), cis-3-hexenol (“leafy-grassy” aroma), IBMP and IPMP (“herbaceous” and “earthy” aromas)⁴¹. Grapes from several American grape cultivars from *V. labrusca* (e.g., Catawba, Concord, Delaware, Isabella, Niagara, as well as some hybrids such as Agawam, Alexander and Onaka) are referred to as “foxy grapes” due to their intense fruity and/or artificial grape aroma/flavor notes in their wines^{30,41}. The term “foxy” is used to describe a “unique, earthy and sweet muskiness” that can be perceived in these grapes. The presence of 2-aminoacetophenone and methyl anthranilate (Fig. 2A and B, respectively) is related to the perception of foxiness in *V. labrusca* grapes^{2,7,41,43}.

In addition to these grape-derived compounds, specific yeast fermentation-derived products, such as volatile phenols, furans and esters, are responsible for some flavor differences between *V. non-vinifera* and *V. vinifera* wines^{35,44}. Some disadvantages of *V. non-vinifera* wines have partially been remedied using alternative yeast and/or bacterial starters^{17,18,37,46}. For example, an acidophilic *I. orientalis* strain, isolated from Korean Campbell Early grape pomace, has been shown to use malic acid efficiently as the sole carbon source³⁷. In mixed fermentations with *S. cerevisiae* W-3 (industrial wine yeast), this *I. orientalis* strain efficiently degraded malic acid of Campbell Early grape must, without significantly influencing alcohol fermentation²⁰. Additionally, an improvement in wine color was observed in these fermented mixed cultures compared to grape musts fermented with *S. cerevisiae* alone²⁰. When the same *I. orientalis* yeast cells were immobilized on oriental oak charcoal and alginate, a 91.6% reduction of malic acid content was observed after 30h treatment of Campbell Early wine¹⁷. In these treatments, however, a decrease in the color of the wine was observed¹⁷. Interestingly, the use of an indigenous *H. uvarum* strain

starter, isolated from spontaneously fermenting Campbell Early grape musts, also improved the sensory profile of Campbell Early wine¹⁸. It has been shown that grapes from *V. non-vinifera* cultivars normally do not reach high total reducing sugar levels, leading to fermented beverages with lower levels of alcohol^{18,35} than *V. vinifera* L. wines. Lower levels of ethanol (~1% v/v) observed at the end of fermentation of Isabella (*V. labrusca*) grape musts, compared to the expected values based on the initial concentrations of total reducing sugars, have repeatedly been observed^{33,34}.

In addition to alternative yeasts, dual starters of *S. cerevisiae* and *Oenococcus oeni* have been used to attempt to reduce the *V. non-vinifera* wine acidity of Campbell Early musts⁴⁷. Although the use of commercial *O. oeni* starters for malolactic fermentation did not result in a significant change of the organic acid profiles, improvements were found in the sensory characteristics of the wines (i.e., higher levels of volatile compounds and an increased synthesis of esters and higher alcohols)⁴⁷.

In a remarkable study, Son et al.³⁸ studied wines obtained with grapes from four different *V. non-vinifera* cultivars in Korea (i.e., Muscat Bailey A –*V. labrusca*-, Campbell Early –*V. labrusca* B.-, Kyoho –*V. labrusca* L.- and Merou –*V. coignetiae*-). As the same starter (i.e., *S. bayanus*) and fermentation conditions were used, this study highlighted the specific characteristics of each of the *V. non-vinifera* grape varieties analyzed. L-proline was noticed as an important metabolite for grape variety differentiation³⁸, as it is relatively non-assimilable by yeast under anaerobic conditions⁴⁰. Anthocyanin profiles, among polyphenols, have also been used for grape varietal differentiation^{9,21,25,35}. In *V. vinifera* red cultivars, only cyanidin, delphinidin, petunidin, peonidin and malvidin 3-monoglucosides (Fig. 2C) occur along with the corresponding acetyl, *p*-coumaroyl and caffeoyl derivatives. In *V. non-vinifera* (i.e. *V. labrusca*, *V. rotundifolia* and their hybrid grapes), on the other hand, glycosylation of these compounds at both positions 3 and 5 is common²⁵ and the presence of malvidin diglucoside (malvidin-3,5-diglucoside) (Fig. 2C) allows recognition of *V. non-vinifera*-derived wines. Wines produced with grapes from some *V. non-vinifera* species may contain high levels of this diglucoside, with 15 mg/l being the maximum acceptable limit according to the international code of enological practices of the OIV (International Organization of Vine and Wine)²⁸.

Conclusions

Extensive research has been conducted on the microbiological communities present in *V. vinifera* L. enological ecosystems as well as the sensory and organoleptic properties of *V. vinifera* L. wines. The few studies conducted on non-*vinifera* *Vitis* ecosystems, however, have identified several chemical, sensory and microbiological characteristics of these fermented beverages with potential interest in enology. Among these characteristics are a great diversity of non-*Saccharomyces* yeasts which may carry fermentation assets of winemaking importance. The apparent specific associations observed between different yeasts and *Vitis* species suggest that some yeast strains may be exclusive to non-*vinifera* *Vitis* ecosystems. Physical and chemical

determinants may favor specific biological interactions between different species of grapes and yeasts, allowing the assembly of specific grapevine microbiotas. In addition to the biological interest of the spontaneous assembly of microbial communities on fruits and plants, the enological microbial ecosystems of *V. non-vinifera* may allow to recognize strains of yeasts or bacteria of interest in enology.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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