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 CLAVES

Levaduras;
Saccharomyces;
Vitis vinifera L.;
Vitis 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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Introduction

Microbial communities present during grape must fermentation largely contribute to the sensory and organoleptic characteristics of wines. 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 species, 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 outcomes, the entire indigenous microbial community present in the must conducts the alcoholic fermentation. 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. 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.

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. Some of these studies suggest that the grape varieties themselves condition the microbial population structure during spontaneous fermentation. 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. 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). Thus, specific structural and/or general physicochemical grape varietal factors appear to influence the structure and fitness of certain yeast microbiota. Apparent specific associations between different Vitis and yeast species have recently been recognized (see below).

Non-Saccharomyces are the predominant yeasts isolated at the early stages of the spontaneous fermentation of V. vinifera grape musts, with the most important genera being Hanseniaspora, Candida, Pichia and Metschnikowia. By mid-fermentation, the population of non-Saccharomyces species decreases and the wine yeast S. cerevisiae completes the fermentation process. A similar pattern of non-Saccharomyces and Saccharomyces yeast species succession was evidenced during the fermentation of V. non-vinifera grapes. Baffi et al. identified Hanseniaspora uvarum as the most frequent non-Saccharomyces yeast species in the Isabel and Bordeaux varietals of Vitis labrusca grapes and must. Additionally, Issatchenkia orientalis 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, respectively. In a later study using Isabel and Bordeaux grapes from the same region, H. uvarum was also a dominant yeast species, both on V. non-vinifera grape surfaces and at the initial stages of spontaneous fermentation. Pichia kluyster was found at the beginning of fermentation while I. orientalis was isolated at the final stages of fermentation. In both studies, S. cerevisiae was the most frequent yeast species during the middle and final phases of spontaneous fermentation.

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. Similar yeast diversity was evidenced in the study of Danish grape varieties. 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 Perle.

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). 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 process. This dominance of S. bacillaris in the same ecosystem, however, was not observed in fermenting Isabella grapes from vintage of year 2017. Additionally, rare non-Saccharomyces yeast species were also recognized in Isabella must at the potential 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. no-vinifera.

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

In the Azores Archipelago, different yeast microfloras were identified on grapes harvested during vintages of years 2009 and 2010 from active versus abandoned *V. labrusca* vineyards (Fig. 1B) as well as on grapes from *V. vinifera* vineyards (Fig. 1C). In these studies, no apparent associations between grapevine and yeast species were found. Climatic conditions and geographic location seem to be the underlying causes for the distribution of the predominant yeast species. 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 Argentina (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. These results suggest that *C. azymoides* and *P. cecembensis* are preferentially associated with *V. labrusca* L. grapes and that specific Vitismicrobial interactions may underlie the assembly of specific grapevine yeast communities. The great diversity of non-*Saccharomyces* species recognized in the *V. labrusca* and *V. vinifera* ecosystems studied by Drumonde-Neves et al. and Raymond Eder et al. 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. Among these, *V. aestivalis*, *V. labrusca*, *V. riparia* and *V. rotundifolia* are widely used to produce wine,
Yeast diversity in _Vitis non-vinifera_ ecosystems

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**Figure 2** Chemical compounds in _Vitis_ spp. 2-aminoacetoephone (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.

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grape juice, table grapes and/or jam\textsuperscript{2,7,18,22,23,31}. Remarkable sensory differences have been observed between wines obtained using _V. vinifera_ and _V. non-vinifera_ grapes\textsuperscript{2,21}. 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 odors\textsuperscript{2,23}. 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)\textsuperscript{3,4}. 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\textsuperscript{2,41}. The term "foxy" is used to describe a "unique, earthy and sweet muskiness" that can be perceived in these grapes. The presence of 2-aminoacetoephone and methyl anthranilate (Fig. 2A and B, respectively) is related to the perception of foxiness in _V. labrusca_ grapes\textsuperscript{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\textsuperscript{38,44}. Some disadvantages of _V. non-vinifera_ wines have partially been remedied using alternative yeast and/or bacterial starters\textsuperscript{17,18,37,46}. For example, an acido philic _I. orientalis_ strain, isolated from Korean Campbell Early grape pomace, has been shown to use malic acid efficiently as the sole carbon source\textsuperscript{17}. 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 fermentation\textsuperscript{19}. Additionally, an improvement in wine color was observed in these fermented mixed cultures compared to grape musts fermented with _S. cerevisiae_ alone\textsuperscript{19}. 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 30 h treatment of Campbell Early wine\textsuperscript{21}. In these treatments, however, a decrease in the color of the wine was observed\textsuperscript{21}. 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 wine\textsuperscript{19}. 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\textsuperscript{39,35} 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\textsuperscript{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 musts\textsuperscript{47}. 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)\textsuperscript{49}.

In a remarkable study, Son et al.\textsuperscript{38} studied wines obtained with grapes from four different _V. non-vinifera_ cultivars in Korea (i.e., Muscat Bailey A \(\sim V. labrusca\)-, Campbell Early \(\sim V. labrusca\)-B., Kyoho \(\sim V. labrusca\)-L. and Merou \(\sim V. coignetiae\)-). 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 differentiation\textsuperscript{38}, as it is relatively non-assimilable by yeast under anaerobic conditions\textsuperscript{40}. Anthocyanin profiles, among polyphenols, have also been used for grape varietal differentiation\textsuperscript{2,41,25,35}. 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 common\textsuperscript{25} 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)\textsuperscript{38}.

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**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- _V. 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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**References**


Yeast diversity in *Vitis* non-*vinifera* ecosystems


