Yeast communities of Nova Scotia wine grapes: characterization and implications for winemaking

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Abstract

Wine grapes host a community of yeasts that reflects the unique geography, climate, and management of a vineyard. After grapes are crushed to produce must, a dynamic succession of yeasts takes place as fermentation unfolds. Initially, many basidiomycete and ascomycete species may be abundant, before one or few fermentative ascomycete yeasts, most often *Saccharomyces cerevisiae* (Desm.) Meyen 1838, become dominant and complete the fermentation. However, all yeasts contribute to the chemistry of a fermentation via interactions with each other and by using nutrients, secreting enzymes, and producing aromatic compounds. The cumulative result is the distinctive aroma and body of the wine. Despite the importance of yeast communities during fermentation, wine grape yeasts in Nova Scotia have yet to be evaluated. This emerging wine region generates >$245 M/year, supports >1100 jobs, and stimulates tourism. Therefore, the yeast communities of L'Acadie blanc grapes, a cool-climate hybrid cultivar, were characterized using high throughput sequencing. Considering rising demand for sustainable products, vineyards using organic and conventional cultivation practices were sampled. Yeast communities in musts were composed of predominantly basidiomycete yeasts and were significantly different among vineyard sites and between cultivation practices. One organic vineyard was selected for further analysis using two sequencing platforms (Illumina MiSeq and PacBio) to address biases and evaluate changes following spontaneous fermentation. Both *S. cerevisiae* and *Saccharomyces uvarum* Beij. 1898 were found to complete fermentations, but discrepancies in the proportions of these and *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak and Phaff ex MT Sm 1956 were detected between the sequencing systems. Finally, considering their abundance, a review of wine grape associated basidiomycete yeasts and their potential applications in winemaking was conducted, highlighting known effects on wine aroma. Understanding the complexity of yeast diversity in wine musts and during fermentation can inform both vineyard management and winemaking.

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List of abbreviations and symbols

°C:	degree(s) Celsius
AAFC:	Agriculture and Agri-Food Canada
ADP:	adenosine diphosphate
ANOVA:	analysis of variance
ATP:	adenosine pyrophosphate
ASV:	amplicon sequence variant
AVA:	American Viticultural Area
bp:	base pair
BLAST:	basic local alignment search tool
CBA:	Canadian Botanical Association
CCOVI:	Cool Climate Oenology and Viticulture Institute
CO_2:	carbon dioxide
CSEE:	Canadian Society for Ecology and Evolution
CYTB:	cytochrome *b*
DGGE:	denaturing gradient gel electrophoresis
DNA:	deoxyribonucleic acid
dNTP:	deoxyribose nucleoside triphosphate
ETS:	external transcribed
g:	gram
Gb:	giga-base pair (1 000 000 000 bp)
GDD:	growing degree days
ha:	hectare
ID:	identification
IGS/NTS:	intergenic spacer/non-transcribed spacer
Illumina:	Illumina cyclic reversible termination
ITS:	internal transcribed spacer
kb:	kilo-base pair (1000 bp)
kg:	kilogram
km:	kilometre
L:	litre
LM:	lysine nutrient medium
LSU:	large sub-unit
M:	million
mg:	milligram
ml:	millilitre
MSA:	Mycological Society of America
MSVU:	Mount Saint Vincent University
n:	nucleic
NAD^+:	nicotinamide adenine dinucleotide (oxidized)
NADH:	nicotinamide adenine dinucleotide (reduced)
NCBI:	National Center for Biotechnology Information
NMDS:	non-metric multidimensional scaling
NS:	Nova Scotia
OH:	hydroxyl group
OTU:	operational taxonomic unit
PacBio:	Pacific Biosciences single-molecule real-time
PCR:	polymerase chain reaction
PERM:	permutational
q:	quantitative
r:	ribosomal
R^2:	coefficient of determination
RFLP:	restriction fragment length polymorphism
RNA:	ribonucleic acid
RPD:	root primordium defective
s:	second
SE:	standard error
SMU:	Saint Mary's University
SO_2:	sulfur dioxide
SRA:	sequence read archive
SSU:	small sub-unit

t:	terminal
TDN:	1,1,5-trimethyl-1,2-dihydronaphthalene
TEF1:	translation elongation factor 1-α
TGGE:	temperature gradient gel electrophoresis
UBC:	University of British Columbia
UK:	United Kingdom
USA:	United States of America
V:	vineyard
VQA:	Vintners Quality Alliance
WL:	Wallerstein laboratory nutrient medium
$\bar{x}$:	mean
YEPD/YPD:	yeast extract peptone dextrose nutrient medium

Thesis preface

Content and formatting

This thesis consists of a general introduction (Chapter 1) which provides background information to justify the research and orient the reader, two data-driven chapters which assess yeast communities in Nova Scotia wine grapes, comparing among vineyards and between cultivation practices (Chapter 2), and before and after spontaneous fermentation and between two sequencing platforms (Chapter 3), a review (Chapter 4) of the presence, roles, and potential applications of basidiomycete yeasts in winemaking, in light of their abundance in the preceding chapters, and a conclusion (Chapter 5) which synthesizes the achievements of this research and describes the aspects that contribute to application and knowledge transfer. Chapter 3 considers the vineyard designated V7 in Chapter 2.

Chapter 2 has been accepted for publication in the Journal of Applied Microbiology and Chapter 3 was published in the Canadian Journal of Microbiology (references follow). These chapters are included as they were formatted for publication except for minimal changes to stylistic formatting to maintain consistency throughout the thesis, the use of unified reference section for the thesis in lieu of the individual reference sections included in each publication, and minor clarifications made upon review by the examining committee. Further specific changes are described in the prefaces of Chapters 2 and 3. Chapter 4 is also intended for publication, but as it has not yet been submitted, it appears here formatted to suit the thesis.

Chapter 2

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2024. Yeast communities of a North American hybrid wine grape differ between organic and conventional vineyards. J Appl Microbiol. lxae092. doi:10.1093/jambio/lxae092.

Chapter 3

Bunbury-Blanchette AL, Fan L, English MM, Kernaghan G. 2023. Yeast communities before and after spontaneous fermentation of wine grapes: a case study from Nova Scotia. Can J Microbiol. 69(1):32-43. doi:10.1139/cjm-2022-0179.

Terminology

All yeast species names were updated from the source material to reflect current taxonomy (Table A1). Exceptions are the use of *Rhodotorula nothofagi* rather than *Curvibasidium nothofagi* in Chapter 3, and *Cryptococcus festucosus* instead of *Holtermanniella festucosa* in Chapter 2, which reflect the taxonomy at the time of writing and in the preparation of those chapters for publication. The status of the genus *Holtermanniella* is indeterminate, but it is otherwise used in this thesis according to Takashima and Sugita (2022).

"Cultivar" is used more often than "variety" to reflect the domestication and breeding of grapevines, but the terms should be considered interchangeable in cases where "variety" is used to best reflect the wording of a reference.

There is considerable overlap in the terms "flavour" and "aroma" in the context of wine attributes. For simplicity, "aroma" is often used in place of "flavour and aroma" or "flavour". "Flavour" is used in some cases in Chapters 2 and 3 when it was decided to be the ideal wording for publication, and throughout the thesis when it best reflects the wording of a reference.

Many studies that consider fungal communities associated with wine grapes, wine musts, or wine fermentations do not distinguish the yeast community from the larger fungal community present. In cases in which I use "yeast community" even when non-yeast fungi were included in

the community as described by the source material, yeasts were present in substantial proportions, and/or I specify the presence and effects of yeasts.

I use “natural” to reflect any non-specific combination of practices that might be perceived as natural, and to describe the wines made using those practices. I use “natural wine” in reference to the definition provided by Legeron (2020) (section 1.54).

The terms “next generation sequencing” and “high throughput sequencing” should be considered interchangeable in this thesis. “High throughput sequencing” is used preferentially in Chapters 1, 4, and 5, reflecting its more accurate and unambiguous nature, while “next generation sequencing” was used at the time of writing and in the publication of Chapters 2 and 3, and remains in the text of these chapters.

The terms “system” and “platform” are used interchangeably in the literature concerning high throughput sequencing technologies (e.g., in Shendure and Ji 2008; Liu et al. 2012; Goodwin et al. 2016; Slatko et al. 2018; Kumar et al. 2019), although “platform” is used more often. In this thesis the terms are also used interchangeably – “system” is used more often in Chapter 3 and when exclusively discussing PacBio sequencing, to reflect this company’s use of the term (PacBio 2024), while “platform” is otherwise used more often.

Chapter 1: Introduction

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1.1 Yeasts in winemaking

1.11 Overview of winemaking

By the most general definition, wine is a beverage made of fermented fruit, most often grapes. To make wine, grapes must be harvested, prepared for fermentation, and allowed to ferment. Further clarification, purification, and aging steps may also take place before bottling, depending on the style of wine desired (Vernhet 2019). The harvest of grapes ideally occurs according to the monitoring of grape characteristics such as ripeness, level of sugar (°Brix), pH, and tannin development, but must also consider external factors like weather (Río Segade et al. 2019; Rolle et al. 2022). Grapes may be harvested and sorted by hand or mechanically, the latter being more economical but less discriminatory (Río Segade et al. 2019)

Grapes may be destemmed in preparation for fermentation, depending on grape cultivar and desired wine outcome, prior to being crushed (Guerrini et al. 2022). Crushing breaks the skins of the berries and releases their contents but does not separate the juice from the solids. The goal of crushing is to allow the skins, pulp, seeds, juice, and stems (if present) to steep together; this mixture is called the must. Depending on the style of wine, the processes of crushing and pressing, which extracts the juice and leaves behind the solids, may take place concurrently before the primary fermentation begins, as is usual for white wines (Del Fresno et al. 2022). Alternatively, grapes may be pressed later, as is usual for red wines, and primary fermentation begins, and may even complete, in the intervening hours or days (Morata et al. 2019). Sulfur dioxide (SO_2) is often added just prior to fermentation, in part to kill undesired spoilage yeasts such as *Brettanomyces bruxellensis* (Giacosa et al. 2019).

In commercial wineries, fermentation generally takes places in large stainless-steel tanks, although other containers including clay vessels, oak barrels or vats, or concrete tanks may be

used (Morata et al. 2019). Yeasts are the main fermentative agents of wine and most commercial wineries inoculate the primary fermentation by adding a commercially developed live dry yeast product to ensure consistent results, although fermentation will generally also occur without inoculation (Ciani et al. 2004). *Saccharomyces cerevisiae* is the yeast responsible for bringing most wine fermentations to completion. It efficiently converts sugars to ethanol, is tolerant of low oxygen, high SO_2, and high ethanol, and will outcompete other yeasts present during the fermentation (Fleet 2008). Hundreds of *S. cerevisiae* strains have been identified and most modern, commercial wineries use one or more strains of *S. cerevisiae* as inoculum to ensure successful and consistent fermentations (Marsit and Dequin 2015).

However, regardless of whether a fermentation is inoculated, many yeast species and strains, including strains of *Saccharomyces*, are present and abundant in the must and earlier stages of fermentation (Jolly et al. 2006). During primary fermentation, the population of highly fermentative yeasts (e.g., species of *Lachancea*, *Saccharomyces*, and *Torulaspora*) grows quickly, and most of the sugars are rapidly converted to alcohol (Fleet 2008; Borren and Tian 2021). There is not a definitive point at which the primary fermentation ends, but after three to five days, the yeast activity will slow and the wine can be considered to have begun its secondary fermentation, which will continue until all the sugars have been converted to alcohol. The fermentation may be moved to another vessel for its secondary fermentation, such as the transfer from a vat to an oak barrel, or in the case of sparkling wine, to bottles. Malolactic fermentation, in which malic acid is converted to lactic acid, most often by the bacteria *Oenococcus oeni*, may also be referred to as a secondary fermentation, especially when a secondary inoculation of malolactic bacteria is performed (Gil-Sánchez et al. 2019).

1.12 Brief historical perspective on yeast fermentation research

Wine has been made by humans for thousands of years, long before there was any understanding of the chemistry or microbiology of fermentation (McGovern et al. 2003). The first milestones leading to current oenological research include the observation of yeast cells by Antonie van Leeuwenhoek in 1680, early descriptions of chemical fermentation by Antoine Lavoisier and Joseph Gay-Lussac in the late 18th and early 19th centuries, and subsequent discoveries concerning fermentative yeast: reproduction by budding, confirmation that yeast and bacteria are responsible for fermentation, the recognition that yeast are fungi, the identification of *Saccharomyces*, and improved yeast cell isolation methods (Chambers and Pretorius 2010). These achievements helped to establish that yeasts are polyphyletic, unicellular fungi. Yeasts of the order Saccharomycetales, including *S. cerevisiae*, are considered "true" yeasts, characterized by asexual reproduction by budding. Eight *Saccharomyces* species and two natural hybrids are currently recognized, hundreds of *S. cerevisiae* strains have been identified, and a multitude of other yeast species and strains have been identified from grape musts that may contribute to fermentation (Jolly et al. 2014; Borneman and Pretorius 2015; Alsammar and Delneri 2020).

By the end of the 19th century, scientific knowledge of fermentative yeasts was being directly applied by winemakers and researchers. In 1890, Hermann Müller-Thurgau proposed the inoculation of musts with selected pure yeast cultures, a technique that was not widely implemented until the 1970's but is now the most common method of fermentation for producing commercial wines (Chambers and Pretorius 2010; Marsit and Dequin 2015). Additional research milestones included the description of the glycolytic pathway and discovery of the mating types of *S. cerevisiae* (Lindegren CC and Lindegren G 1943; Chambers and Pretorius 2010). The emergence of molecular biology in the 1970's led to the establishment of *S. cerevisiae* as a model

organism following use as a vector for foreign DNA, and *S. cerevisiae* was the first eukaryotic organism to have its genome sequenced (Beggs 1978; Hinnen et al. 1978; Goffeau et al. 1996). This centuries-in-the-making body of research has made *S. cerevisiae* one of the best understood organisms in all aspects of its biology.

For example, the mechanism by which *S. cerevisiae* ferments sugar into CO_2 and alcohol is well known. The yeast will split a six-carbon glucose into two three-carbon pyruvates and use the resulting energy to convert ADP to ATP and NAD^+ to NADH. The pyruvates break down to two acetaldehyde molecules, releasing CO_2. Under anaerobic conditions, the yeast will further reduce acetaldehyde to ethanol, and a lesser amount of acetic acid, via the oxidation of NADH. The factors that most impact the progression of wine fermentation are the sugar content of the must, the strain(s) of yeasts (and bacteria), fermentation temperature, level of oxygen present at the start of fermentation, the fermentation vessel, and nutrient levels in the must (Fleet 2003; Marsit and Dequin 2015). During fermentation, yeasts, including *S. cerevisiae*, produce a variety of secondary metabolites, dependent on strain(s), that influence wine taste and mouthfeel (Fleet 2003).

1.13 Yeast population dynamics during wine fermentation

Many diverse non-*Saccharomyces* yeasts are abundant during the early stages of fermentation, although most do not persist to the completion of fermentation due to intolerance to low oxygen levels, high SO_2 levels, high ethanol concentration, or competition with *S. cerevisiae* (Ciani et al. 2010). These yeasts are generally termed “non-fermentative”, “non-*Saccharomyces*”, or “non-traditional”, despite having varying levels of fermentative capacity and contributing to the characteristics of the wine. Those most often identified from grape musts belong to the genera *Aureobasidium*, *Candida*, *Cryptococcus* (in original citations, now

reclassified *Filobasidium*, *Vishniacozyma*, and other genera), *Hanseniaspora*, *Lachancea*, *Metschnikowia*, *Naganishia*, *Pichia*, *Rhodotorula*, *Sporobolomyces*, *Starmerella*, *Torulaspora*, and *Zygosaccharomyces*, along with *Saccharomyces* species other than *S. cerevisiae* (Jolly et al. 2014; Capozzi et al. 2015; Borren and Tian 2021). As fermentation continues, there is an increasing abundance of one or more yeasts with strong fermentative capacity, most often *S. cerevisiae* but sometimes *Torulaspora* or *Lachancea*, until no or few other yeast species remain (Fleet et al. 1984; Heard & Fleet 1985; Borren and Tian 2021). These general yeast population fermentation dynamics occur in both inoculated fermentations and in fermentations in which no yeasts are added (termed "spontaneous fermentations"), although non-*Saccharomyces* yeasts seem to be more likely to remain active for a greater length of time in spontaneous than inoculated fermentations.

For example, Torija et al. (2001) sampled musts and wines at three points during spontaneous fermentation and found non-*Saccharomyces* yeasts were most abundant in the must and earlier stage of fermentation while *Saccharomyces* was most abundant in the later stages of fermentation. However, some non-*Saccharomyces* species persisted into the later stages, albeit at lower concentrations, as well as a large variety of non-dominant *S. cerevisiae* strains (Torija et al. 2001). Similarly, Combina et al. (2005a) sampled three spontaneous fermentations frequently to find that the non-*Saccharomyces* yeasts *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, and *Starmerella stellata* were most common in musts, along with other, somewhat less abundant, non-*Saccharomyces* species. These yeasts continued to proliferate during the first eight days of fermentation, while ethanol levels remained below 6-7%, before being replaced by *S. cerevisiae* (Combina et al. 2005a). Bougreau et al. (2019) also found that non-*Saccharomyces* yeasts dominated early spontaneous fermentations, namely *Aureobasidium pullulans*, *Hanseniaspora*

sp. and *Lachancea thermotolerans*, and that of these, *Hanseniaspora* sp. and *L. thermotolerans* persisted until mid-fermentation, with *L. thermotolerans* remaining as the dominant species until day 15 in some batches before *Saccharomyces* completed the fermentation (Bougreau et al. 2019).

As spontaneous fermentations may allow a more diverse community of yeasts to exist longer throughout the fermentation than in inoculated fermentations, it is reasonable to expect that these yeasts could exert a stronger influence on the resulting wine than if *S. cerevisiae* had been added (Ciani et al. 2004; Čuš et al. 2017; Lu et al. 2020). This effect may be even stronger in fermentations without the addition of SO_2 (Sumby et al. 2021). However, even inoculated fermentations experience effects linked to the initially diverse community of must inhabiting yeasts, along with the additional influences of the chosen inoculant(s).

1.14 Metabolic influence of yeasts on wine characteristics

While the aroma, mouthfeel, and appearance of a wine are determined by factors such as grape cultivar, winemaking methods, and overall microbial activity during fermentation (each of which can be broken down into additional interacting sub-factors), yeast metabolism plays an essential role. Depending on cultivar attributes such as sugar level and winemaking variables such as temperature, yeasts produce a variety of volatile compounds during fermentation (Bordet et al. 2020), the effects of which have been extensively reviewed and are summarized here from Lambrechts and Pretorius (2000), Swiegers et al. (2005), and He et al. (2023):

The key components that make up wine aroma are acidity, levels of glycerol and alcohol, and concentrations of aldehydes, volatile phenols, esters, sulfur compounds (sulfides, polysulfides, heterocyclic compounds, thioesters, and thiols), and terpenoid compounds. The overall acidity is composed of non-volatile components, including the pleasant tartness of tartaric

and malic acids (considered a sour and spoiled attribute if concentrations are too high), and volatile acids such as acetic, propionic, hexanoic, octanoic, decanoic and tridecanoic acids. The volatile acids also contribute tartness, and may modulate other characteristics of the wine, although high levels result in negative pungent, vinegar, rancid, cheesy, or fatty aromas. The alcoholic component of wine is composed of ethanol, which may enhance other aromas but is overwhelming at high levels, and higher alcohols (having two or more carbons and a higher molecular weight and boiling point than ethanol), which may contribute a variety of pleasant or pungent aromas. The higher alcohols common in wine include isoamyl alcohol, propanol, isobutyl alcohol, and phenethyl alcohol. Glycerol contributes a slightly sweet flavour and may impact the smoothness and fullness of mouthfeel. Aldehydes may contribute a variety of pleasant aromas, although high levels result in negative pungent, rancid, acrid, or musty attributes. Common aldehydes are acetaldehyde (green apple aroma), diacetyl (buttery or butterscotch aroma), benzaldehyde (almond aroma), propanal (apple aroma), and pentanal (cocoa or coffee aroma). Common volatile phenols may be unpleasant, such as the medicinal and farmyard aromas of 4-vinylphenol and 4-ethylphenol, respectively, but some, such as vinylguiacol may be positive (smoky, vanilla or clove aroma). Esters often contribute a variety of pleasant attributes, for example, the fruity aromas of ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl hexanoate, and floral and honey aromas of 2-phenylethyl acetate. Many others provide attributes distinctive to certain wines, such as ethyl anthranilate, which provides the sweet, fruity, grape aroma of Pinot noir wines. Sulfur compounds such as some thiols may contribute positive attributes (e.g., furfurylthiol provides a toasty aroma in Bordeaux wines, and 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol and 3-mercapto-hexyl acetate provide distinctive box tree, passionfruit, grapefruit, gooseberry, and guava aromas in Sauvignon blanc wines), but many are primarily

negative, such as hydrogen sulfide (rotten eggs aroma), dimethyl sulfide and disulfide (vegetable aromas), and ethyl and methyl mercaptan (onion, rubber, rotten eggs, or cabbage aromas). Terpenoid compounds generally contribute floral or fruity characteristics, such as the rose and geranium aromas of nerol and geraniol.

Although there is variation among strains (Romano et al. 2003; Bisson and Karpel 2010; Capece et al. 2012; Scacco et al. 2012), commercial *S. cerevisiae* inoculum will generally result in predictable aroma formation outcomes (Comitini et al. 2011; Capozzi et al. 2015). For example, many *S. cerevisiae* strains produce relatively high levels of isoamyl alcohol without exceeding an upper threshold (Romano et al. 2003; Scacco et al. 2012; Capozzi et al. 2015). Most *S. cerevisiae* fermentations result in consistent levels of acetic acid, acetaldehydes, and various desirable esters and sulfur compounds, all within acceptable limits (Scacco et al. 2012; Capozzi et al. 2015). Also, wine strains of *S. cerevisiae* almost universally demonstrate SO_2 resistance and so are unaffected by the early addition of SO_2 to decrease levels of undesirable microorganisms (Comitini et al. 2011).

Yeasts other than *S. cerevisiae* were traditionally thought to produce an excess of compounds that negatively impact wine character, but a variety of ascomycete yeasts are increasingly being developed for use in inoculations (Capozzi et al. 2015). Inoculum may be composed of a single yeast strain or a mix of two or strains (mixed inoculum). Mixed inoculations generally incorporate *S. cerevisiae* and may occur by adding the strains simultaneously, typically using a higher proportion of the yeast with the weaker fermentative metabolism, or the inoculation may be sequential, in which the non-*Saccharomyces* strain is added some hours or days before the addition of *Saccharomyces* (Wang et al. 2023b). Both

methods of mixed inoculation may be manipulated to achieve a variety of outcomes (Bordet et al. 2020; Wang et al. 2023b).

Indeed, the effects and interactions of alternate yeasts in wine fermentations may be used to improve aspects of wine character (Jolly et al. 2014; Lappa et al. 2020; Borren and Tian 2021), although strains of non-*Saccharomyces* yeast species can vary significantly in their production of secondary compounds during fermentation, thereby having variable effects on wine, meaning that results may vary within a species (Capozzi et al. 2015; Rossouw and Bauer 2016). Variable production of compounds depending on strain and inoculation methods can be advantageous, however, allowing for a wide range of effects to be exploited by winemakers (Capozzi et al. 2015; Sumby et al. 2021). These effects are generally known from mixed inoculum fermentations, although the production of various secondary compounds has also been evaluated in individual culture for some yeasts.

At an optimum ratio with *S. cerevisiae*, inoculation with *Torulaspora delbrueckii* may produce wines with a desirable reduction in acetic acid (Bely et al. 2008; Renault et al. 2009), as may *Zygosaccharomyces florentina* (Domizio et al. 2011). *Hanseniaspora osmophila*, *Hanseniaspora guilliermondii* and *Wickerhamomyces anomalus* produce acetate esters, including 2-phenylethyl acetate and isoamyl acetate in pure culture (Rojas et al. 2001; Viana et al. 2008), and, along with *H. uvarum* and *Hanseniaspora vineae*, also increase acetate ester formation in mixed inoculum fermentations (Rojas et al. 2003; Moreira et al. 2005; Moreira et al. 2008; Viana et al. 2009; Rossouw and Bauer 2016). Increased production of favourable esters is similarly a result of mixed inoculations using *M. pulcherrima* (Rodríguez et al. 2010; Sadoudi et al. 2012), *L. thermotolerans* (Whitener et al. 2017), and *T. delbrueckii* (Renault et al. 2015). Higher alcohols, which also have a substantial impact on wine aroma, are produced in a range of

concentrations by *H. guilliermondii*, *H. uvarum* and *T. delbrueckii*, known from growth in synthetic media (Romano et al. 1992) as well as mixed inoculum wine fermentations (Herraiz et al. 1990). Comprehensive testing of higher alcohol production by non-*Saccharomyces* yeasts, including those previously listed as well as *W. anomalus*, *Starmerella bacillaris*, *Kurtzmaniella quercitrusa*, *Pichia kluyveri*, *H. vineae*, *H. uvarum*, *Candida railenensis*, *M. pulcherrima*, has found variable production of higher alcohols depending on single vs mixed inoculation with *S. cerevisiae* (Lee et al. 2019). Relatively high levels of glycerol may also be produced by many non-*Saccharomyces* yeasts (Rossouw and Bauer 2016), including *L. thermotolerans* (Kapsopoulou et al. 2007; Comitini et al. 2011; Gobbi et al. 2013).

Non-*Saccharomyces* yeasts generally also produce substantially higher levels of extracellular enzymes than *Saccharomyces* species, and these can impact the production of many of the compounds responsible for wine character, thus indirectly affecting wine stability, quality, and aroma (Strauss et al. 2001; Capozzi et al. 2015). Isolates of *Candida*, *Hanseniaspora*, *Lachancea*, *Metschnikowia*, *Pichia*, and *Torulaspora* may produce high levels of a wide variety of enzymes including amylases, cellulases, esterases, pectinases, proteases, or xylanases (Charoenchai et al. 1997; Dizy and Bisson 2000; Strauss et al. 2001; Comitini et al. 2011; Maturano et al. 2012; Capozzi et al. 2015), while glucosidase or glycosidase activities are high in many strains of *Candida*, *Hanseniaspora*, *Lachancea*, *Metschnikowia*, *Pichia*, *Schizosaccharomyces*, *Saccharomycodes*, *Torulaspora*, *Wickerhamomyces* and *Zygosaccharomyces* (Charoenchai et al. 1997; Fernández et al. 2000; Mendes Ferreira et al. 2001; Strauss et al. 2001; Comitini et al. 2011; Maturano et al. 2012). These activities can release high concentrations of terpenoid compounds and aromatic thiols (Mendes Ferreira et al. 2001; Čuš et al. 2017).

Fermentations using mixed inoculation that includes a non-*Saccharomyces* yeast generally produce wines that vary from those inoculated only with *S. cerevisiae*, not only in chemical concentrations, but also in the perceived sensory attributes, even when using the same grape cultivar and vintage from a single vineyard (Ciani et al. 2010). Overall positive influences of experimental mixed fermentations with *S. cerevisiae* have been found by incorporating *H. uvarum* (Zohre & Erten 2002; Moreira et al. 2008; Padilla et al. 2017), *M. pulcherrima* (Zohre & Erten 2002; Moreira et al. 2008; Comitini et al. 2011; González-Royo et al. 2015; Padilla et al. 2017), *H. guilliermondii* (Moreira et al. 2008), *H. osmophila* (Viana et al. 2008), *S. bacillaris* (Comitini et al. 2011), *L. thermotolerans* (Comitini et al. 2011; Gobbi et al. 2013; Whitener et al. 2017), and *T. delbrueckii* (Comitini et al. 2011; González-Royo et al. 2015; Renault et al. 2015; Padilla et al. 2017), *S. bacillaris* (Padilla et al. 2017). Rossouw and Bauer (2016) also found positive outcomes using multiple species of *Candida*, *Cryptococcus*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Aureobasidium*, and other less common non-*Saccharomyces* yeasts. Results are dependent on the grape cultivar, mix of species used, inoculation timing and ratios, and fermentation parameters (Comitini et al. 2011; Gobbi et al. 2013).

Non-*Saccharomyces* yeasts may also be used to produce lower-alcohol wines to meet consumer demand, especially in warm-climates where grapes ripen for a longer period and reach higher sugar levels, which is likely to be of increasing concern as climates warm (Kutyna et al. 2010; Ivit et al. 2020). Larger populations of non-*S. cerevisiae* yeasts can deplete excess sugar via aerobic respiration and/or lower-efficiency fermentation, resulting in a lower final ethanol concentration (Kutyna et al. 2010; Gonzalez et al. 2013; Ivit et al. 2020). The growth of these non-*Saccharomyces* yeasts can also be encouraged by aeration practices during the early stages

of fermentation (Gonzalez et al. 2013; Aplin and Edwards 2021; Jolly et al. 2022). Numerous non-*Saccharomyces*, including *M. pulcherrima*, have been identified as candidates for reduced alcohol wine fermentations, being able to grow in high-sugar fermentation conditions, producing little ethanol, and having desirable oenological traits (Quirós et al. 2014; Mestre Furlani et al. 2017; Varela et al. 2016; García et al. 2020; Aplin and Edwards 2021; Jolly et al. 2022).

The use of a non-*Saccharomyces* yeast during wine fermentation may, however, result in unfavourable aromas, as any aromatic compound will be distasteful if concentrations are too elevated, even those generally considered pleasant. It is also essential that *S. cerevisiae*, or another fermentative yeast, completes the fermentation and so it must not be hindered by competition with the non-*Saccharomyces* yeast. Successful mixed inoculation fermentations combine the benefits of *S. cerevisiae* (predictable results, strong fermentative capability, agreeable rather than unpleasant aromas) with those of non-*Saccharomyces* yeasts (added complexity comprised of aromas that are difficult to achieve with *S. cerevisiae* alone, as well as reduced alcohol content if desired) (Comitini et al. 2017; Lappa et al. 2020). For example, most non-*Saccharomyces* yeasts produce lower levels of higher alcohols than *S. cerevisiae*, but this result is generally not observed in a mixed fermentation (Moreira et al. 2008). The presence of multiple yeasts will also result in interactions between yeast species/strains that impact the formation of aromatic compounds (Ciani et al. 2010).

1.2 Yeast community characterization methods

Characterizing the yeast community present in environmental samples such as on grape surfaces, in grape musts, and throughout fermentations to produce wines, relies on the accuracy and precision of taxonomic identification methods. Broadly, yeasts may be identified following

the isolation of individual cultures from a substrate, or by methods that directly use substrate samples for DNA analysis without an intermediary culturing step.

1.21 Culture dependent yeast community characterization

Individual yeasts may be identified from a pure culture, generally grown on a synthetic medium that selects for desired functional or taxonomic groups. The methods of identification that are currently most used, often in combination, are (1) assessing morphology or physiological traits, and (2) DNA extraction and PCR (polymerase chain reaction) followed by restriction fragment length polymorphism (PCR-RFLP) and/or Sanger sequencing. However, culture-based methods are intrinsically biased by the selective composition of the media as well as by competitive interactions among species present in a sample, and some yeasts may be viable but non-culturable (Serpaggi et al. 2012; Boynton et al. 2019). Furthermore, physiological and physical traits are often insufficient to accurately identify yeasts, due to effects such as evolutionary phenotype reversals and parallelisms (Stefanini and Cavalieri 2018). For example, the genus *Candida* is currently being revised to address the polyphyletic nature of its initial description based on phenotypic traits (Daniel et al. 2014; Kidd et al. 2023). However, culturing methods are valuable in that they provide pure, living cultures that can be used in physiological analyses and physical applications such as experimental wine fermentations. Yeasts intended for use as wine inoculum may be characterized for SO_2 and ethanol resistance, enzyme activity, aromatic compound and hydrogen sulfide production, and killer yeast activity (production of toxins lethal to other yeasts) and selected for use in fermentations based on these traits (Domizio et al. 2011; Mannazzu et al. 2019).

1.22 Culture independent yeast community characterization

Identification methods that extract (and then generally amplify by PCR) DNA directly from an environmental sample, such as grape must or wine, circumvent the biases of cultivation and are generally less time consuming. The first methods developed to do this physically separated DNA by taxa, which were then individually identified by matching to known DNA fragment patterns or by Sanger sequencing. To briefly summarize some of the methods used in wine grape and fermentation community research, denaturing gradient gel electrophoresis (DGGE) and RFLP analyses visualize DNA using gel electrophoresis. PCR products are separated by PCR-DGGE using a temperature/chemical gradient, while PCR-RFLP applies restriction enzymes to PCR products create DNA fragments. These methods have considerable drawbacks for community analysis and are declining in use as high throughput sequencing becomes increasingly accessible. For example, PCR-DGGE was determined to be less sensitive than media culture for the characterization of grape yeasts (Prakitchaiwattana et al. 2004).

Next-generation, or high-throughput, sequencing is a newer, accepted, and successful method to assess grape yeast communities and delivers more accurate population data than other approaches (David et al. 2014; Portillo and Mas 2016; Belda et al. 2017). High throughput sequencing uses cyclic-array technology: repeat cycles in which multiple DNA fragments are sequenced in parallel (Shendure and Ji 2008); and generates large amounts of sequence data quickly at low cost per base, compared to the more traditional Sanger sequencing. The information that follows provides context for the metabarcoding approach used for this thesis, in which a shared DNA region is compared to distinguish taxa, although much of the underlying methodology is the same for the other metagenomic approaches used for various applications.

There are several commercial systems using various methods to complete high throughput sequencing, but the general components, following DNA extraction, are consistent: (1) preparation of a library of DNA fragments, (2) distribution or tethering of DNA fragments to wells or locations on a solid substrate, (3) amplification incorporating labeled nucleotides, and (4) enzyme-driven sequence detection and data acquisition (Slatko et al. 2018). Although now largely overcome, initial drawbacks to high throughput sequencing compared to Sanger sequencing were overall project costs (although not cost per base), shorter sequence read length, and up to 15-fold lower base-call accuracy (Shendure and Ji 2008; Goodwin et al. 2016).

Two high throughput sequencing platforms that have endured as reliable and provide high-quality data that enable comparison among studies are short-read (~25-300 bp depending on the instrument) Illumina cyclic reversible termination sequencing by synthesis (Illumina sequencing) and long-read (~10-100 kb) Pacific Biosciences single-molecule real-time sequencing (PacBio sequencing) (Slatko et al. 2018; Kumar et al. 2019). Briefly, as summarized from Goodwin et al. (2016) and Bleidorn (2017), these systems operate as follows: Illumina sequencing creates the DNA library of clonal "clusters" on a slide or flow cell with the initial DNA molecules and primers, which are elongated via PCR with fluorophore labelled, 3'-OH blocked dNTPs. Following the addition of each single dNTP to a strand, the slide surface is imaged for dNTP identification, then the fluorophore and block are removed, and a new cycle begins. In PacBio sequencing, DNA polymerase is fixed in the bottoms of wells with transparent bottoms, in a specialized flow cell. A single DNA molecule is built in each cell, for which fluorophore labelled dNTP incorporation is continuously visualized as nucleotides are added and fluorophores are removed. PacBio sequencing typically uses a DNA library made of circular

double-stranded DNA templates but may use a linear DNA template for longer reads, albeit with lower accuracy (Kumar et al. 2019)

Recent improvements in high throughput sequencing and analysis technologies have made significant strides in addressing the drawbacks previously associated with these methods. Illumina sequencing is less expensive per base than other platforms, has low error rates, offers multiple types of sequencers to best suit the needs of a lab, and typical sequence read lengths have increased from up to ~100 bp to up to ~300 bp (Kumar et al. 2019; Fadiji and Babalola 2020). Enduring drawbacks of Illumina sequencing include difficulties in assigning accurate read abundances and in resolving repetitive genome regions, as well as the incorrect assignment of sequences to samples, or "tag-switching" (Thomas et al. 2017; Kumar et al. 2019). Methods are in development to address these issues, such as incorporating a synthetic mock community of pseudo sequences to quantify tag-switching (Jusino et al. 2016) and improved technology in newer Illumina machines to improve accuracy (Singer et al. 2019). PacBio sequencing, meanwhile, can resolve sequence repeats due to the longer read length, error rates have fallen from ~13% in older systems to ~3% in current systems, and cost per base has also decreased (Kumar et al. 2019).

There are other drawbacks inherent to all methods of high throughput sequencing. For example, the capture of noncellular "relic" DNA that is not associated with living organisms, which may inflate estimates of diversity and make it difficult to assess which results are ecologically relevant (Carini et al. 2016), although there is evidence that even high levels of relic DNA may not significantly alter diversity estimates, at least in some environments (Lennon et al. 2018). Most research that considers fungal relic DNA does so in the context of soil or aquatic samples, which differ substantially from the ephemeral environment of grapes, which cannot

accumulate nonliving biomass over a time span of years. Although further research is needed to explore this issue, it is possible that the seasonal nature of grapes reduces the level of relic DNA in must samples. It is more feasible to imagine that the nonliving fungal and yeast cells that accumulate throughout wine fermentation may potentially bias community characterization by high throughput sequencing. However, quantitative PCR (qPCR) during fermentation suggests that the DNA of dead species is not detected, and furthermore, that the considerable increase in living yeasts as fermentation progresses would function to limit the detection of DNA from dead cells (Stefanini et al. 2016).

The increased accessibility of high throughput sequencing analysis has also led to potentially reduced reliability of published data. Analytic pipelines allow researchers without specialization in bioinformatics to analyse data, increasing the likelihood of errors when choosing the best procedures, adapting to unexpected outcomes, and accurately interpreting results (Stefanini and Cavalieri 2018). Data may be rarefied to normalize library size of all samples for accurate comparison, a topic on which there is debate not only concerning best methods but also whether the practice should be done at all (McMurdie and Holmes 2014; Weiss et al. 2017; McKnight et al. 2019; Beule and Karlovsky 2020; Cameron et al. 2021). McMurdie and Holmes (2014) emphatically maintain that microbiome data should not be rarefied, to prevent the deliberate "omission of available valid data" and that the underlying issues due to different library sizes are masked by rarefying, not amended. Various methods of rarefying may also introduce biases that impact the intended statistical analyses (Weiss et al. 2017; McKnight et al. 2019), although alternate methods are gaining traction and may ultimately prove to be reliable and advantageous (Beule and Karlovsky 2020; Cameron et al. 2021).

There are multiple approaches to operational taxonomic unit (OTU) picking (clustering sequences into species proxies), each of which may be achieved by numerous algorithms used by different software. These algorithms are generally less effective when using fungal ITS sequences than bacterial 16S rRNA amplicons and vary in their ability to identify fungal ITS OTUs on default settings, therefore reducing comparability among studies (Oulas et al. 2015; Callahan et al. 2017; Halwachs et al. 2017). Algorithms such as DADA2 are intended to correct sequencing errors through modelling and produce amplicon sequence variants (ASVs), which are more readily compared among studies and avoid masking relevant, fine-scale variation, but split some species into multiple ASVs. (Callahan et al. 2016; Callahan et al. 2017).

1.23 DNA barcoding regions and associated challenges

The internal transcribed spacer (ITS) gene region is accepted as the universal fungal barcode sequence and is used both for Sanger sequencing and high throughput sequencing (Schoch et al. 2012). The ITS region consists of two spacer regions (ITS1 and ITS2) of nuclear DNA between the *18S* small sub-unit (SSU), *5.8S* and *26S* large sub-unit (LSU) rRNA genes in eukaryotes and is adjacent to the external transcribed (ETS) region. This region occurs in thousands of copies of tandem repeats separated by non-transcribed intergenic spacer (IGS or NTS) regions. The degree of variation in the ITS region is often high enough to allow for distinction between sequences at the species level, without being so high as to have divergent sequences within species (Schoch et al. 2012). This region is readily amplified by PCR and sequenced due to its high copy numbers, small size, and location between more highly conserved regions of DNA (Schoch et al. 2012; Stielow et al. 2015).

Using solely the ITS rDNA gene region for species identification may not be sufficient in genera with low interspecific or high intraspecific variability of this region, or for taxa lacking

barcoding data, including some groups of ascomycete and basidiomycete yeasts (Scorzetti et al. 2002; Vu et al. 2016). Additional genetic markers, such as the D1/D2 domain of ribosomal 26S DNA and the intergenic spacer (IGS) can be used to increase confidence (Kurtzman and Robnett 1998; Fell et al. 2000; Scorzetti et al. 2002; Nilsson et al. 2008). Additionally, the small subunit of the ribosomal DNA (nSSU), subunits of the RNA polymerase II (*RPD1* and *RPD2*), the translation elongation factor 1-α (*TEF1α*) and the mitochondrial cytochrome *b* (*CYTB* gene) can be used to create more accurate yeast phylogenies (Scorzetti et al. 2002; Liu et al. 2015). High throughput sequencing facilities, however, generally offer default protocols using the ITS region to target the broadest range of fungi, and the cost of including sequencing using additional primers for the same samples may be prohibitive for many studies.

Furthermore, due to the shorter maximum read lengths obtained using Illumina and other short-read sequencing platforms, ITS metabarcoding may be limited to either the ITS1 or ITS2 region rather than full-length ITS sequences. There is ongoing disagreement as to whether the ITS1 or ITS2 region provides better species resolution for fungi; some authors do not find a considerable advantage to either (Blaalid et al. 2013; Badotti et al. 2017), some support the use of ITS1 (Nilsson et al. 2008; Bellemain et al. 2010; Wang et al. 2015c), while others advocate for ITS2 (Bazzicalupo et al. 2013; Yang et al. 2018). Although some research may benefit from the use of one or the other region, in practice, many studies are likely to obtain adequate data using either and it remains difficult to make an informed choice based on the existing research. For example, while Leaw et al. (2006) suggest using the ITS2 region to best identify clinically relevant yeasts, Bokolich and Mills (2013) suggest using the ITS1 region for wine fermentation fungi. Moreover, differential amplification of genera or even species within a group of interest, such as yeasts, may bias results even if the selected region is generally suitable. Long-read

platforms are not so restricted and allow for full ITS region sequencing, but this itself may result in preferential amplification of shorter fragments among groups in which the full region is more or less conserved, such as the 400 bp ITS region of *M. pulcherrima* compared to the 800 bp of *S. cerevisiae* (Esteve-Zarzoso et al. 1999; Stefanini and Cavlieri 2018), and may also favour amplification of ascomycete over basidiomycete fungi (Bellemain et al. 2010).

There are further concerns related to DNA barcoding and identification methods that are universal to all sequencing methods. For example, different primer pairs, even among studies that use the same barcoding region, may favour amplification of different taxa (Op De Beeck et al. 2014; Stielow et al. 2015), also confounding the comparison of gene regions best suited to different taxonomic groups. The wine grape and must yeast communities offer particular challenges, given the high abundance and diversity of both ascomycete and basidiomycete yeasts, which represent divergent taxonomic groups that are both of interest but that may be preferentially amplified by different primers (Bellemain et al. 2010). However, some newer ITS rDNA region primers designed for short amplicon high throughput sequencing may reduce biases (Toju et al. 2012; Bokulich and Mills 2013; Usyk et al. 2017).

Once sequences have been obtained, identification protocols that rely on database comparison introduce additional limitations. The commonly used BLAST algorithm has well documented identification accuracy issues and online databases, including the National Center for Biotechnology Information (NCBI) GenBank database, contain incorrectly identified species, poor quality sequences, and out of date information (Nilsson et al. 2006; Xu 2016; Lücking et al. 2020), although this can be mitigated using GenBank by selecting search parameters that limit results to type and curated reference sequences within the RefSeq database (O'Leary et al. 2016). Databases contributing to the International Nucleotide Sequence Database Collaboration

(INSDC), including the European Nucleotide Archive (ENA), the DNA Data Bank of Japan (DDBJ), as well as GenBank and others, also contain large numbers of sequences lacking taxonomic description, while simultaneously lacking sequences for many described fungal species (Xu 2016; Lücking et al. 2020). Authors such as Halwachs et al. (2017) and Lücking et al. (2020) recommend that taxonomic assignment should be confirmed using strategies that involve checking multiple databases or phylogenetic analysis. However, despite the limitations, DNA sequencing is an essential tool for exploring microbial and yeast diversity in vineyard and wine fermentation environments, and high throughput sequencing has provided previously unattainable insights into the microbial aspects of terroir.

1.3 Microbial terroir of vineyards and wines

1.31 Concept of microbial terroir

Terroir is a term that refers to both the set of factors that influence the character of a crop and the character of the resulting product itself. Traditionally, factors contributing to the terroir of a wine included climate and weather, landform features such as slope and surrounding geography, soil properties (e.g., mineral and nutrient contents), and agricultural practices (Vaudour 2002; Van Leeuwen and Seguin 2006). Although there is not a strict consensus on the definition or scale of sub-factors that define terroir, it is an important concept in the description and marketing of wines (Vaudour 2002; Van Leeuwen and Seguin 2006).

Microbes, including yeasts and other fungi indigenous to grapes in the vineyard, influence the aroma and mouthfeel of wine via their metabolism during fermentation (see section 1.14). These yeast communities are in turn reflective of traditional components of terroir, such as location (Bokulich et al. 2014; Bokulich et al. 2016), climate (Bokulich et al. 2014; Brilli et al.

2014), and vineyard cultivation practices (Setati et al. 2012; Setati et al. 2015), as well as crop specific factors including grape cultivar (Mezzasalma et al. 2018), stage of grape growth (Martins et al. 2014), and condition of the grapes (Barata et al. 2012). Numerous studies have furthermore demonstrated links between yeast community composition as determined by vineyard and grape characteristics, and wine character (Knight et al. 2015; Bokulich et al. 2016; Liu et al. 2021; Wang et al. 2021a; Li et al. 2022; Perpetuini et al. 2022; Rossetti et al. 2023). Considering this body of evidence, microbial factors are now an accepted component of terroir in viticulture; the microbial terroir (Stefani and Cavalieri 2018; Liu et al. 2019).

1.32 Impacts of geographical location on vineyard yeast communities

While "location" does not specify the aspects responsible for differences (i.e., climate, landform, and soil properties are intrinsic to location yet distinct from each other), studies often do not link observed differences in grape yeast communities to these or other more specific variables. Gayevskiy and Goddard (2012) found significantly different grape yeast populations (including *S. cerevisiae* strains) between three wine growing regions in New Zealand. Paired studies undertaken by Bokulich et al. (2014) and Bokulich et al. (2016) found differences in the community structure of grape fungi among four California wine regions as well among individual vineyards within regions. Kioroglou et al. (2019) and Milanović et al. (2022) also found both regional and local differences in the fungal communities of Australian and Croatian vineyards, respectively. Many similar studies worldwide have correlated vineyard fungal and yeast communities with different geographical regions (Garofalo et al. 2016; Kamilari et al. 2021; Li et al. 2021; Castrillo and Blanco 2022; Tronchoni et al. 2022), as well as with individual vineyards or wineries (Stefanini et al. 2016; Miura et al. 2017; Cureau et al. 2021b; Liu et al. 2021), and even at finer scales of nearby individual vines (Setati et al. 2012; Martiniuk et al.

2023). However, in some cases, one or more variables may eclipse the effect of geographic location. For example, Vigentini et al. (2015) found that year of sampling was a stronger predictor of vineyard yeast communities than location when comparing two Italian regions over three years.

1.33 Impacts of climate on vineyard yeast communities

Several studies have isolated the important effects of climate variables on vineyard fungal and yeast communities. In general, yeasts and filamentous fungi likely prefer a minimum level of humidity, especially as warmth and humidity may promote nutrient release from grapes (Combina et al. 2005b; Čadež et al. 2010). Brilli et al. (2014) analysed 16 years of yeast composition and meteorological data (temperature, humidity, and rainfall) from a vineyard in Tuscany and determined that total yeast abundance was positively correlated with increased rainfall, and yeast community composition was positively correlated with temperature. Bokulich et al. (2014) suggest average evapotranspiration, net wind run, minimum and maximum temperatures, relative humidity, latitude, longitude, average high temperature, average soil temperature, and net precipitation to be the environmental conditions correlated with differences in grape microbial community between Californian wine regions, although their analyses point to confounding interactions with other factors such as microbial species.

Li et al. (2021) also correlated multiple climatic factors, including rainfall, temperature, solar radiation, elevation, and relative humidity to the composition of vineyard fungal communities across nine Chinese regions, noting that species of *Pichia* and *Hanseniaspora* had strong positive relationships with temperature and rainfall, and negative relationships with elevation and solar radiation. Additional correlations are provided between numerous yeast species and these climatic variables, and the cumulative effect of climate is estimated to account

for approximately 42% of fungal community variation (Li et al. 2021). Chalvantzi et al. (2021) determined that temperature, elevation, and precipitation were most highly correlated with yeast community biogeographic patterns, while factors such as wind speed may influence the population size of individual yeast species. However, these authors also emphasized that climatic variables can only partially explain the composition of the vineyard yeast community (Chalvantzi et al. 2021). Other, less expansive studies also suggest relationships between variables including relative humidity, precipitation, and temperature (Jara et al. 2016; Steenwerth et al. 2021). Year of sampling, which is correlated to climatic factors, is also highlighted as influencing yeast community composition in multiple studies (Bokulich et al. 2014; Garofalo et al. 2016; Cureau et al. 2021b; Wang et al. 2021b; Castrillo and Blanco 2022).

1.34 Impacts of surrounding environment on vineyard yeast communities

The diversity of plants and insects in the regions surrounding the vineyard is another factor inherent to location. *S. cerevisiae* strains show genetic similarity among wineries, originative vineyards, and surrounding forests (Goddard et al. 2010; Knight and Goddard 2015; Börlin et al. 2016), and the larger community of filamentous fungi and yeasts show similar patterns (Morrison-Whittle and Goddard 2018). Vineyard soil may act as a key source of microbial diversity for grapes (Sabate et al. 2002; Setati et al. 2012; Burns et al. 2015; Morrison-Whittle and Goddard 2018), although fermentative ascomycete yeasts may disperse more so from other proximate flora by air, rain or irrigation water, and insects (Valero et al. 2005; Garijo et al. 2011; Valentini et al. 2022).

1.35 Vineyard cultivation practices and impacts on vineyard yeast communities

Decisions made about agricultural growing practices in vineyards such as type, amount, and timing of application of pesticides, herbicides, and fungicides; type, amount, and timing of

application fertilizers; use and timing of tilling; and choices concerning factors including training systems, pruning, and cover crops, will impact the growth of grapevines and their associated microorganisms (Provost and Pedneault 2016; Karimi et al. 2020; Giffard et al. 2022). Commonly employed vineyard cultivation systems are no-treatment, organic, conventional, biodynamic, and integrated. Organic vineyards may use copper and sulfur fungicides, as well as biocontrol products, while conventional vineyards may use the products available in organic cultivation as well as any of a wide variety of commercially available synthetic pesticides, and generally apply many products to target a wide range of pests (Craig 2022). Organic certification is now regulated by legislature in most wine producing countries, although the history of certification is complex and there are variations among countries (Jones and Grandjean 2018). Biodynamic vineyard management employs similar fungicide and chemical use as organic agriculture and is further guided by the philosophy that humans, all living things, the earth, and the spiritual world are interconnected and should be managed holistically (Castellini et al. 2017). A biodynamic vineyard should meet the general requirements of organic cultivation but may or may not choose to seek organic certification; biodynamic certification is governed by the non-profit Demeter International organization (Castellini et al. 2017). Integrated agriculture is an intermediate between organic and conventional styles that emphasizes sustainability and high-quality farm management.

The correlations between vineyard yeast diversity, community composition, and the relative abundance of individual yeast species with cultivation practice are inconsistent, likely depending on variation in management within the larger designations (e.g., “organic”, “biodynamic”, “conventional”) and on interactions with factors such as climate (Barata et al. 2012). For example, Cordero-Bueso et al. (2011) found *S. cerevisiae* genotype diversity and

richness were higher in an organic vineyard than a conventional vineyard in the Madrid region, but overall yeast species richness was higher in the conventional vineyard. Tello et al. (2012) found differences in species assemblages between organic and conventional vineyards, and that organic vineyards had higher yeast species and *S. cerevisiae* strain richness and diversity and lower dominance than conventionally managed vineyards, but only three additional species were found in the organic vineyards, which also benefitted from a greater number of samples.

Martins et al. (2014) found higher population counts, species diversity, and species richness of yeasts in an organic vineyard compared to a nearby conventional vineyard that had higher copper levels in cell suspensions obtained from grapes at time of harvest. Similarly, biodynamic vineyard cultivation in South Africa is consistently found to result in higher yeast species diversity and richness than integrated and conventional cultivation (Setati et al. 2012; Bagheri et al. 2015; Setati et al. 2015). Other studies have also found higher yeast diversity correlated with organic cultivation (Castrillo et al. 2019).

In other cases, however, diversity can be higher in conventional vineyards than organic vineyards, as was found in Italian vineyards by Milanović et al. (2013), both for yeast species and *S. cerevisiae* strains. Sometimes, no differences are detected. Castrillo and Blanco (2022) found no effect of farming system on yeast counts or species richness among Spanish wine denominations, Kecskeméti et al. (2016) found no significant differences in yeasts species abundances or fungal diversity based on management, and Comitini and Ciani (2008) found that diversity was comparably reduced by both organic and conventional fungicides compared to no treatment. Grapes sampled from 12 experimental fungicide treatments, including untreated and conventional controls, did not show significant differences in fungal diversity or abundances of individual species (Englezos et al. 2022).

Differing fungicide regimes or cultivation practices are in some cases directly attributed to differences in the relative abundance on individual yeast species. For example, Agarbati et al. (2019) found *H. uvarum* abundance decreased with exposure to either organic or conventional fungicides, *Pichia terricola* abundance was lower under conventional cultivation but did well in organic vineyards, while *M. pulcherrima* showed the opposite pattern. In accordance, Comitini and Ciani (2008) also noted significant decreases of *H. uvarum* and other fermentative yeasts in response to both organic and conventional fungicides, while Milanović et al. (2013) found a higher incidence of *M. pulcherrima* in conventional vineyard samples. Xu et al. (2020) found that *H. uvarum* was abundant in musts of both organically and conventionally cultivated grapes, although their samples originated from a single vineyard experiencing different experimental treatments.

Abundance of the ubiquitous and often highly abundant *A. pullulans* further demonstrates the difficulty in correlating individual species with cultivation practice. Numerous studies note higher abundance in organic vineyards (Martins et al. 2014; Castañeda et al. 2018; Perpetuini et al. 2022; Xu et al. 2022), while several others describe the opposite (Comitini et al. 2008; Castrillo et al. 2019; Rossetti et al. 2023). In both scenarios, authors comment on the lack of statistical power given that *A. pullulans* is generally abundant in all samples (Castañeda et al. 2018; Rossetti et al. 2023). Inclusion of a no-treatment vineyard by Agarbati et al. (2019) for comparison to both organic and conventional cultivation practices suggests that the omission of this control may confound results; *A. pullulans* was more abundant in both treated vineyards compared to the no-treatment vineyard. Indeed, some authors find that *A. pullulans* is relatively unaffected by all fungicides tested (Čadež et al. 2010; Grangeteau et al. 2017; Rantsiou et al.

2020) or is comparably abundant in vineyards employing different cultivation practices (Setati et al. 2012; Setati et al. 2015).

Differentiation between or among vineyards experiencing different cultivation practices is increasingly determined by yeast species community composition rather than solely by diversity metrics such as species richness. Castañeda et al. (2018) did not find differences in fungal diversity between organically and conventionally cultivated grapes but did find that community composition was statistically different. Setati et al. (2012) found that the yeast communities of integrated and biodynamic vineyards were more alike than either was to a conventional vineyard, and Bagheri et al. (2015) noted differences in the assemblages of abundant yeasts among biodynamic, integrated, and conventional vineyards. Additional research such as the comparison of fungal communities among Italian vineyards with conventional, organic, and biodynamic cultivation have also revealed differences in community composition (Perpetuini et al. 2022; Rossetti et al. 2023). Evidently, the relationship between vineyard cultivation practices and the diversity of grape yeasts is nuanced.

1.36 Impacts of grape cultivar on vineyard yeast communities

Microbial terroir is also shaped by the qualities of the grape cultivar itself, although few studies have attempted to experimentally address the causes underlying cultivar discriminative yeasts or yeast community differences between cultivars. Yeast communities on grapes of four different cultivars were determined to be significantly different by Belessi et al. (2022) but the authors did not state any correlations of community with the measured chemical characteristics of the grape musts. Bokulich et al. (2014) proposed that differences in vine growth habits and stress responses may impact fungal communities, while Yanagida et al. (1992) suggested that differences among cultivars may be driven by duration of ripening. Mezzasalma et al. (2018)

provide a summary of cultivar factors that may influence yeast species composition, suggesting that differences in fruit skin characteristics of thickness and waxiness may restrict the access of yeast species unable to permeate tougher defences, that higher levels of anthocyanins may have antifungal effects on some yeast species, and that bunch characteristics such as density, size, and shape may play a role. Mezzasalma et al. (2018) noted that these characteristics differed between the cultivars they sampled.

Fungal community composition varied among the grape cultivars sampled by Cureau et al. (2021b), who highlighted differences in the abundances of *Meyerozyma*, which was unique to Zinfandel and at high relative abundances in both years of sampling. Zhang et al. (2019) found that fungal communities were different among the six red cultivars tested, but not among the three white grape cultivars, and differences were driven by 35 taxa including the Cystofilobasidiaceae in Syrah and *Cryptococcus* in Longan. Effects of cultivar on fungal communities were also found to moderate the influences of other variables, for instance, country of origin (Tronchoni et al. 2022), vineyard site within Slovenian (Raspor et al. 2006) and Australian (Liu et al. 2021) regions, climate among Japanese (Yanagida et al. 1992) and Chinese (Li et al. 2021) wine regions, and year of sampling (Nemcová et al. 2015). Comparison of samples taken from South Africa and three European countries even suggest that some cultivars, based on the similarities among Cabernet Sauvignon samples, may carry a shared, distinct fungal community (Tronchoni et al. 2022).

1.37 Impacts of grape ripeness and condition on vineyard yeast communities

Yeast communities on grapes are also formed by the physical condition of the grapes, as determined by ripeness and damage to the berries. Grapes that are less ripe are generally described as supporting larger proportions of aerobic yeasts such as the basidiomycetes

Rhodotorula and *Cryptococcus* and the ubiquitous vineyard resident *Aureobasidium* (Fleet 2003). These species may also be found on ripe grapes, but the increase of sugars available either on or just under the surface of the grape skin as ripening progresses attracts an increase in diversity driven by ascomycete yeasts such as *Hanseniaspora* and *Metschnikowia* (Fleet 2003; Barata et al. 2012). Although these patterns are often confirmed by sampling grapes throughout ripening (Renouf et al. 2005; Liu and Howell 2021; Constantini et al. 2022), multiple studies find a succession of species that is alternative, or even seemingly opposite (Mateo et al. 2020; Ding et al. 2021; Wang et al. 2021b; Zhu et al. 2021), indicating that further research is needed in this area.

Regardless, the effects are likely related to the damage level of grapes, as the softening associated with ripening makes the berries more susceptible to damage (Fleet et al. 2002; Barata et al. 2012). Indeed, Nemcová et al. (2015) found higher levels of the basidiomycete yeast *Sporobolomyces* on intact grapes, compared to increased *Pichia* and *Saccharomyces* on damaged (but not rotten) grapes. The effects of damage may also explain results in which the typical species succession is not observed throughout ripening, for example, Zhu et al. (2021) found mainly basidiomycete yeasts (predominantly *Vishniacozyma*) on both unripe and ripe grapes in Xinjiang, China, but took care to sample only undamaged grapes. However, *Vishniacozyma* may represent a reliable anomaly, also increasing from veraison (change of colour due to ripening) to harvest in a vineyard in Washington State, USA (Wang et al. 2021b).

1.38 Connections between vineyard yeast communities and wine characteristics

Knight et al. (2015) and Bokulich et al. (2016) were the first to explicitly and directly link grape microbial communities, including yeasts, to wine character. Microbial consortia were correlated to vineyard geography at multiple scales (Bokulich et al. 2014), and wine metabolite

profiles were connected to the grape microbiota of viticultural areas and individual vineyards (Bokulich et al. 2016). In addition, the aroma profiles of inoculated fermentations of sterile grape juice correlated to the indigenous *S. cerevisiae* strain selected to represent each of six geographic regions (Knight & Goddard 2015, Knight et al. 2015).

Other research complements these seminal publications to improve our understanding of microbial terroir and yeast-specific effects. Varela et al. (2009) compared wines made from the same must fermented either spontaneously or with a *S. cerevisiae* inoculation and found significant differences in the yeast-derived fermentation products identified as contributing to wine aroma in the resulting wines. In this example, the spontaneous fermentations represent the grape yeast community in comparison to fermentations altered by the addition of *S. cerevisiae*, an approach that has been repeated many times with similar results: the wines produced from spontaneous fermentations compared to that of equivalent inoculated fermentations using the same grape cultivar in the same setting, have differing concentrations of esters, terpenoid compounds, and other aromatics, and can score higher ratings for positive aromas, flavour complexity, and overall impression (Egli et al. 1998; Garde-Cerdán & Ancín-Azpilicueta 2006; Varela et al. 2009; Medina et al. 2013; Liu et al. 2016; Çelebi Uzkuç et al. 2020; Lu et al. 2020). Volatile profiles of spontaneously fermented wines also consistently show that naturally occurring yeasts contribute positive compounds at appropriate levels (Clemente-Jimenez 2004; Čuš et al. 2017; Chen et al. 2020).

Studies that employ mixed inoculum fermentations also provide support for the concept of microbial terroir by showing that the inclusion of non-*Saccharomyces* yeasts affects wine character (see section 1.14). Comitini et al. (2011) demonstrated that mixed inoculations of *S. cerevisiae* and each of *S. bacillaris*, *L. thermotolerans*, *T. delbrueckii*, and *M. pulcherrima*

resulted in different wine character profiles regarding acidity and concentrations of ethanol, glycerol, higher alcohol contents, acetaldehyde, and various esters. González-Royo et al. (2015) found variable effects on wine aroma and mouthfeel when using only *S. cerevisiae* as inoculum compared to mixed inoculation with *T. delbrueckii* or *M. pulcherrima*. Fermentations with *Hanseniaspora opuntiae* and *H. uvarum* have also brought about positive aspects like hazelnut, coffee, cherry, and caramel aromas, while *Papiliotrema flavescens* and *Pichia kudriavzevii* may result in oaky, floral, and earthy aromas (Rossouw and Bauer 2016). Padilla et al. (2017) and Whitener et al. (2017) likewise found differences in character among wines using mixed inocula of *H. uvarum*, *S. bacillaris*, *T. delbrueckii*, *L. thermotolerans*, *P. kluyveri*, *M. pulcherrima*, *Kazachstania aerobia*, and multiple *S. cerevisiae* strains, vs. inoculations containing only *S. cerevisiae*.

Finally, at least one study has isolated yeasts from spontaneous fermentations of grapes from different regions to create representative yeast communities for use in inoculated fermentations. Hawkins et al. (2023) sampled fermentations from seventeen vineyards in total, from three New Zealand regions, and prepared mixed yeast communities (n=96) for each. Analyses confirmed that yeast communities were regionally distinct and significantly impacted wine aromatic chemical profiles of inoculated fermentations of sterilized musts (Hawkins et al. 2023).

However, the concept of microbial terroir is not beyond critique. The number of variables that may influence the trajectory of a fermentation, starting in the vineyard and continuing in the winery, are many, and their interactions are complex and difficult to predict. Bordet et al. (2020) emphasized that the diversity of methodologies used to assess the activities of yeasts during

fermentation are incredibly diverse and seemingly contribute to a great deal of variation in population dynamics, fermentation kinetics, and metabolite production results.

And, although wine fermentations and the yeasts involved have long been of interest, microbial terroir research underpinned by high throughput sequencing is a relatively new field that is still developing. Alexandre (2020) advises that additional experimental research is warranted (in addition to the many existing correlational studies) to support whether the contribution of vineyard microbes truly plays a role in wine characteristics, highlighting uncertainty regarding the influence of winery-resident yeasts, and of *S. cerevisiae* strains vs. the total yeast community. However, this author uses a definition of "terroir" that reflects the French appellation system (defined geographical areas) which does not correspond with more general definitions used in most research, or with the approach to microbial terroir taken in this thesis.

1.4 Vineyard and wine yeast research: Canada and cool-climate American states

1.41 British Columbia

The Wine Research Centre at the University of British Columbia (UBC) has generated and supported Canadian wine research for the past 25 years with a focus to grow the wine industry in British Columbia. There are nine VQA (Vintners Quality Alliance – the regulatory and appellation system for British Columbia and Ontario wines) defined viticultural areas in British Columbia, including the Okanagan Valley and Fraser Valley, both near UBC campuses. Numerous UBC theses and publications have addressed the role of yeast communities in winemaking, finding that community differences are associated with variables including year of sampling, vineyard, grape cultivar, winery, inoculation method (e.g., spontaneous vs. inoculated with *S. cerevisiae*), and stage of fermentation (Lange et al. 2014; Neuner 2016; Martiniuk et al.

2023). Many different commercial, commercial-derived (similar genotypes to commercial strains) and indigenous *S. cerevisiae* strains are residents of British Columbia wineries (Hall et al. 2011; Lange et al. 2014; Martiniuk et al. 2016; Scholl et al. 2016; Martiniuk 2020). Common wine fermentation yeast species are also represented, including *H. uvarum*, *W. anomalus*, *M. pulcherrima*, and *T. delbrueckii*, which follow expected successional patterns starting with a larger proportion of non-*Saccharomyces* yeasts and shifting to a predominantly *Saccharomyces* community following successful fermentation (Lange 2012; Neuner 2016).

The first North American report of wine fermentations dominated by indigenous *S. uvarum* strains, rather than *S. cerevisiae*, resulted from comparisons of microbial communities among different SO_2 and inoculation treatments at a winery in British Columbia (Morgan et al. 2019). It is notable that fermentations that experienced *pied de cuve* inoculation (in this case, inoculum derived from spontaneously fermenting must) and to which no SO_2 was added, experienced changes in the diversity of microorganisms, including yeasts, and produced wines with increased fruity and desirable aromas, and improved body and sweetness (Morgan et al. 2019). Furthermore, the diversity of *S. uvarum* strains, confirmed to be abundant and to dominate spontaneous fermentations of grapes from multiple vineyards (Morgan et al. 2019; McCarthy et al. 2021) may ultimately be of great interest to the Canadian winemaking community. Characterization of indigenous *S. uvarum* strains has since revealed strong fermentative and competitive abilities, especially at lower temperatures, persistence throughout fermentation even when *S. cerevisiae* is dominant, high production of a strain-dependent variety of volatile compounds that contribute to fruity and floral aromas, and production of distinctive wines in both single and co-inoculations (Morgan et al. 2020; Lyons et al. 2021).

High throughput sequencing and microsatellite strain typing have further characterized the fungal terroir of British Columbia vineyards. At the scales of regions and wineries, assemblages of *S. cerevisiae* strains are distinctive and interact with cultivar effects (Scholl et al. 2016; Cheng et al. 2020). Even at the fine scale of nearby vineyards growing the same cultivar, managed by the same winery, different fungal communities are present which persist throughout fermentation, suggesting that "micro" terroir may influence single-vineyard wines (Martiniuk et al. 2023).

Other vineyard and wine yeast research from UBC has covered topics including various aspects of *S. cerevisiae* metabolism (Erasmus et al. 2004; Whitmore et al. 2021), discovery of a unique Pacific West Coast Wine clade of *S. cerevisiae* (Marr et al. 2023), and development of a novel method for typing *Saccharomyces* strains (Wang et al. 2023a). A collection of studies has also examined malolactic fermentation in wine, ranging from testing different malolactic inoculation cultures (Delaquis et al. 2000), to genetically engineering a malolactic yeast strain (Husnik et al. 2006), and most recently assessing the interactions between *S. cerevisiae* and the malolactic fermentation bacteria *O. oeni* under different methods of spontaneous and inoculated fermentations (Tantikachornkiat et al. 2020).

1.42 Ontario and Quebec

The prominent wine regions in central Canada are in Quebec and in Southern Ontario, where there are three VQA defined viticultural areas including the Niagara Peninsula. Brock University's Cool Climate Oenology and Viticulture Institute (CCOVI) takes advantage of the Niagara region to conduct research that prioritizes the Canadian grape and wine industry. These more eastern regions also support an icewine industry, which has motived research from various groups including CCOVI.

For example, several common grape-associated yeast species are known from icewine grapes, including *Starmerella stellata*, *Papiliotrema laurentii*, *M. pulcherrima*, and *Pichia kluyveri*, some persisting even in cold temperatures (Chamberlain et al. 1997). Icewine musts support a community of yeasts similar to other Canadian wine musts, with high proportions of *A. pullulans* and the basidiomycete yeasts *Naganishia albida*, *Rhodotorula* sp., and *Sporobolomyces* spp. including *Sporobolomyces roseus* (Subden et al. 2003). This community may contribute to the unique regional sensory profile of Ontario icewines compared to icewines worldwide (Nurgel et al. 2004; Huang et al. 2018). Other wine yeast research programs in Ontario explore topics including the assessment of different *S. cerevisiae* inoculation and metabolic parameters in icewine, table wine, and sparkling wine fermentations (Kontkanen et al. 2004; Pickering et al. 2008; Yang et al. 2017; Muysson et al. 2019; Kemp et al. 2020), and the evaluation and development of genetically engineered *S. cerevisiae* strains for various improved metabolic processes during fermentation (Coulon et al. 2006; Bellon et al. 2015).

CCOVI research has also reinforced the role of *S. uvarum* in Canadian vineyards and wineries established in British Columbia. A strain of *S. uvarum* isolated from a spontaneous icewine fermentation (Nurgel et al. 2004) performs well in test fermentations for production of Vin de Curé (a sweet wine made from partially dehydrated grapes) and other appassimento style wines, producing comparable levels of ethanol to a commercial *S. cerevisiae* strain, higher levels of glycerol, and different aromatic profiles (Kelly et al. 2018; Kelly et al. 2020; Inglis et al. 2020). Collaborations between researchers have further resulted in useful reviews that cover topics such as the impact of non-*Saccharomyces* yeasts on sparkling wine (Ivit and Kemp 2018) and the effects of fermentative and semi-fermentative ascomycete yeasts other than *S. cerevisiae* on ethanol and glycerol levels in wine (Ivit et al. 2020).

In Quebec, vineyard and wine research is generally not yeast-related, although Lallemand, a Canadian company that develops, produces, and markets yeasts and bacteria for use in the food and drink industries, conducts research in Montreal to develop new yeasts for wine fermentations (Lallemand Oenology 2024). However, Lallemand's research and development team is based in Toulouse, France and primarily collaborates with larger, international wine industries (Lallemand Oenology 2024). Wine grape and winemaking studies from research institutions in Quebec have focused on topics including the northern distribution of *Saccharomyces* in natural habitats (Charron et al. 2014), cultivar-specific volatile compounds from grape skin, juice, and wine (Slegers et al. 2015), improvements in organic vineyard management (Provost and Pedneault 2016), and isotope tracking to monitor nutrient absorption and use by grape vines and transfer to wine (Guibourdenche et al. 2020).

1.43 Northwestern states

On the American west coast, vineyards in Washington State and Oregon may experience similar conditions to British Columbia wine regions and these states possess 17 and 20 American Viticultural Areas (AVAs), respectively, in addition to three AVAs that overlap both states (Alcohol and Tobacco Tax and Trade Bureau 2023). Non-*Saccharomyces* yeasts, including *M. pulcherrima*, *Candida*, and *Meyerozyma* isolated from Washington vineyards have been evaluated for use in wine fermentations, considering parameters of utilization of fermentable sugars and amino acids (Aplin et al. 2019), and ethanol and acetic acid production (Aplin and Edwards 2021). These candidate yeasts were selected from an extensive number of cultures obtained from several Washington State vineyards, also including common vineyard ascomycete yeasts *Candida* spp., *H. uvarum*, *Pichia* spp., and *A. pullulans*, and basidiomycete yeasts *Rhodotorula* spp., *Sporobolomyces* spp., *Naganishia* spp., and *Filobasidium* spp. (Bourret et al.

2013). Native yeast communities have also since been characterized by Wang et al. (2021b), finding effects of vineyard location on diversity, and abundant populations of *A. pullulans*, *F. magnum*, *M. pulcherrima*, *Vishniacozyma victoriae*, and *Udeniomyces puniceus* on grapes, succeeded by *S. cerevisiae*, *H. uvarum*, and *Metschnikowia* spp. during fermentation. Complementary studies tested the biocontrol potential of vineyard yeasts against the grape pathogen *Botrytis cinerea* (Wang et al. 2018) and developed real-time PCR assays to quantify yeasts in grape and fermentation samples (Wang et al. 2020).

One of the earliest records of vineyard yeasts in northern North America exists in the form of a master's thesis from Oregon State College (now Oregon State University) from 1940, titled "A study of some yeasts occurring on Oregon grapes", identifying *S. cerevisiae*, and *H. uvarum* (McBee 1940). More recent preliminary results of yeast community compositions in Oregon vineyards anticipate differences based on management style (conventional vs biodynamic), considering differences in metabolite profiles independent of geography or grape cultivar (Spencer et al. 2022). Other Oregon vineyard and wine yeast research has evaluated aroma of wines made with local Pinor noir grapes in experimental fermentations inoculated with selected strains of *S. cerevisiae* and *L. thermotolerans* (Takush and Osborne 2012) and demonstrated an enrichment culturing method to isolate rare vineyard yeasts (Piago et al. 2021).

1.44 Central and northeastern states

Wisconsin (3 AVAs), Michigan (5 AVAs), and Ohio (5 AVAs) may experience similar conditions to wine regions in Ontario (Alcohol and Tobacco Tax and Trade Bureau 2023). Two complementary studies characterized yeasts of the Lake Eerie Appellation in Ohio on grapes: *A. pullulans* and *R. glutinis* were highly abundant (Panagopoulos 2002), and during fermentation, a succession from *H. uvarum* to *Saccharomyces* was documented (Thomas 2002). More recent

studies from Wisconsin and Michigan have assessed stress sensitivity phenotypes of vineyard and oak tree associated *S. cerevisiae* populations to distinguish the genetic basis for their growth in grape juice and reduced gene flow between the populations (Clowers et al. 2015) and evaluated treatments to reduce cluster rots (Neugebauer et al. 2024).

Vineyards in the northeastern states of New York (11 AVAs), Pennsylvania (5 AVAs), New Jersey (4 AVAs), Massachusetts (2 AVA), Connecticut (3 AVAs) and Rhode Island (1 AVA) may experience conditions similar to either Ontario or more eastern Canadian vineyards (Alcohol and Tobacco Tax and Trade Bureau 2023). Much of the New York vineyard research focuses on grapevine diseases (Fuchs et al. 2009; Sun et al. 2019; DeKrey et al. 2022), although The Northern Grapes Project, which took place from 2011-2016 at Cornell University, included studies that tested commercial yeasts in fermentation trials (Mansfield [2015]) and methods to predict yeast assimilable nitrogen levels at harvest (Nisbet et al. 2014). Research from New Jersey, on the other hand, has concentrated on perceptions of local wines (Ashton 2014), and on sustainability in viticulture and wine tourism. Gottlieb and Moscovici (2015) found positive correlations between sustainability practices, vineyard size, and output and suggest that vineyard and winery managers implement sustainable practices to increase product quality rather than due to pressure from visitors. Subsequent studies highlight opportunities in collaborative sustainability marketing and suggest resources such as certification or guidelines to support these efforts (Villaneuva and Moscovici 2017; Moscovici and Gottlieb 2017).

A separate research group has investigated the invasive vineyard pest *Lycorma delicatula* (spotted lanternfly) in New Jersey vineyards, which may eventually also be a concern in central and eastern Canadian regions (Allen et al. 2021; Madalinska et al. 2022). Spotted lanternfly is also a pertinent research topic in Pennsylvanian vineyards (Leach A and Leach H 2020; Baker et

al. 2021), but some assessments of vineyard filamentous fungi and yeasts have also taken place. A thorough examination of the fungal community associated with hybrid Chambourcin grapes in Pennsylvania found abundant populations of the common vineyard yeasts *H. uvarum*, *P. kluyveri*, *P. kudriavzevii*, *A. pullulans*, *Sporobolomyces shibatanus*, and *S. bacillaris*, along with several additional species of *Hanseniaspora* and *Pichia*, the composition of which varied among the three vineyards sampled (Feng et al. 2021). Five abundant species were selected for further analysis of chemical activities during fermentation; strains of *P. kudriavzevii*, *H. uvarum*, and *H. opuntiae* had high ethanol tolerance, both *Hanseniaspora* strains were positively correlated with increased production of higher alcohols, and *P. kudriavzevii* was positively correlated with many esters and acetals not associated with the other strains, including a control *S. cerevisiae* strain (Feng et al. 2021). Wang et al. (2021a) also characterized yeast communities of the hybrid cultivar Chambourcin cultivar during fermentation of Pennsylvania grapes, noting populations of *Starmerella* and *Aureobasidium* early in fermentation.

Although Lehman and Nawrocki (2011) provide an entertaining read concerning the history of wine production in Connecticut, vineyard research in this region is limited. However, commercial yeasts were tested in micro-fermentations using grapes from Massachusetts and Rhode Island, and preferred candidates were suggested (Lagassey 2014).

1.5 The Nova Scotia wine region

1.51 Characteristics of the Nova Scotia wine region

As a cool climate wine growing region, Nova Scotia is characterized by lower temperatures and a shorter growing season compared to other wine regions worldwide and carries a risk of spring and fall frosts (Shaw 1999; Jones and Schultz 2016). Although the local

climate will undoubtedly shift as global climate change continues (average trend of 0.13°C warming/decade; Jones and Schultz 2016), regions within Nova Scotia traditionally experience a continuum from a moderate continental climate, with larger daily and seasonal temperature changes, to a more temperate maritime climate with cooler springs and summers, depending on elevation and proximity to the ocean (Museum of Natural History Staff 1996; Vasseur and Catto 2008). Mean annual temperatures range from 5°C-7°C and mean total annual precipitation ranges from approximately 12.5-16 cm/year with a slight bias toward heavier precipitation in cooler months (Museum of Natural History Staff 1996). There may be significant variations in weather, and therefore grape growing, conditions from year to year and variations in microclimate of individual vineyards, although the province generally experiences approximately 1000 growing degree days (GDD) (Shaw 1999; Wine of Nova Scotia 2024).

Within Nova Scotia, several geographic and climatic factors favour the Annapolis Valley region for grape growing. There are several areas in the Annapolis Valley with fine loamy or fine loamy-gravelly soils over slate and shale, which have optimal drainage and heat retention for grape growing (Shaw 1999). The Annapolis Valley also experiences the longest growing season (approximately 210 days) and some of the warmest temperatures in the province, relatively low precipitation, and shelter from fog and cool winds (Museum of Natural History Staff 1996). Spring arrives late, especially at the eastern end of the valley due to the moderating effect of the Minas Basin, which delays spring budding, however, fall also arrives late, allowing for continued crop maturation (Shaw 1999). Although cold winters limit grape varieties that can be grown and require careful consideration in vineyard site selection, temperatures below -8°C in early winter allow for a small ice wine industry (Shaw 1999).

Hybrid grape cultivars (European *Vitis vinifera* crossed with native North American species) that have been bred for cool climates are grown here with the most success, including white wine cultivars L'Acadie blanc, New York Muscat, Seyval blanc, and Vidal blanc, and red wine cultivars Baco noir, Leon Millet, Lucie Kuhlmann and Marechal Foch (Cameron et al. 2012). White hybrid cultivars flourish in Nova Scotia and produce the wines considered to be most distinctive of the region. More widely known cultivars of *V. vinifera*, including Chardonnay, Riesling, Pinot gris, and Sauvignon blanc (whites), and Pinot noir (red) are also grown in Nova Scotia, although in smaller quantities (Cameron et al. 2012). As climate change has moderated and warmed the Nova Scotia climate in recent years, and the wine industry has become more established, growers have been experimenting with these and other grape varieties once thought to not be suited to Nova Scotia.

Nova Scotia has one appellation wine, Tidal Bay, designated in 2012. Tidal Bay wines are defined as those produced from only Nova Scotian grown grapes and possessing the Tidal Bay profile: a fresh, crisp, off-dry, still, white wine, with bright notes of fruit, acidity, and minerality (Wines of Nova Scotia 2024). A selection of L'Acadie blanc, Seyval blanc, Vidal and/or Geisenheim grapes must make up most of the final blend, with an additional 24 secondary and tertiary varieties allowed, although wineries may apply to have additional varieties considered (Wines of Nova Scotia 2024). Additional regulations regarding bottling yield, winemaking methods, and final alcohol, acidity, and sugar contents are also in place. Tidal Bays are approved by an independent tasting panel.

More broadly, Nova Scotian wines have been suggested to express terroir distinct from other wine regions. The accumulation of organic acids, concentrations of metals, and sugar

contents differ between Nova Scotian wines and wines from other Canadian regions and eight other major wine producing countries (Gjelaj 2019).

1.52 Vineyard and wine yeast research in Nova Scotia

There was little consideration of the Nova Scotia wine region in the decade and a half following the description of eastern Canadian wine regions by Shaw (1999). However, as the industry has expanded, research interest has followed. Cover crop and fertility treatments have been assessed, finding benefits to yield and sugar levels from several cropping and amendment combinations (Messiga et al. 2015; Messiga et al. 2016). Diez-Zamudio et al. (2021b) compared the performance of cold-hardy *Vitis* hybrid cultivars to *V. vinifera* cultivars, finding tolerance to spring frost in L'Acadie blanc. Yield, bunch weight, and berry weight of locally grown cultivars were additionally found to be related to soil nutrients; higher tissue accumulations of boron, potassium, and magnesium were correlated with increased yields of L'Acadie blanc (Diez-Zamudio et al. 2021a). Control of pests in local vineyards has also been explored (Hillier and Lefebvre 2012; Poojari et al. 2020; Forge et al. 2022).

Regarding the potential microbial terroir of the region, Kernaghan et al. (2017) surveyed fungal endophytes of locally grown *Vitis* leaves and found substantial populations of the yeasts *Rhodotorula*, *Dioszegia*, *Sporobolomyces*, and *Aureobasidium* in vineyards, as well as several fungal isolates that inhibited grapevine pathogens. Subsequent research has identified additional foliar fungal endophytes with bioactivity against *B. cinerea* from "feral" wine grape vines growing in Nova Scotia (Ali et al. 2024). The vineyard root microbiome in the Annapolis Valley region of Nova Scotia has also been evaluated, uncovering large proportions of the fungal groups *Fusarium* (as *Gibberella*) and *Mycena* in cover crop roots, Nectriaceae in grape roots, and *Pseudaleuria*, *Heydenia*, *Trichsporon*, and Mortierellaceae and *Mortierella* in the surrounding

soils (Wright et al. 2022). These aspects of the microbial community may be linked to the chemical composition of Nova Scotia wines as described above by Gjelaj (2019).

1.53 Economic contributions of the wine industry in Nova Scotia

Winemaking is a rapidly growing industry in Nova Scotia. As of 2024, there were 24 commercial wineries, the oldest of which was established only 40 years ago, and 13 began operations within the past 15 years (Table S1.1). More than 485 ha of vineyards produced approximately 1442 metric tonnes of wine grapes at harvest in 2020, to make almost 2 million litres of wine (Wines of Nova Scotia 2024). Over $163 million in business revenue is generated yearly by the Nova Scotia wine and grape industry, with an additional $32 million in tax revenues and more than 1100 people provided full-time employment (Wines of Nova Scotia 2024). Local wine sales within Nova Scotia have been continuously rising for at least the past decade, increasing by 23.5% between 2021 and 2023 (Nova Scotia Liquor Corporation 2021; 2023).

Local wineries attract more than 150 000 visitors to the region each year, generating additional tourism revenue (Wines of Nova Scotia 2024). There are numerous wine tour packages, events, and experiences offered in the province, as well as the "NS Wines Explorer App" (Nova Scotia 2023). The Nova Scotia Ministry of Business has identified local wineries as a world-class tourism strength, but scientific research has so far trailed the accelerated growth of the industry (Nova Scotia 2023). Innovations in winemaking have the potential to benefit economic profits, job creation, and tourism in Nova Scotia, especially in the Annapolis Valley region where 18 of the 24 wineries are located.

1.54 Opportunities for continued growth of the Nova Scotia wine industry

There is an ongoing push by consumers and government for food products that are more sustainable and health conscious in their production, and wine is no exception (Tait et al. 2019; Fabbrizzi et al. 2021; Valenzuela et al. 2022). For wine, environmental sustainability is primarily addressed at the level of cultivation, by choosing cultivation practices that use less water, less fuel, and fewer and less pesticides. Hybrid grape cultivars (i.e., hybrids of American vines and *V. vinifera*, as well as complex hybrids), which constitute most vines planted in Nova Scotia (Diez-Zamudio et al. 2021b), are more resistant than *V. vinifera* against multiple grapevine diseases including powdery mildew, phylloxera, Pierce's disease, and Esca (Duley et al. 2023), reducing the need for pesticides. Nova Scotia wines produced using hybrid grapes could be well positioned to appeal to consumers in countries with increasing environmental regulations, such as those impacted by the European Green Deal 2050 (Töpfer and Trapp 2022) and the European Union's "Farm to Fork" strategy (Rossi 2020).

Organic cultivation, including that of grapes, also aims to be ecologically sustainable and sustain and enhance human health (Provost and Pedneault 2016; Standards Council of Canada 2021). Although specifics may vary among countries, organic wines are reliably made from grapes grown without synthetic pesticides and fertilizers, and because "organic" is a legal designation in many countries and labelling is well regulated, consumers can consistently identify organic wines (Jones and Grandjean 2018; Schäufele and Hamm 2018). Segments of consumers in many countries are willing to pay more for, or preferentially seek out, organic wines, including in Brazil (Araujo et al. 2017), Chile (Valenzuela et al. 2022), Germany (Schäufele and Hamm 2018), Italy (Pagliarini et al. 2013; D'Amico et al. 2016; Boncinelli et al. 2019; Vecchio et al. 2023) Switzerland (Mann et al. 2012; Deneulin and Dupraz 2018) and the

USA (Tait et al. 2019), often citing that organic wines are perceived as “healthier” or more premium (Cravero 2019). Indeed, organic wines may contain lower levels of pesticide residues, copper and other metals, sulfites, and other contaminants compared to conventional wines, although results are inconsistent (Provost and Pedneault 2016; Cravero 2019).

In Nova Scotia, organic production seemingly has a neutral effect on the preference of local wine consumers (Jantzi and McSweeney 2019). However, organic farming has suffered from delayed development in North America compared to Europe and preferences for organic products take longer to become apparent in rural regions than urban centres (Gagnon 1999). Furthermore, members of the millennial generation, who may place higher importance on sustainability attributes, are still gaining market share (Galati et al. 2019; Tait et al. 2019). Therefore, it is likely the demand will increase locally and that organic Nova Scotia wines could increasingly appeal to more urban North American and European markets.

Consumers driven by health concerns also demand food products that are, or seem to be, more “natural”, by virtue of containing fewer additives or having simplified production methods (Rozin et al. 2004; Renner et al. 2012; Fabbrizzi et al. 2021). Wines marketed in this manner may vary as wineries choose to implement different “natural” methods. However, generally, the term “natural wine” is understood to refer to a wine made with organic grapes and minimal winemaking interventions, including no or minimal physical processes such as filtration and clarification, and no additives (Legeron 2020). Although there is debate regarding the labelling and marketing of such wines, they may carry the legal designation “natural method wines” in France as of 2020 (Alonso González et al. 2022), and Canada may ultimately also implement an official description, as wineries in Nova Scotia are already using terms like “natural”, “no sulfites added”, “low/no intervention”, “nothing added”, “least manipulated”, “raw”, “wild

ferment", and "living wine", even declaring the "absence of unnecessary processing interventions" and "most natural wine in Canada" (Benjamin Bridge 2024; Nova Scotia L'Acadie Vineyards 2024).

As with the organic designation, some consumers prefer "natural wines" or wines they simply perceive as more "natural" in some way, especially in Italian markets (Galati et al. 2019; Migliore et al. 2020; Palmieri et al. 2023; Vecchio et al. 2023). For example, sulfites are often singled out as a negative component to be avoided in wines, and consumer surveys consistently reveal a higher willingness to pay for wines without added sulfites (Costanigro et al. 2014; D'Amico et al. 2016; Amato et al. 2017; Deneulin and Dupraz 2018; Migliore et al. 2020). This is mainly due to perceptions that sulfites are related to negative health effects such as headaches (Costanigro et al. 2014).

Finally, there are also consumers willing to pay more for "original" wines and wines that express terroir (Capitello et al. 2021), especially when provided multiple levels of information such as an added in-person description that mentions terroir (Angelini et al. 2023). These consumers may be attracted to "natural" wines for the influence of microbial terroir. The marketing of wines regarding consumer preferences, sustainability, and health is complex, as organic wines, "natural" wines, and "natural wines" overlap in their production methods but different consumers value different components of those methods (Tait et al. 2019). Furthermore, consumers consistently rate price as an essential consideration when purchasing a bottle of wine, so other preferences are moderated by cost (Mann et al. 2010; Costanigro et al. 2014; Galati et al. 2019; Jantzi and McSweeney 2019; Tait et al. 2019). However, the Nova Scotia wine industry can take cues from shifting consumer preferences worldwide and adapt that knowledge to better produce and market wines locally and in other regions.

1.6 Objectives of this PhD research

1.61 Summary of gaps in knowledge

The relatively new establishment of the Annapolis Valley and other Nova Scotian winemaking regions corresponds with an absence of research regarding yeast communities in our local cool climate vineyards growing hybrid vines. We lack information concerning the community composition of grape yeasts in Nova Scotia vineyards, and how traditional components of terroir, such as cultivation practice, impact the microbial terroir. Increased knowledge in this field would help local vineyards and wineries make management decisions and should inform new inoculation options that result in novel, high-quality wines unique to the region.

1.62 Thesis objectives

To resolve:

1. Considering L'Acadie blanc grape musts of the Annapolis Valley, NS, how do measures of yeast species diversity, richness, and the community composition differ (a) between years, (b) between organic and conventional cultivation practices, and (c) among vineyards?
2. How does the yeast community in L'Acadie blanc grape must from an organic vineyard in the Annapolis Valley, NS, change with fermentation, as assessed by two sequencing platforms (Illumina and PacBio)?
3. Regarding basidiomycete yeasts associated with wine grapes, (a) what is the record of species reported from grapes, musts, and fermentations, (b) what are their current and potential applications in winemaking, and (c) what challenges exist in evaluating their community composition patterns?

1.7 Supplementary materials

Table S1.1. Operational commercial wineries in Nova Scotia as of February 2024. Year of establishment is provided according to best available public information.

Region	Winery	Year Established
Acadian Shore	Maison Meuse et Fils	2014
Annapolis Valley	1365 Church Street Vineyard & Winery	2021
	Beausoleil Farmstead	2019
	Bent Ridge Winery	2018
	Mercator Vineyards	2018
	Gaspereau Vineyards	2012
	Muwin Estate Wines	2011
	Luckett Vineyards	2011
	Planters Ridge Winery	2011
	Avondale Sky Winery	2009
	Lightfoot & Wolfville Vineyards	2009
	Beaver Creek Vineyard	2008
	Blomidon Estate Winery	2007
	Bear River Vineyards	2005
	Casa Nova Fine Beverages	2005
	L'Acadie Vineyards	2004
	Grand Pré Winery	2000
	Benjamin Bridge Vineyards	1999
	Lost Bell Winery (formerly Sainte-Famille Winery)	1990
Cape Breton	Eileanan Brèagha Vineyards	2012
Northumberland	Jost Vineyards	1983
South Shore	District 33 Winery	2019
	Petite Rivière Vineyards	2004
	Lunenburg Country Winery	1993

Chapter 2: Yeast communities of a North American hybrid wine grape differ between organic and conventional vineyards

Table of Contents

Chapter 2 preface

Formatting

Information in the “Materials and methods” (section 2.2) that was not included for publication but that is essential to Chapter 2 is included in the format of a block quotation, copied from Chapter 3. This is due to the chronology of the associated publications: the later Chapter 2 publication (2024) cites methods that were included in the earlier Chapter 3 publication (2023).

2.1 Introduction

Wine grape skins in the vineyard host a diverse community of yeasts, which remain metabolically active during fermentation of the must, contributing to the flavour and aroma of the resulting wine – the "microbial terroir" (Bokulich et al. 2014; Knight et al. 2015). Oxidative yeasts like *Rhodotorula* spp. and semi-fermentative yeasts such as *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* are initially abundant in fermentations, but their growth is inhibited as alcohol concentration rises and oxygen level decreases. Highly fermentative yeasts, however, such as species of *Lachancea*, *Saccharomyces*, and *Torulaspora*, are more competitive and can persist throughout the fermentation (Borren and Tian 2021). The final wine is therefore influenced by the entire community of yeasts present, both directly, through the production of volatile compounds, and indirectly through competitive interactions (Bokulich et al. 2016; Knight et al. 2018; Liu et al. 2021).

However, most commercial wineries alter the diversity and abundance of yeasts when they inoculate with *Saccharomyces cerevisiae* and add SO_2 to achieve consistent fermentation and reduce spoilage microorganisms (Raspor et al. 2002; Lange et al. 2014; Windholtz et al. 2021b). These practices may modify wine flavour profiles (Varela et al. 2009; Liu et al. 2016), possibly even reducing appeal (Çelebi Uzkuc et al. 2020; Lu et al. 2020), and are at odds with the increased consumer demand for so-called "natural wines" produced using grapes from organic vineyards with minimal interventions (González and Parga-Dans 2020; Palmieri et al. 2023). These wines are allowed to spontaneously ferment without additives that influence the naturally existing microbiota present in musts. Although these fermentations are often still completed by *Saccharomyces* (which may persist within wineries despite existing in low

numbers in the vineyard), other yeasts that are present may exert a more profound influence on wine flavour and aroma in “natural wines” (Ciani et al. 2004; Lu et al. 2020).

Prior to grape harvest, vineyard yeast communities are impacted by interacting factors such as climate, which varies from year to year, soil properties, geographic location, and cultivar (Bokulich et al. 2014; Steenwerth et al. 2021). The use of organic vs. conventional cultivation practices is also a major distinction in vineyard management that impacts vineyard yeast communities (Bagheri et al. 2015; Agarbati et al. 2019). Organic vineyards are more likely to employ methods to improve the overall biodiversity of the agricultural system, such as the use of mulches, cover crops, and organic fertilizers (Provost and Pedneault 2016), and may attempt to control pests with a combination of copper and sulfur based fungicides (Craig 2022). Conventional vineyards, on the other hand, may use a wide variety of commercially available synthetic fungicides, pesticides, and herbicides, often applying several different products to target a range of pests (Craig 2022).

Tolerance to fungicides varies among yeasts. Some, like the generalist *Aureobasidium pullulans*, seem to be broadly tolerant. while others, like the common semi-fermentative yeast *H. uvarum*, may be sensitive to fungicides used in both conventional and organic vineyards (Setati et al. 2015; Grangeteau et al. 2017; Agarbati et al. 2019). Still other yeasts may be more susceptible to either an organic or a conventional fungicide regime, such as *Pichia terricola* and *M. pulcherrima*, which can be more abundant under organic cultivation or in conventionally cultivated vineyards, respectively (Milanović et al. 2013; Agarbati et al. 2019).

The impact of cultivation practices on wine grape yeasts is further moderated by interactions with the grape cultivar (Griggs et al. 2021; Sumby et al. 2021), which has its own independent effects on the yeast community (Nemcová et al. 2015; Tronchoni et al. 2022).

Despite this, almost all wine grape yeast community research has been in relation to cultivars of *Vitis vinifera* (Drumonde-Neves et al. 2021), as opposed to other *Vitis* species and hybrids. However, due to their improved disease resistance, hybrid cultivars are projected to be an essential component of the shift to more sustainable agriculture, demanded by both consumers and changing pesticide regulations (Bavaresco and Squeri 2022). *Vitis* hybrids also facilitate the development of emerging cool climate wine regions due to their improved cold tolerance (Bradshaw et al. 2018); for example, they are the most planted cultivars in Canada and in the American mid-western and northern states (McCole 2022). To the best of our knowledge, grape yeast communities have not been compared between organic and conventionally cultivated hybrid vines in North America.

Also, the body of work that has examined the influence of cultivation practices on vineyard yeast communities remains largely based on pure cultures (Sumby et al. 2021), despite the increased accuracy and depth of community analyses provided by next generation sequencing technologies (Belda et al. 2017). Furthermore, studies that have used next generation sequencing methods have generally considered yeasts as only one component of the entire fungal community (including filamentous fungi), potentially underestimating differences in low abundance yeasts.

Our objective was to use Illumina amplicon sequencing data to compare the species diversity and community composition of the yeast communities of L'Acadie blanc hybrid grapes collected over multiple years from conventionally and organically cultivated vineyards in the Annapolis Valley, Nova Scotia, Canada.

2.2 Materials and methods

2.21 Sample collection. preparation. and processing

From 2018 to 2020, L'Acadie blanc grapes (a cold-hardy hybrid white variety commonly grown in Nova Scotia and used in the Tidal Bay appellation) were supplied at commercial harvest maturity by eight actively functioning vineyards in the Annapolis Valley region of Nova Scotia (Table 2.1). Two vineyards (V3 & V8) shared the same management, but otherwise all vineyards were independent. We classified vineyards as using organic (n=3) or conventional (n=5) cultivation practices, although there was variation within these designations. The greatest distance between vineyards is approximately 70 km (Table S2.1), and therefore all sites experienced similar environmental and climatic conditions. The region is a lowland valley sheltered from coastal wind and fog, characterized by a relatively short and cool growing season (Table S2.2), with coarse to fine loamy-gravelly soils (Shaw 1999).

Table 2.1. Cultivation practice (organic or conventional) of each vineyard (V1A-V8) and grape collections contributing to the multi-year (2018-2020), and 2021 datasets.

		Organic				Conventional				
		V1A	V1B	V6	V7	V2	V3	V4	V5	V8
Each collection = 1 sample (divided into 4 technical sequencing replicates)	2018				●	●		●	●	●
	2019	●		●	●	●	●	●	●	
	2020			●						●
Each collection = 3 replicate samples	2021		●	●	●	●	●	●	●	●

For the first three years of the study, a bulk sample of grapes (minimum 5 kg) was supplied from each vineyard (total of 14 samples; Table 2.1) and sorted for ripeness, destemmed by hand while wearing clean gloves, transferred to sterile bags, and crushed using a stomacher blender (Seward 400 C, Fermionx Ltd., UK) to obtain unfiltered juice (must; the substrate used in winemaking). Four 10 ml sub-samples were taken from each sample as technical sequencing replicates for DNA analysis.

As the grape samples available from 2018-2020 were limited to a single location within each vineyard by practical constraints on vineyard participation, vineyards were resampled in 2021 to determine the within-vineyard variability. During the 2021 sampling, three 0.5 kg samples of grapes were collected from random locations within each vineyard (total of 24 samples), and musts obtained using the same methods as above (i.e., sorted, destemmed, and crushed) for DNA analysis. No technical sequencing replicates were produced in 2021. The area of L'Acadie blanc vines represented by the three samples (as well as by each bulk sample collected from 2018-2020) was <6 ha for each vineyard (Table S2.1). Vineyard 1 could not be resampled so a geographically separate vineyard, managed by the same organic winery, was sampled in 2021. These vineyards are referred to as V1A (2019 sampling) and V1B (2021 sampling).

Our results comprise two independent datasets due to the different type of replication in each sampling regime. The 2018-2020 sampling allowed for comparisons between cultivation practices and years, while the 2021 sampling allowed for comparisons between cultivation practices and among vineyards.

2.22 DNA extraction, PCR, sequencing, and sequence data processing

DNA was extracted from 1.8 ml aliquots of all grape must samples using the DNeasy UltraClean Microbial Kit following the manufacturer's protocol (Qiagen 2017). PCR reactions using the forward ITS86 (Turenne et al. 1999) and reverse ITS4 (White et al. 1990) primers were performed on all samples to ensure presence of amplifiable fungal DNA. A single 2020 V8 technical replicate was omitted from sequencing due to poor amplification. All remaining samples underwent short amplicon sequencing at the Integrated Microbiome Resource genomics facility at Dalhousie University, according to standard protocols (Op De Beeck et al. 2014; Comeau et al. 2017), using the Illumina MiSeq platform (paired-end mode). Typical sequencing output is approximately 20-22 M raw reads and 13 Gb of sequences, or ~50 000 reads per sample. Sequencing covered the ITS2 region, again using primer pair ITS86(F) and ITS4(R), resulting in sequences of approximately 400-500 bp (300+300 bp with 100-200 bp overlap). All sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database with the BioProject accession number PRJNA860361. Sequencing data were processed and assessed using FastQC 0.11.8 (Andrews et al. 2017) and the SEED 2 analysis pipeline (Seed2.1_64bit; Větrovský et al. 2018) and filtered for yeast species as described in Bunbury-Blanchette et al. (2023):

> All sequences in each file were trimmed to the length at which the median quality value fell below 28 (Sanger/Illumina 1.9 encoding). […] Briefly, the following steps were completed using SEED 2 for Illumina files. (1) Sequences in each file were trimmed according to the quality value identified in FastQC, (2) pair-end data files were joined, (3) all individual sequences with a quality value lower than 30 or a base pair quality value

lower than 10 were removed, (4) sequences were de-replicated, the fungal ITS2 region was extracted, and sequences were re-replicated, (5) sequences were clustered into operational taxonomic units (OTUs) by complete-link clustering (UPARSE) and the most abundant sequence from each cluster, excluding singletons, was found, and (6) OTUs were identified as the top NCBI GenBank BLASTn hit.

Manual phylogenetic binning was used to further clarify the taxonomy of *Saccharomyces* sequences acquired by Illumina sequencing. A selection of sequences of currently recognized *Saccharomyces sensu stricto* species (*S. arboricola*, *S. cerevisiae*. *S. eubayanus*, *S. jurei*. *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. uvarum*; Alsammar and Delneri 2020) were obtained from the NCBI GenBank database and used along with a selection of our PacBio *Saccharomyces* sequences to create a maximum parsimony tree using PAUP* 4.0b10 (Swofford 2003). Illumina sequences initially identified as representing *Saccharomyces* species through BLASTn were then added to the tree for clarification of their species assignment.

Species assignments of all sequences making up ≥1% of any replicate were also confirmed by manual NCBI GenBank BLASTn searches. Sequences that could not be identified below the phylum level were considered "unassigned". For analysis of yeasts, Kurtzman and Fell (2006), Sterflinger (2006), Choudhary and Johri (2009), Boekhout et al. (2011), Kurtzman (2011), Kurtzman et al. (2015), and Li et al. (2020) were consulted to create a list of fungal classes, orders, families, and genera with a yeast growth form. The names of all yeasts, and of other fungal species making up ≥1% of any replicate, were checked and updated according to www.mycobank.org and www.indexfungorum.org. In cases of disagreement on current species name, additional

references were consulted on a case-by-case basis. A final list of 406 yeast genera and 113 higher order taxonomic divisions was produced and used to filter yeasts from filamentous fungi (Table A2; Table A3). (Section 3.23)

The OTU rarefaction curves of all 2018-2020 technical replicates and of all 2021 samples were asymptotic, and all libraries were retained without being normalized, in order to conserve all data (McMurdie and Holmes 2014; Weiss et al. 2017).

2.23 Statistical analyses and data visualization

All statistical analyses were run, and all figures were created, using R version 4.2.2 (R Core Team 2022). Data from technical sequencing replicates within the multiple year sampling dataset (2018-2020) were pooled to reflect the 14 samples collected, an approach that was supported by the similarity of yeast community composition among replicates originating from the same sample (differences among samples assessed by PERMANOVA: R^2=0.724, p=0.001).

Plots were made using the GGPLOT2 package (Wickham 2016), in addition to the "metaMDS" function in the VEGAN package (Oksanen et al. 2022) for the non-metric multidimensional (NMDS) ordinations. Both datasets were visualized using stacked bar plots and NMDS biplots based on Bray-Curtis dissimilarities to display community composition. Boxplots were used to show differences in relative sequence abundance of certain taxonomic groups.

Both datasets were assessed by PERMANOVA based on Bray-Curtis dissimilarity and distance matrices with 999 permutations with the "adonis2" function in VEGAN to assess statistically significant differences in community compositions. Species diversity was determined by the "fisher.alpha" function in VEGAN, and statistical differences in species diversity and richness were determined by t-tests. Indicator species were determined using the "multipatt"

function specified by func = "r.g." within the INDICSPECIES package (De Cáceres and Legendre 2009).

Using the 2018-2020 dataset (sampling over multiple years), differences in community composition, species diversity, and species richness were assessed between years and cultivation practices. As there were only two samples taken in 2020, these were omitted and differences according to year were assessed by comparing the 2018 and 2019 samples.

The 2021 dataset (with within vineyard replication) was assessed for differences in community composition between cultivation practices and among vineyards (sites). Differences in species diversity and richness between conventional and organic samples were also determined.

The relative abundance of selected taxonomic groups was also compared between cultivation practices for both datasets by t-tests. Selected groups adhered to the following criteria, in either dataset: (1) at least one species belonging to the group was present in at least two samples of either cultivation practice at a proportion of ≥3% of total sequences, (2) considering samples, at least one species belonging to the group was present in its lowest proportion at ≥2X its highest proportion in the other cultivation practice, (3) no species belonging to the group adhered to criterion "2" with the cultivation types reversed.

2.3 Results

2.31 Sampling over multiple years (2018-2020)

The total number of sequences per sample ranged from 189 135 to 1 006 795 before filtering for yeast species, and from 88 618 to 705 259 after filtering, with a median sample size of 197 953 yeast sequences. Of the 300 yeast species present, 209 were identified to species, 67

to genus, and 24 could only be identified to family, order, or class. Overall, the most diverse yeast genera were *Dioszegia*, *Papiliotrema*, *Candida*, *Mrakia*, *Rhodotorula*, and *Sporobolomyces*, each with ≥10 species. The most common species considering overall number of sequences was *A. pullulans* (1 567 408), followed by *Sporobolomyces shibatanus* (397 369), *Vishniacozyma carnescens* (268 299), *Filobasidium magnum* (249 533), *Filobasidium globisporum* (223 027), and *Rhodotorula glutinis* (191 793).

Of the abundant species (≥3% of the total yeast community in any one sample), *F. globisporum* was exclusively found in organic vineyards, while *F. magnum* and members of the genera *Sporobolomyces* and *Rhodotorula* were more abundant in conventionally cultivated vineyards (Figure 2.1). All abundant fermentative yeasts (*Lachancea thermotolerans*, *Saccharomyces uvarum* and *Torulaspora delbrueckii*) were restricted to samples from a single vineyard (V8).

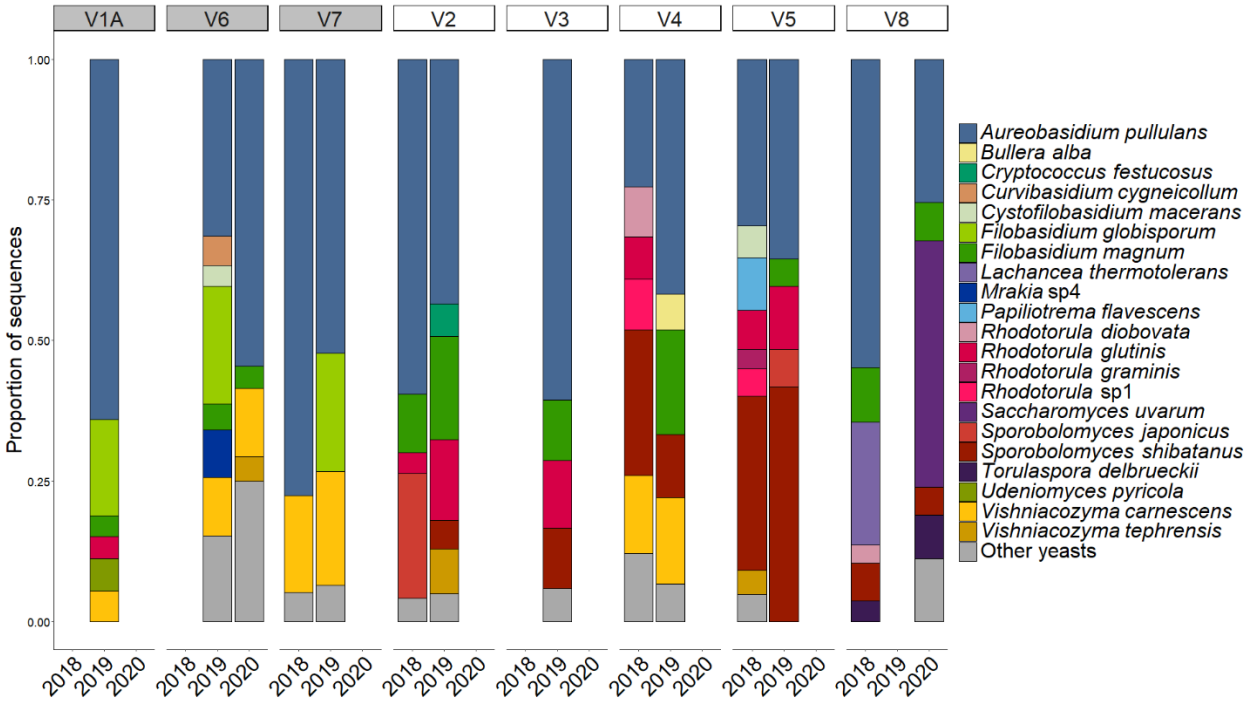

Figure 2.1. Yeast community composition of samples collected from 2018-2020. Only species making up ≥3% of the community in any individual sample are shown. Organic vineyards have gray headings (V1A, V6, V7) and conventional vineyard have white headings (V2, V3, V4, V5, V8).

Species diversity (t-test p=0.030) and richness (t-test p=0.020) differed between 2018 and 2019 but did not differ between organic and conventional cultivation. However, there was a significant effect of cultivation practice (PERMANOVA R^2=0.171, p=0.016) as well as sampling year (PERMANOVA R^2=0.142, p=0.035) on yeast community composition (Figure 2.2).

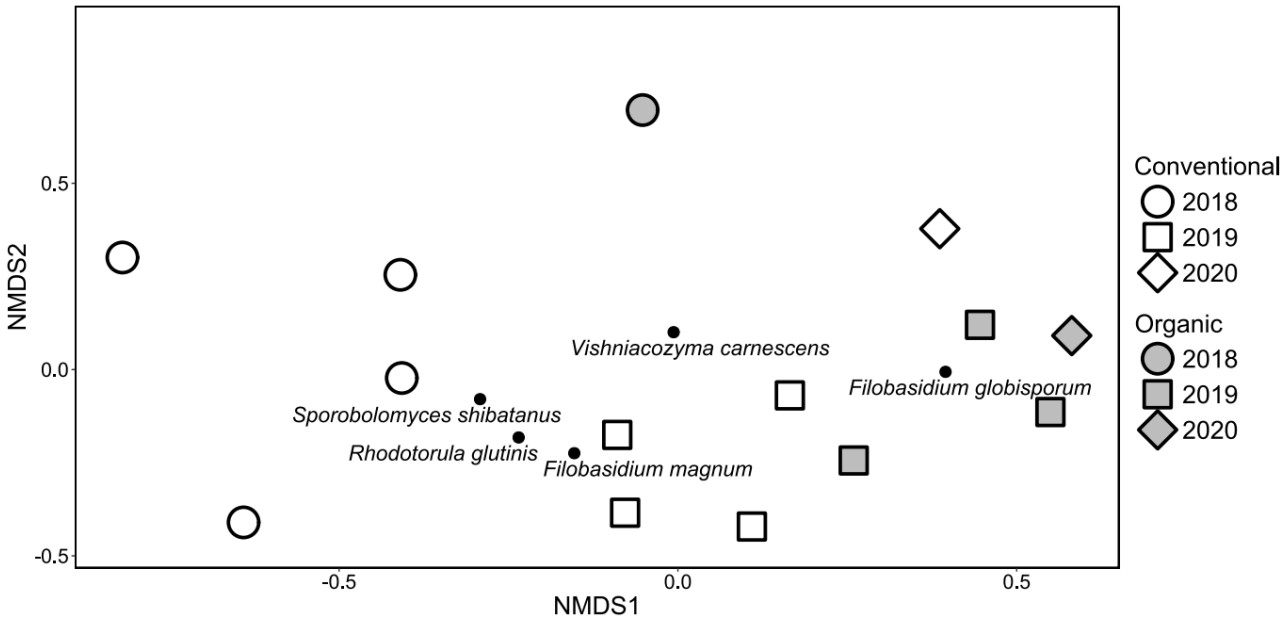

Figure 2.2. NMDS plot of vineyard yeast communities of samples collected from 2018-2020. Each point represents a unique vineyard and year combination. Representative species of taxonomic groups that differed in abundance by cultivation practice are shown.

The relative abundance of Sporidiobolales (*Rhodosporidiobolus*, *Rhodotorula* and *Sporobolomyces*) sequences (species listed in Table S2.3) was significantly higher (t-test p=0.002) in conventional vineyards ($\bar{x}$=28.6% of sequences) than in organic vineyards ($\bar{x}$=2.4%) (Figure 2.3A). At the species level, the abundance of *F. magnum* was higher in conventional vineyards (conventional $\bar{x}$=8.8%, organic $\bar{x}$=2.6%, t-test p=0.013) (Figure 2.3B). In contrast, *F. globisporum* was significantly more abundant in organic vineyards (conventional $\bar{x}$=0.05%, organic $\bar{x}$=12.1%, t-test p=0.038) (Figure 2.3C).

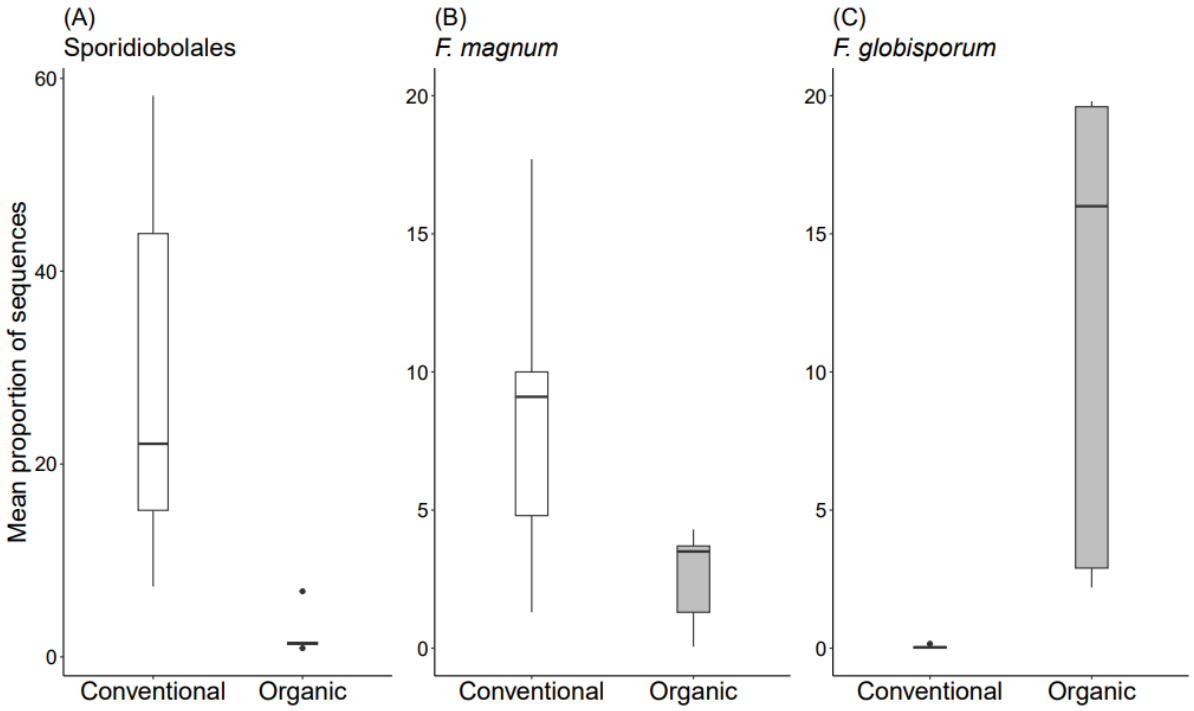

Figure 2.3A-C. Differences in mean proportions of (A) Sporidiobolales, (B) *F. magnum* and (C) *F. globisporum* sequences per sample for conventional and organic vineyards, of samples collected from 2018-2020.

Indicator species analysis determined that *R. glutinis* and *S. shibatanus* were significantly associated with conventional cultivation (Table S2.4). Twenty-three species were identified as indicator species of organic cultivation (Table S2.4), but of those, only *F. globisporum* and *V. carnescens* were present at ≥3% of the community in any one sample. Of all vineyards, only V6 (organic cultivation) had indicator species, and none were present at ≥3% of the community: *Metschnikowia* sp1, *Symmetrospora symmetrica*. and *Vishniacozyma* sp1 (Table S2.5).

2.32 Sampling for within vineyard variation (2021)

Within vineyard samples ranged from 4131-71 151 total sequences and 2090-70 700 yeast sequences. The median sample size was 15 640 yeast sequences. Yeasts were represented by 156 species, 127 of which were identified to the species level, 27 to the genus level, and two

which could only be identified to class or order. The most diverse genera, considering all samples, were *Papiliotrema*, *Rhodotorula*, *Sporobolomyces*, *Cystobasidium*, *Filobasidium*, and *Holtermanniella*, each with ≥5 species. The most common species by overall sequence count were *S. shibatanus* (151 000), *F. magnum* (96 633), *Rhodotorula babjevae* (75 004), and *Tilletiopsis washingtonensis* (23 595).

Filobasidium chernovii, *Filobasidium stepposum*, and the genus *Symmetrospora* were found only in organic vineyards. Other highly abundant species were found in both vineyard types but were more abundant in one or the other; *F. globisporum* and *T. washingtonensis* were more abundant in organic vineyards, while *F. magnum*, *S. shibatanus*, and the genus *Rhodotorula* were more abundant in conventional vineyards (Figure 2.4).

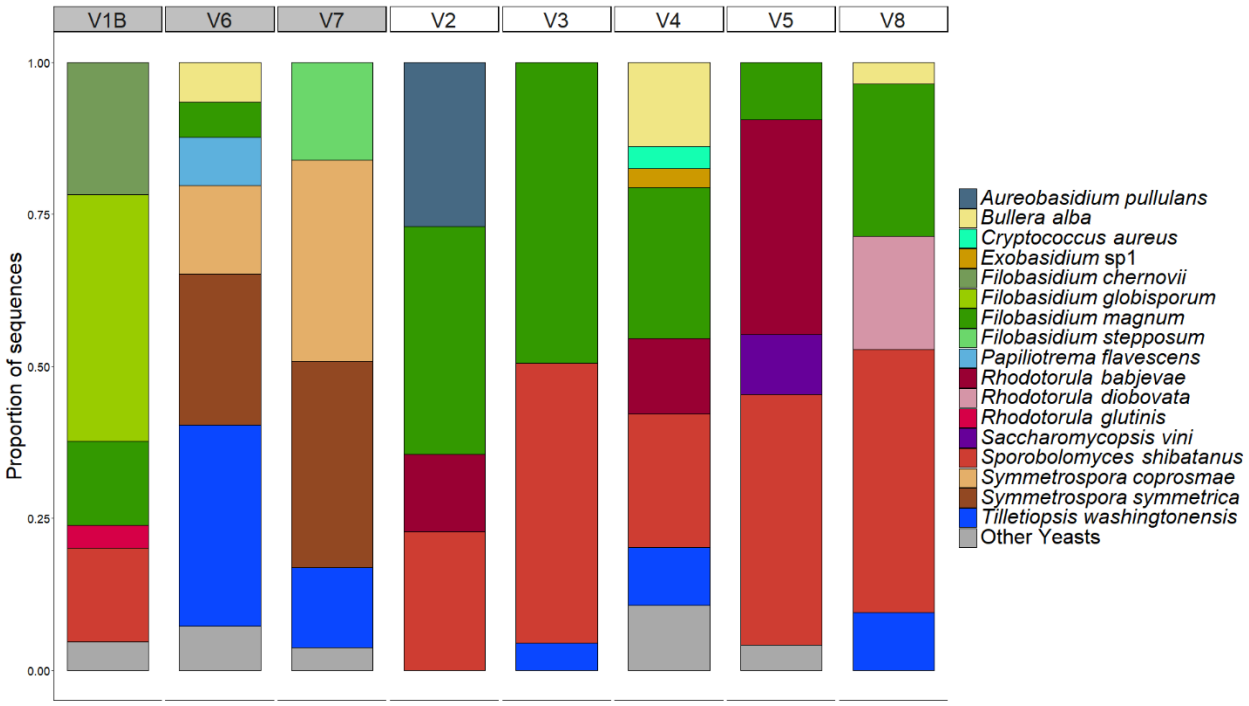

Figure 2.4. Yeast community composition of each vineyard, based on samples from 2021. Only species comprising ≥3% of the yeast community in any individual vineyard are included. Organic vineyards have gray headings (V1B, V6, V7) and conventional vineyards have white headings (V2, V3, V4, V5, V8).

Yeast community composition was significantly different among individual vineyard sites (PERMANOVA R^2=0.702, p=0.001) as well as between organic and conventionally cultivated vineyards (PERMANOVA R^2=0.262, p=0.001) (Figure 2.5).

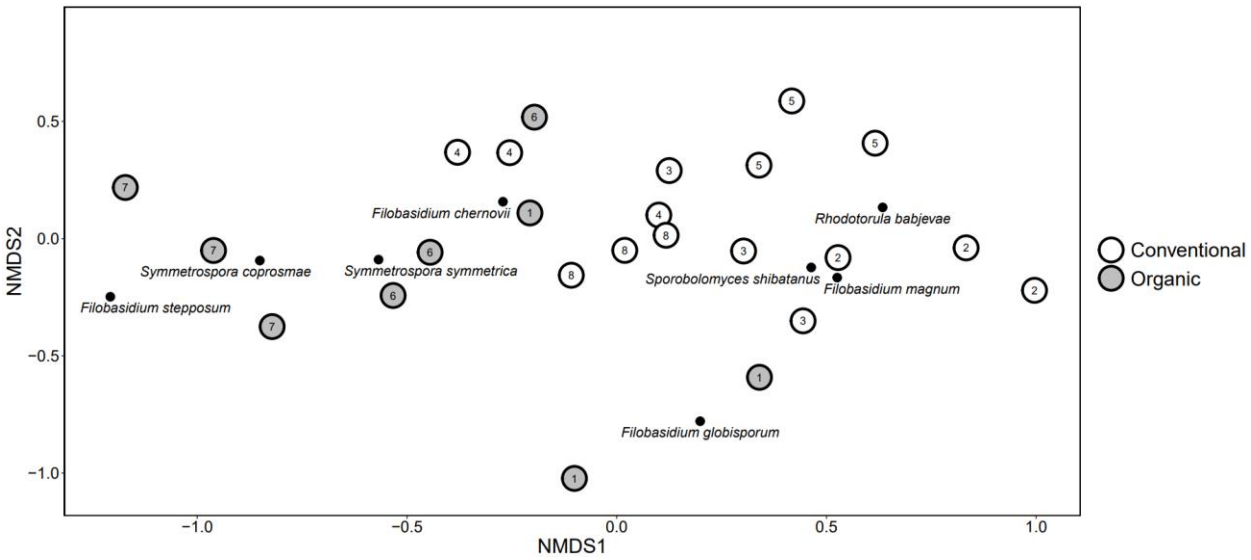

Figure 2.5. NMDS plot of vineyard yeast communities. Each point represents one of three replicate samples taken from each of the eight vineyards (V1B-V8 abbreviated to 1-8) in 2021. Representative species of taxonomic groups that differed in abundance by cultivation practice are shown.

As with multi-year sampling data, neither species diversity nor richness differed significantly with cultivation practice, and the abundance of sequences from the Sporidiobolales (Table S2.3) was again significantly higher (t-test p<0.001) in conventional vineyards ($\bar{x}$=51.1% of sequences) than in organic vineyards ($\bar{x}$=9.0%) (Figure 2.6A), as was the abundance of *F. magnum* sequences (conventional $\bar{x}$=27.5%, organic $\bar{x}$=8.4%, t-test p=0.002) (Figure 2.6B). Also as in the multi-year data, other *Filobasidium* species (*F. chernovii*, *F. globisporum* and *F. stepposum*) were more abundant (p=0.019) in organic vineyards ($\bar{x}$=23.5%) than conventional vineyards ($\bar{x}$=0.5%) (Figure 2.6C). The abundance of sequences belonging to the genus

Symmetrospora was also significantly higher (t-test p=0.012) in organic vineyards ($\bar{x}$=31.6%) than in conventional vineyards ($\bar{x}$=0.1%) (Figure 2.6D). This trend was also present in the multi-year data, although the difference was not significant (t-test p=0.16).

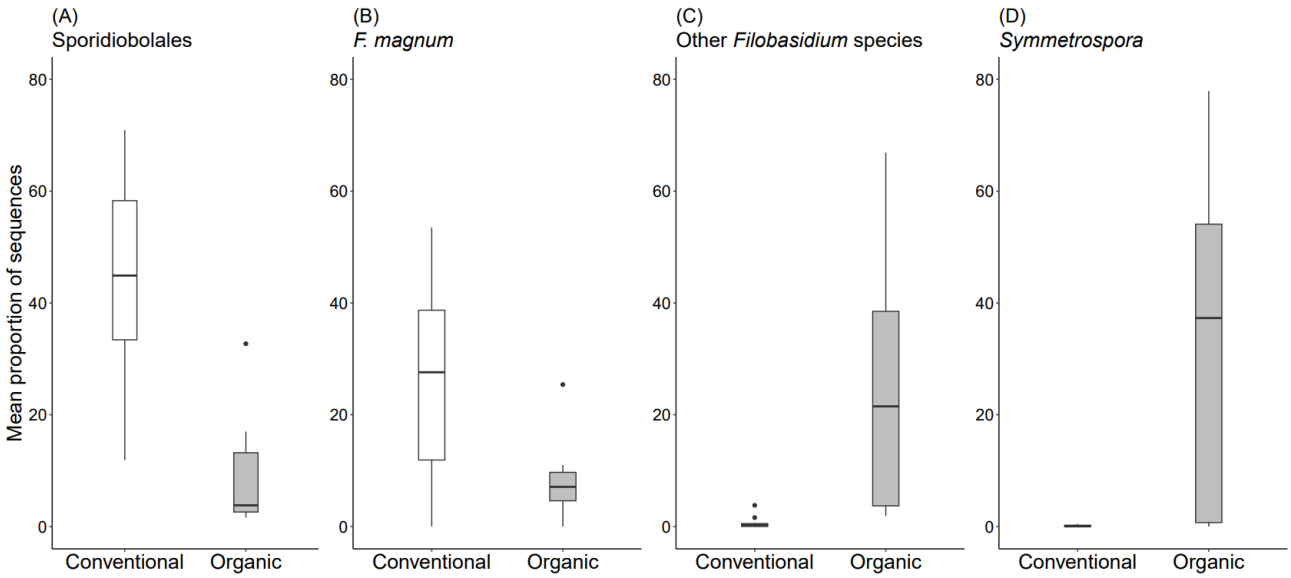

Figure 2.6A-D. Differences in mean proportions of (A) Sporidiobolales, (B) *F. magnum*, (C) other *Filobasidium* species, and (D) *Symmetrospora* sequences per sample, for conventional vs. organic vineyards.

Eight and three yeast species were indicators of conventionally and organically cultivated vineyards, respectively (Table S2.6). The indicator species present at ≥3% of the community were *F. magnum*, *S. shibatanus*, and *R. babjevae* in conventional vineyards, and *Symmetrospora coprosmae* and *S. symmetrica* in organic vineyards (Table S2.6). Five of the eight vineyards sampled also had indicator yeast species; those present at ≥3% were *B. alba* in V4, *R. babjevae* in V5, and *S. coprosmae* and *F. stepposum* in V7 (Table S2.7).

2.4 Discussion

Our assessment of hybrid L'Acadie blanc grapes in Nova Scotia detected novel differences between the yeast communities of organic and conventionally cultivated vineyards, despite variation in procedures within these designations, as well as between sampling years and among individual vineyards. These differences were driven by basidiomycete yeasts, with conventional vineyards characterized by larger populations of Sporidiobolales and *F. magnum*, and organic vineyards supporting *Filobasidium* species other than *F. magnum*, and larger populations of *Symmetrospora*.

Aside from these differences, the patterns of yeast species diversity detected on Nova Scotia hybrid grapes are broadly comparable to that of vineyards producing other cultivars in other locations. Studies that use next generation sequencing methods consistently detect high proportions of *A. pullulans* and *H. uvarum*, followed by relatively high, but more varied, proportions of the basidiomycete yeast genera *Filobasidium*, *Sporobolomyces*, *Rhodotorula* and *Vishniacozyma*, as well as the ascomycete genera *Saccharomyces* and *Starmerella* (Table S2.8). Similarly, *A. pullulans* and the genus *Vishniacozyma* were present in high proportions in our multiple year dataset, and the genera *Filobasidium*, *Sporobolomyces*, and *Rhodotorula* were abundant in both the multiple year and 2021 datasets. The recently described genus *Symmetrospora* (Wang et al. 2015b) was also abundant in our 2021 data. This genus also appears in the literature under the previous classifications *Sporobolomyces* and *Rhodotorula*.

The notable absence of *H. uvarum*, a semi-fermentative ascomycete typically reported in moderate to high abundances on grapes (Drumonde-Neves et al. 2021), may be due to reduced detection by some next generation sequencing methods (Costantini et al. 2022; Bunbury-Blanchette et al. 2023), or may reflect the fact that our grapes were mostly of good health and

remained intact until pressing, as overripe and damaged grapes promote the growth of fermentative yeasts (Barata et al. 2012). Conversely, *T. washingtonensis* was abundant in our study but is rarely reported from other vineyard environments. A single vineyard (V8) from the multiple year data supported relatively large populations of fermentative ascomycete yeasts, but this was also the only vineyard that used mechanical harvesting methods, resulting in a visible increase in damage prior to grape pressing, which appeared to lead to the onset of fermentation prior to DNA extraction.

Although additional sampling involving increased biological replication within vineyards and across multiple years would have been desirable, as in other studies comparing vineyard yeast community data over time (Steenwerth et al. 2021; Castrillo and Blanco 2022), we detected significant year to year variation in yeast diversity, species richness, and community composition (between 2018 and 2019). We also observed differences in community composition between the multiple year samples and 2021 samples. This variation is likely linked to fluctuations in yearly climatic conditions, as temperature, evapotranspiration, wind, and relative humidity influence vineyard yeast communities (Bokulich et al. 2014; Brilli et al. 2014). For example, the Annapolis Valley region experienced warmer conditions throughout the summer months of 2018, compared to 2019 (Environment and Climate Change Canada).

Yeast community composition was also significantly different among individual vineyards, as suggested by the visible clustering of vineyards in the multiple year data and confirmed to have a strong influence by the high PERMANOVA R^2 value (0.702) assigned to vineyards in our 2021 data. Yeast community composition is consistently correlated with individual vineyards (Cureau et al. 2021b; Wang et al. 2021a; Li et al. 2022), regardless of the distance between them (Jiraska et al. 2023).

Although several studies have reported higher diversity in organic vineyards relative to conventionally managed vineyards (Tello et al. 2012; Setati et al. 2012; Martins et al. 2014; Bagheri et al. 2015; Setati et al. 2015), we found no significant difference in either yeast species diversity or species richness between vineyard types from our multiple year or our 2021 sampling. However, some studies agree with our findings, showing no difference in yeast diversity with cultivation practice (Kecskeméti et al. 2016; Castañeda et al. 2018; Rantsiou et al. 2020; Castrillo and Blanco 2022) or even report higher yeast diversity in conventionally managed vineyards (Milanović et al. 2013). Collectively, these inconsistent results indicate that cultivation practice is likely not a primary influence on vineyard yeast species counts or diversity index values.

However, unlike species diversity and richness, species composition of yeast communities differed significantly between the vineyards using organic and conventional cultivation practices, based on both of our data sets (multiple year and 2021). This is consistent with research in other regions, although the assemblages of species associated with either organic or conventional cultivation are highly variable, likely due to factors such as climate, geography, and grape cultivar (Sumby et al. 2021). For example, Perpetiuni et al. (2022) found that the genera *Zygoascus*, *Zygosaccharomyces*, and *Candida* were absent from organic grape samples, while *Saccharomyces* and *Vishniacozyma* were only in organic and not conventional grape samples, while Rossetti et al. (2023) found nearly opposite results in the same Italian municipality when sampling a different cultivar. However, in both these cases, as well as other similar studies (Setati et al. 2015; Castañeda et al. 2018), vineyard yeast community composition, unlike species diversity, was significantly influenced by cultivation practice. It is notable that this pattern also held true for our data, even though we did not assign causality to

specific methods within the designations of organic and conventional, and a high proportion (>80%) of the abundant yeasts were basidiomycetes.

The differences in yeast species composition between our organic and conventional vineyards were attributed to the abundances of three basidiomycete taxonomic groups: (1) the Sporidiobolales, including species of *Rhodosporidiobolus*. *Rhodotorula*, and *Sporobolomyces*, (2) the *Symmetrospora* species *S. coprosmae* and *S. symmetrica*, and (3) the *Filobasidium* species *F. magnum*. *F. chernovii*, *F. globisporum*, and *F. stepposum*. Species of Sporidiobolales (class Microbotryomycetes) were more abundant in conventional vineyards and were identified as indicator species. A similar result was found in a comparison of grape yeast communities between organic and conventional vineyards in the south of France, in which the genus *Sporidiobolus* was more frequently isolated from conventional vineyards (Martins et al. 2014). These authors suggested that *Sporidiobolus* may be sensitive to copper, and increased copper levels in organic vineyards may reduce its incidence. Similarly, Čadež et al. (2010) found no long-term effects of the systemic fungicides pyrimethanil and cyprodinil on populations of the generally abundant Sporidiobolales species *R. glutinis* and *S. shibatanus* (as *Sporidiobolus pararoseus*) or on *F. magnum* (as *Cryptococcus magnus*), and Kernaghan et al. (2017) found larger populations of *Sporidiobolus* sp. and *Rhodotorula mucilaginosa* on grapevine leaves in conventional vineyards relative to organic vineyards.

Conversely, we found that species of *Symmetrospora* (class Cystobasidiomycetes) were more abundant in, and were indicator species of, organic vineyards. A similar result was reported by Englezos et al. (2022), in which *Symmetrospora* sp. was identified more frequently under treatment conditions akin to organic cultivation, containing both sulphur and copper fungicides but excluding the synthetic fungicide Metiram, and *Symmetrospora oryzicola* was most

frequently identified in a no treatment condition, when compared to conventional fungicide regimes against downy and powdery mildew. Finally, the abundance of the genus *Filobasidium* (class Tremellomycetes) in the two cultivation practices differed at the species level. *F. magnum* was more abundant in, and was an indicator species of, conventional vineyards. All others (*F. globisporum*, *F. chernovii*, and *F. stepposum*) were more abundant in organic vineyards, and *F. globisporum* was an indicator species. These differences are supported by Čadež et al. (2010), described above, and Rantsiou et al. (2020), who found that the abundance of *F. magnum* was reduced under a sulfur-intensive anti-fungal regime similar to conventional cultivation, compared to regimes in which sulfur was applied fewer times and in combination with other products.

We note that while the yeast community composition of an organic site (V7) sampled in 2018 did not group closely in the NMDS plot with the other organic vineyards, it also did not group with the conventional vineyards, and exhibited a low proportion of Sporidiobolales and the presence of *F. globisporum* rather than *F. magnum* (features that defined organic vineyards). The dissimilarity to the other organic samples seems to be best explained by the lower diversity of this sample rather than by an increased abundance of any defining species or taxonomic group. The NMDS plot of the 2021 data also displayed two outlier samples from V1B (organic), which may be explained by their high proportions of *F. globisporum*, characteristic of organic vineyards. While one of these grouped somewhat more closely to the conventional samples due to higher levels of *S. shibatanus* and *F. magnum*, the other V1B outlier did not group closely with any other samples.

As with fermentative yeasts, the species composition of basidiomycete yeasts within a vineyard will impact the wine flavour and aroma. However, the influence of basidiomycete yeasts is often not considered (Tempère et al. 2018; Lappa et al. 2020; Borren and Tian 2021;

Comitini et al. 2021). For example, there are no data available on the influence of individual *Filobasidium* species on wine fermentation, although members of the order Filobasidiales produce pectinases (*Piskurozyma capsuligena*; Merín et al. 2014) and lipoxygenases (*F. magnum*; Wu et al. 2023), which improve several organoleptic aspects of winemaking, including the release of desirable flavour compounds. The importance of other basidiomycete yeasts, even those that are well known from vineyards and wineries, has not been thoroughly explored (Drumonde-Neves et al. 2021). However, *Sporobolomyces roseus* isolated from vineyard grapes produces multiple volatile compounds that confer pleasant sensory qualities to wine (Verginer et al. 2010), *S. shibatanus* is of interest for winemaking due to its β-glucosidase activity (Kot et al. 2021), and *R. mucilaginosa* has been used in mixed inoculations with *S. cerevisiae* to enhance wine aroma due to its high glycosidase activity (Wang et al. 2017).

To date, research that has evaluated wine grape yeasts in relation to cultivation practices has either only considered culturable yeasts, and often with a secondary focus, e.g., fermentative yeasts or strains of *Saccharomyces* (Cordero-Bueso et al. 2011; Tello et al. 2012) or, when next generation sequencing was used, yeasts have often not been evaluated independently from the broader fungal community, including filamentous fungi. Furthermore, there has been no previous comparison of yeast communities between the conventional and organic cultivation of North American hybrid grape cultivars, despite an expected increase in the need for hybrid vines in expanding cool climate regions, as well as an increasing demand for disease resistant hybrid vines worldwide.

Our description of yeast communities associated with the cold hardy, disease resistant L'Acadie blanc hybrid grape addresses this gap in research. The consistencies between our datasets, and between comparisons of abundant taxonomic groups and indicator species analysis,

suggest that we have revealed patterns in basidiomycete yeasts communities that reflect differences between organic and conventional vineyards and among vineyard sites. The potential importance of basidiomycete yeasts in winemaking is not well understood and we propose further exploration of their presence in vineyards and their oenological relevance.

2.5 Supplementary materials

Table S2.1. Latitude, longitude and the approximate total contiguous area of each vineyard, and area of L'Acadie blanc sampling.

Vineyard	Latitude & longitude	Approx. total area (ha)	Approx. sample area (ha)
V1A	45.16 N 64.42 W	16.9	5.4
V1B	45.06 N 64.38 W	16.8	<1.0
V2	44.92 N 65.16 W	9.0	4.9
V3	45.06 N 64.35 W	8.7	3.4
V4	45.10 N 64.30 W	7.5	<1.0
V5	45.06 N 64.48 W	2.7	[a]
V6	45.06 N 64.33 W	4.5	2.6
V7	45.10 N 64.33 W	1.0	<1.0
V8	45.10 N 64.32 W	17.5	5.5

[a]Rows of L'Acadie blanc are interspersed throughout V5.

Table S2.2. Monthly mean temperature (°C) and cumulative precipitation (mm) recorded by the Kentville CDA CS climate station, Nova Scotia, for each year in which sampling took place (Environment and Climate Change Canada).

	Monthly mean temperature (°C)					Monthly cumulative precipitation (mm)				
	May	Jun	Jul	Aug	Sep	May	Jun	Jul	Aug	Sep
2018	11.0	13.9	21.4	21.0	15.1	65.8	116.6	47.1	146.6	103.8
2019	8.1	15.4	20.0	19.6	14.2	108.4	72.1	49.8	137.2	134.0
2020	10.5	17.3	20.2	20.2	15.3	78.0	49.1	114.9	70.6	121.8
2021	10.8	18.5	18.9	20.4	16.7	108.9	32.8	164.3	121.7	168.7

Table S2.3. Species of Sporidiobolales present in our multi-year (2018-2020) and 2021 data sets.

Multi-year (2018-2020) data			2021 data		
Rhodosporidiobolus	*Rhodotorula*	*Sporobolomyces*	*Rhodosporidiobolus*	*Rhodotorula*	*Sporobolomyces*
R. azoricus	*R. babjevae*	*S. cellobiolyticus*	*R. colostri*	*R. babjevae*	*S. lactucae*
R. colostri	*R. dairenensis*	*S. japonicus*	*R. odoratus*	*R. chungnamensis*	*S. phaffii*
R. odoratus	*R. diobovata*	*S. musae*		*R. dairenensis*	*S. roseus*
R. oreadorum	*R. glutinis*	*S. phaffii*		*R. diobovata*	*S. ruberrimus*
	R. graminis	*S. roseus*		*R. glutinis*	*S. shibatanus*
	R. mucilaginosa	*S. ruberrimus*		*R. graminis*	*S.* species 1
	R. svalbardensis	*S. shibatanus*		*R. mucilaginosa*	*S.* species 2
	R. species 1	*S.* species 1		*R. nothofagi*	
	R. species 2	*S.* species 2		*R.* species 1	
	R. species 4	*S.* species 3		*R.* species 2	
Sporidiobolales sp1					

Table S2.4. Yeast indicator species (indicator values >0.400) associated with conventional and organic vineyard management, based on our data from 2018-2020.

Management	≥3%	Species	Indicator value	*p* value	
Conventional	Y	*Rhodotorula glutinis*	0.550	0.0301	
	Y	*Sporobolomyces shibatanus*	0.472	0.0401	
Organic	Y	*Aureobasidium subglaciale*	0.664	0.0053	*
		Mrakia aquatica	0.656	0.0053	*
		Vishniacozyma dimennae	0.641	0.0115	
		Occultifur species 1	0.637	0.0285	
		Genolevuria species 1	0.630	0.0194	
		Symmetrospora coprosmae	0.629	0.0066	*
		Dioszegia crocea	0.590	0.0152	
		Globoramichloridium indicum	0.584	0.0099	*
		Metschnikowia species 1	0.537	0.0285	
		Protomyces inouyei	0.531	0.0010	**
		Symmetrospora symmetrica	0.529	0.0032	*
		Filobasidium globisporum	0.524	0.0010	**
		Chrysozyma griseoflava	0.496	0.0285	
		Curvibasidium rogersii	0.483	0.0497	
		Filobasidium oeirense	0.482	0.0285	
		Tremellomycetes species 1	0.480	0.0094	*
		Cystobasidium laryngis	0.476	0.0295	
		Taphrina species 1	0.473	0.0285	
		Mrakia frigida	0.448	0.0171	
	Y	*Vishniacozyma carnescens*	0.444	0.0417	
		Tilletiopsis washingtonensis	0.434	0.0400	
		Vishniacozyma foliicola	0.422	0.0162	
		Tremellales species 4	0.415	0.0093	*

All *p* values <0.05. *p<0.01. **p<0.001.

Table S2.5. Yeast indicator species (indicator values >0.400) associated with vineyards, based on our data from 2018-2020.

Vineyard	≥3%	Species	Indicator value	*p* value	
V6		*Metschnikowia* species 1	0.988	0.0219	
		Symmetrospora symmetrica	0.948	0.0010	**
		Vishniacozyma species 1	0.936	0.0183	

All *p* values <0.05. *p<0.01. **p<0.001.

Table S2.6. Yeast indicator species (indicator values >0.400) associated with conventional and organic vineyard management considering our samples from 2021.

Management	≥3%	Species	Indicator value	*P* value	
Conventional	Y	*Filobasidium magnum*	0.725	0.0002	**
	Y	*Sporobolomyces shibatanus*	0.572	0.0002	**
		Saccharomyces cerevisiae	0.521	0.0061	*
		Erythrobasidium hasegawae	0.447	0.0116	
	Y	*Rhodotorula* species 1	0.417	0.0089	*
		Papiliotrema fusca	0.417	0.0121	
	Y	*Rhodotorula babjevae*	0.404	0.0229	
		Bullera alba	0.402	0.0441	
Organic		*Cystobasidium layrngis*	0.622	0.0001	**
	Y	*Symmetrospora coprosmae*	0.569	0.0004	**
	Y	*Symmetrospora symmetrica*	0.516	0.0001	**

All *p* values <0.05. **p*<0.01. ***p*<0.001.

Table S2.7. Yeast indicator species (indicator values >0.400) associated with vineyards, considering our samples from 2021.

Vineyard	≥3%	Species	Indicator value	*P* value	
V2		*Curvibasidium pallidicorallinum*	0.642	0.0049	*
V4	Y	*Pseudomicrostroma phylloplanum*	0.835	0.0122	
		Sporobolomyces roseus	0.820	0.0042	*
		Bullera alba	0.792	0.0117	
		Papiliotrema wisconsinensis	0.718	0.0108	
		Erythrobasidium hasegawae	0.675	0.0477	
		Holtermanniella species 1	0.580	0.0107	
V5	Y	*Rhodotorula babjevae*	0.917	0.0035	*
		Kondoa species 1	0.681	0.0122	
V6		*Taphrina* species 1	0.809	0.0038	*
V7	Y	*Symmetrospora coprosmae*	0.927	0.0035	*
		Occultifur species 1	0.899	0.0035	*
	Y	*Filobasidium stepposum*	0.846	0.0035	*
		Protomyces inouyei	0.755	0.0129	
		Meniscomyces layueensis	0.736	0.0112	

All *p* values <0.05. **p*<0.01. ***p*<0.001.

Table S2.8. Reports of genera present in >1 reference cited in this paper that assessed yeast communities of grapes or musts using NGS. Species reported by >1 reference are named.

Reported by	Genus and species
16	*Aureobasidium*
	A. pullulans (10)
2	*Buckleyzyma*
4	*Candida*
5	*Cryptococcus*
2	*Curvibasidium*
2	*Cystobasidium*
3	*Cystofilobasidium*
12	*Filobasidium*
	F. magnum (2)
	F. stepposum (2)
17	*Hanseniaspora*
	H. uvarum (8)
2	*Holtermanniella*
2	*Kazachastania*
4	*Lachancea*
	L. thermotolerans (2)
5	*Metschnikowia*
4	*Meyerozyma*
3	*Naganishia*
4	*Papiliotrema*
5	*Pichia*
	P. terricola (3)
2	*Rhodosporidiobolus*
10	*Rhodotorula*
	R. babjevae (2)
	R. glutinis (3)
12	*Saccharomyces*
	Sa. cerevisiae (8)
11	*Sporobolomyces*
	Sp. roseus (3)
8	*Starmerella*
	St. apicola (2)
	St. bacillaris (4)
2	*Symmetrospora*
3	*Torulaspora*
3	*Udeniomyces*
9	*Vishniacozyma*
	V. carnescens (2)
	V. victoriae (4)
2	*Wickerhamomyces*
	W. anomalus (2)
3	*Zygosaccharomyces*

Chapter 3: Yeast communities before and after spontaneous fermentation of wine grapes: a case study from Nova Scotia

Table of contents

Chapter 3 preface

Formatting

A figure and supplemental table that were not included in the published version of this chapter have been added here to demonstrate the phylogenetic binning method used to assign *Saccharomyces* taxonomy (Figure 3.1; Table S3.1) and subsequent figure and supplementary table numbers were amended accordingly.

3.1 Introduction

Wine fermentations are generally completed by the yeast *Saccharomyces cerevisiae*, due to its fermentation efficiency and tolerance to high alcohol and low oxygen (Fleet 2008). This is true not only for inoculated wines but also for spontaneous fermentations, even though *S. cerevisiae* may be present on only one of every thousand grapes (Mortimer and Polsinelli 1999; Fleet 2003). However, before *S. cerevisiae* dominates the fermentation, diverse communities of indigenous yeasts present on the grape skins can produce alcohols, glycerol, sulphur compounds, phenols and hundreds of volatile metabolites, influencing wine flavour, aroma, and texture (Verginer et al. 2010; Rossouw and Bauer 2016). Yeasts commonly found in the early stages of wine fermentation include the non-fermentative genera *Cryptococcus*, *Pichia*, and *Rhodotorula*, as well as the fermentative genera *Hanseniaspora*, *Metschnikowia*, *Kluyveromyces*, *Zygosaccharomyces*, and *Torulaspora* (Drumonde-Neves et al. 2021). Although these yeasts were once thought to negatively impact wine character, a growing body of research has established that spontaneous fermentation with indigenous yeasts can produce complex wines with positive sensory attributes (e.g., Çelebi Uzkuç et al. 2020; Lu et al. 2020). Furthermore, in some cases non-traditional fermentative yeasts such as *Saccharomyces uvarum* can complete the fermentation to produce appealing wines distinct from those dominated by *S. cerevisiae* (Tosi et al. 2009; Csoma et al. 2010).

The assemblage of indigenous yeasts supported by a given vineyard is governed by a complex set of interacting factors including geography, soil, and climate (Liu et al. 2019). Vineyard management factors such as grape variety, vine age, plant heath, and fungicide application are also important drivers of vineyard yeast communities (Pinto et al. 2014; Sumby et al. 2021). It is therefore not surprising that sampling detects significant site-to-site variation of

indigenous yeast communities among distant wine-making regions (Gayevskiy and Goddard 2012; Li et al. 2021; Steenwerth et al. 2021) as well as among more local vineyards (Garofalo et al. 2016; Mendes et al. 2017). Within Canada, vineyard yeast communities have been documented in the Niagara region of Ontario (Nurgel et al. 2004) and in the Okanagan Valley of British Columbia (Martiniuk 2020; Lyons 2021). However, even though Nova Scotia has a growing wine industry that cultivates unconventional cold tolerant hybrid grapes for the short growing season and cool climate, including the Tidal Bay appellation variety, L'Acadie blanc, there has yet to be any systematic evaluation of indigenous vineyard yeasts in Atlantic Canada.

The connections between the composition of a vineyard's indigenous yeast community and the characteristics of a wine from that vineyard have led to the concept of "microbial terroir" (Knight et al. 2015; Bokulich et al. 2016), and consumers are showing increased interest in wines defined by characteristic local yeasts through spontaneous fermentation (Jantzi and McSweeney 2019). Next generation sequencing is accepted as the best approach to characterize vineyard microbial communities, including low-abundance and difficult to culture yeasts (Belda et al. 2017). We used two different metagenomic sequencing systems (Illumina MiSeq and PacBio Sequel II) to help overcome inherent biases in next generation sequencing and obtain a clearer picture of the indigenous vineyard yeast communities. The PacBio system targets a longer rDNA region than the Illumina system, thus facilitating more accurate identification, but yields fewer sequences overall, resulting in lower sequencing depth that detects fewer rare species than Illumina sequencing (Furneaux et al. 2021).

A better understanding of Nova Scotia vineyard yeast communities would allow winemakers to make better use of non-traditional yeasts and spontaneous fermentations to produce wines with increased complexity and stronger regional identity. We therefore set out to

document yeast communities before and after the spontaneous fermentation of grape musts from an organic vineyard in the Annapolis Valley region of Nova Scotia. This approach provided information on the initial vineyard yeast community as well as the yeasts most likely involved in the fermentation process.

3.2 Materials and methods

3.21 Sample collection. preparation. and processing

L'Acadie blanc grapes (a cold-hardy hybrid white variety commonly grown in Nova Scotia and used in the Tidal Bay appellation) were supplied in September, at harvest, from a vineyard under organic management in the Annapolis Valley region of Nova Scotia. A minimum of 5 kg of grapes were collected in each 2018 and 2019. Grapes were sorted for ripeness, destemmed by hand while wearing sterile gloves, and then transferred to sterile bags and crushed using a stomacher blender (Seward 400 C, Fermionx Ltd., UK) to obtain unfiltered juice (must) which was then divided into four 0.5 L replicate volumes for fermentation. Prior to fermentation, a 10 ml sample was taken from each of these four replicates for DNA extraction and next generation sequencing. A fifth replicate from 2018 and 2019 musts was produced for pre-fermentation chemical evaluation. All replicates were then allowed to undergo spontaneous fermentation at 23°C.

One replicate fermenter included a container of desiccant attached to the airlock to retain water vapour and allow for loss of only CO_2. This fermenter was weighed every 3-4 days until stabilization, at which point fermentation for all replicates was considered complete. Wines stabilized after one week, with most fermentative activity occurring from days 2-7. Following

fermentation, a second 10 ml sample was taken from each of the same four fermenters initially sampled, for next generation sequencing. Samples were stored at -80°C until DNA extraction.

As the grape samples available in 2018 and 2019 were limited to a single location within the vineyard, a further three 0.5 kg samples were collected at different random locations within the vineyard in 2021 to determine variation in the yeast community within the vineyard. Musts were obtained using the same methods as above to produce 15 ml per sample, from which DNA was extracted for Illumina sequencing.

3.22 DNA extraction and PCR

DNA was extracted from the fresh musts and corresponding post-fermentation products using the DNeasy UltraClean Microbial Kit following the manufacturer's protocol (Qiagen 2017). Samples were vortexed for 5 s immediately before removing a 1.8 ml aliquot for DNA extraction. PCR reactions with forward (ITS86: GTGAATCATCGAATCTTTGAAC, Turenne et al. 1999) and reverse (ITS4: TCCTCCGCTTATTGATATGC, White et al. 1990) primers were performed on all samples to ensure presence of amplifiable DNA.

3.23 Sequencing and sequence data processing

Yeast communities present in grape musts and fermented products were characterized by next generation sequencing at the Integrated Microbiome Resource genomics facility at Dalhousie University, according to standard protocols (Op De Beeck et al. 2014; Comeau et al. 2017). All samples underwent short amplicon sequencing using the Illumina MiSeq system (paired-end mode), covering the ITS2 region using primer pair ITS86 and ITS4, resulting in sequences of approximately 400-500 bp (300+300 bp with 100-200 bp overlap). Typical sequencing output is approximately 20-22 M raw reads and 13 Gb of sequences, or ~50 000 reads per sample. Samples from 2019 also underwent full length amplicon sequencing covering

the ITS region using the PacBio Sequel system using forward and reverse primers ITS1FKYO2 and ITS4KYO1 (TAGAGGAAGTAAAAGTCGTAA; TCCTCCGCTTWTTGWTWTGC; Toju et al. 2012). Typical sequencing output is ~240-320 Gb of sequence per cell. All sequence data has been deposited in the NCBI SRA database with the BioProject accession number PRJNA860361.

FastQC 0.11.8 (Andrews et al. 2017) was used to view quality values across all bases at each position for each Illumina file. The SEED 2 analysis pipeline (Seed2.1_64bit; Větrovský et al. 2018) was used to assess sequencing data. All sequences in each file were trimmed to the length at which the median quality value fell below 28 (Sanger/Illumina 1.9 encoding). All PacBio sequences retained a sequence quality value above 28 throughout and so were not trimmed. Briefly, the following steps were completed using SEED 2 for Illumina files. (1) Sequences in each file were trimmed according to the quality value identified in FastQC, (2) pair-end data files were joined, (3) all individual sequences with a quality value lower than 30 or a base pair quality value lower than 10 were removed, (4) sequences were de-replicated, the fungal ITS2 region was extracted, and sequences were re-replicated, (5) sequences were clustered into operational taxonomic units (OTUs) by complete-link clustering (UPARSE) and the most abundant sequence from each cluster, excluding singletons, was found, (6) OTUs were identified as the top NCBI GenBank BLASTn hit. PacBio files were processed starting at step (3), using a base pair quality cut-off of 8. The OTU rarefaction curves of all replicates were asymptotic and differences among library sizes were considered relatively small, so all libraries were retained, and were not normalized in order to conserve all data (McMurdie and Holmes 2014; Weiss et al. 2017).

Manual phylogenetic binning was used to further clarify the taxonomy of *Saccharomyces* sequences acquired by Illumina sequencing. A selection of sequences of currently recognized *Saccharomyces sensu stricto* species (*S. arboricola*, *S. cerevisiae*. *S. eubayanus*, *S. jurei*. *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. uvarum*; Alsammar and Delneri 2020) were obtained from the NCBI GenBank database and used along with a selection of our PacBio *Saccharomyces* sequences to create a maximum parsimony tree using PAUP* 4.0b10 (Swofford 2003). Illumina sequences initially identified as representing *Saccharomyces* species through NCBI GenBank BLASTn were then added to the tree for clarification of their species assignment (Figure 3.1).

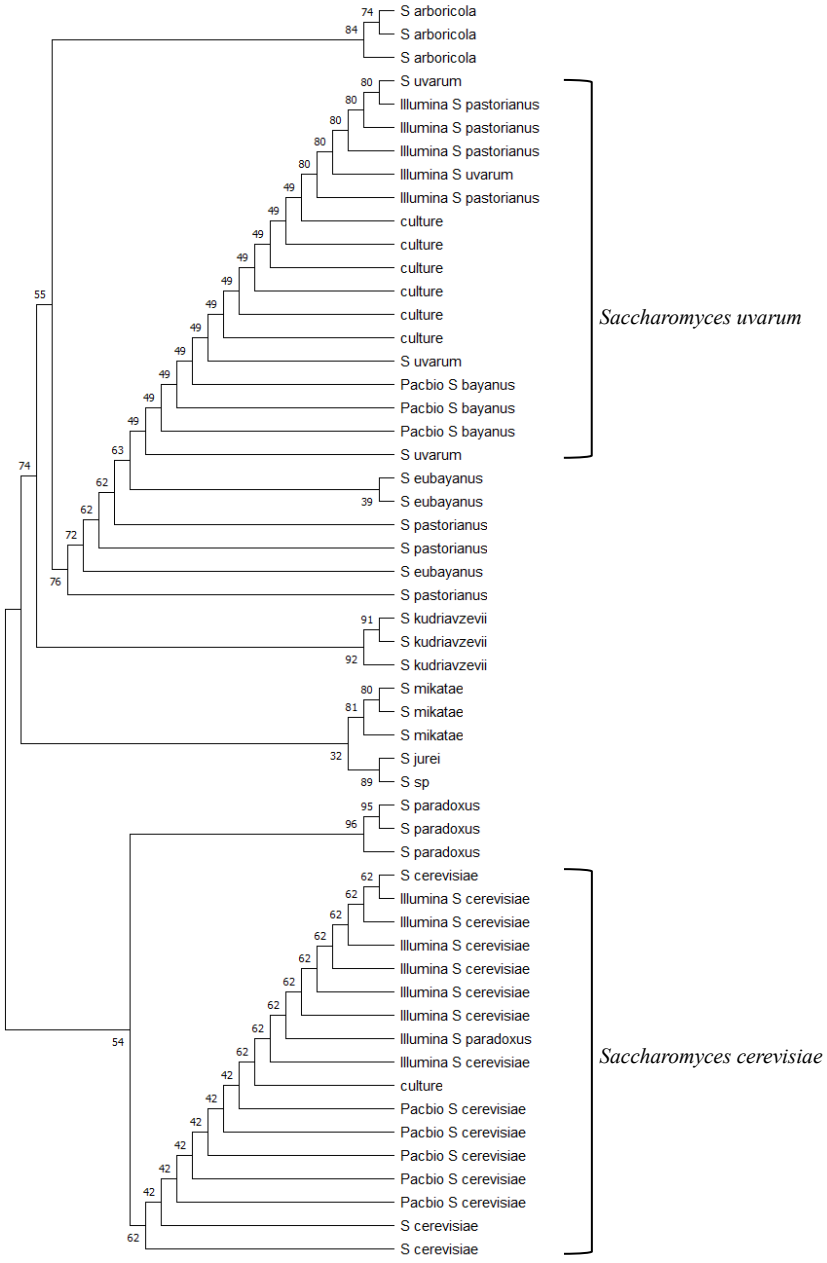

Figure 3.1. Maximum parsimony phylogenetic tree constructed using PAUP* 4.0b10 (Swofford 2003) using (1) sequences of the complete ITS rDNA region (ITS 1 & 2) of *Saccharomyces* species obtained from NCBI GenBank database (Table S3.1), (2) ITS 2 sequences obtained from Illumina sequencing of thesis samples, named according to their GenBank BLASTn assignment, (3) complete ITS sequences obtained from PacBio sequencing of thesis samples, named according to their GenBank BLASTn assignment, and (4) ITS 2 sequences obtained from individual cultures isolated from thesis samples. Assignments reflecting relevant species in the *Saccharomyces* species complex are shown to the right (Alsammar and Delneri 2020). Species placement informed the reassignment of sequences initially identified by BLASTn of thesis samples: *S. pastorianus* and *S. bayanus* were reassigned to *S. uvarum*, and *S. paradoxus* was reassigned to *S. cerevisiae*.

Species assignments of all sequences making up ≥1% of any replicate were also confirmed by manual NCBI GenBank BLASTn searches. Sequences that could not be identified below the phylum level were considered "unassigned". For analysis of yeasts, Kurtzman and Fell (2006), Sterflinger (2006), Choudhary and Johri (2009), Boekhout et al. (2011), Kurtzman (2011), Kurtzman et al. (2015), and Li et al. (2020) were consulted to create a list of fungal classes, orders, families, and genera with a yeast growth form. The names of all yeasts, and of other fungal species making up ≥1% of any replicate, were checked and updated according to www.mycobank.org and www.indexfungorum.org. In cases of disagreement on current species name, additional references were consulted on a case-by-case basis. A final list of 406 yeast genera and 113 higher order taxonomic divisions was produced and used to filter yeasts from filamentous fungi (Table A2; Table A3).

3.24 Chemical and sensory evaluation of wines

In 2018 and 2019, sub-samples from each of the four replicates were assessed for pH, % titratable acidity, % ethanol, and fructose and glucose contents before and after fermentation. The fifth replicate (not used in fermentation) was assessed by the same metrics to provide additional pre-fermentation data. Following spontaneous fermentation, the three replicates from the fermentations that did not have the air lock with desiccant (replicates 1, 2, & 3 in 2018; replicates 2, 3, & 4 in 2019) were filtered, transferred to sterile glass bottles, and stored at 4°C. No treatments were conducted to prevent oxidization. Panels of three (for 2018 samples) and nine (for 2019 samples) volunteers then tasted each sample and rated them for overall acceptability on a 9-point scale, noted sensory attributes, including floral, sweet, sour, vinegar, fruity, bitter, pungent, earthy, vanilla, and grassy, and ranked replicates from best to worst. Volunteers were not professionals and were not trained in tasting.

3.25 Statistical analyses and data visualization

All statistical analyses were run, and all figures were created, using R version 4.2.1 (R Core Team 2022). Statistically significant differences in community compositions were assessed by PERMANOVA based on Bray-Curtis dissimilarity using distance matrices with 999 permutations, with the "adonis2" function in the VEGAN package (Oksanen et al. 2022). Species diversity was determined by the "fisher.alpha" function in VEGAN and statistical differences in species diversity and richness were determined by paired t-tests. Community composition was visualised with stacked bar plots and alluvial plots, as well as NMDS ordinations showing differences based on Bray-Curtis dissimilarities. All plots were made using the GGPLOT2 package (Wickham 2016), in addition to the GGALLUVIAL package (Brunson and Read 2020) for the alluvial plots and the "metaMDS" function in VEGAN for the NMDS plots. To compare year to year differences in community compositions (2018-19) with differences among sampling locations (2021), average dispersions were determined using the "betadisper" function in VEGAN. The average dispersion of samples between years (one randomly chosen pre-fermentation replicate from each of 2018 and 2019) was then compared to the average dispersion of the 2021 within-site samples by analysis of variance (ANOVA). Regarding the sensory analysis, the overall acceptability of each replicate and the final rankings replicates per year were determined by calculating averages using each panelist's scores.

3.3 Results

3.31 Yeast community composition before and after spontaneous fermentation based on Illumina sequencing

After removal of low-quality sequences, a total of 2 543 340 sequences were obtained by Illumina sequencing from the 2018 and 2019 replicates taken before and after spontaneous fermentation. Replicate library sizes ranged from 69 615 to 423 420 sequences before fermentation, and from 106 154 to 198 184 sequences after fermentation. When all sequences were filtered for yeasts, 161 putative species were present, with 152 identified to at least genus, six to class, and three to order. Nine genera of the order Saccharomycetales were represented, along with six additional genera of other ascomycete yeasts including the "black" yeasts (e.g., *Aureobasidium*), contributing to a total of 28 ascomycete yeast species. Basidiomycete yeasts of at least 50 genera were also present, including the red yeasts (e.g., *Sporobolomyces*), smuts (e.g., *Ustilago*), and other fungi with an anamorphic yeast state (e.g., *Tremella*). A total of 133 basidiomycete yeast species were identified.

Prior to fermentation 155 yeast species were present (Table 3.1). The largest diversity of species in pre-fermentation replicates belonged to the genus *Dioszegia* (Table 3.2). A greater number of yeast species were present before fermentation in 2019 (134) than 2018 (76), although 58 species were common to both years, including all species of *Vishniacozyma*. After spontaneous fermentation, the number of yeast species declined to 70. At this stage, the genus with the greatest species diversity was *Vishniacozyma*, for which all pre-fermentation persisted into post-fermentation. A total of six species were found after fermentation that were not detected before fermentation, although five were represented by only 2-3 sequences, and the sixth

(*Rhodotorula graminis*) by 13 sequences. Again, more species were present in 2019 (63) than 2018 (24), with 18 species common to both years.

Table 3.1. Number of Basidiomycete and Ascomycete yeast species identified from Illumina and PacBio sequencing, and corresponding percentages of sequences that these groups represent.

		Pre-fermentation		Post-fermentation	
		Basidiomycete	Ascomycete	Basidiomycete	Ascomycete
Illumina	Number of species	132	23	58	12
	% sequences	46	54	2	98
PacBio	Number of species	29	4	15	7
	% sequences	16	84	7	93

Table 3.2. Species identified before and after spontaneous fermentation based on Illumina and PacBio sequencing (genera with >3 species[a]).

Pre-fermentation	Post-fermentation	Pre-fermentation	Post-fermentation
Cryptococcus	*Cryptococcus*	*Mrakia*	*Mrakia*
aureus	*aureus*	*aquatica*	*aquatica*
cellulolyticus	*cellulolyticus*	*cryoconiti*	*cryoconiti*
sp1		*frigida*	*frigida*
sp2		*hoshinonsis*	*hoshinonsis*
Cystobasidium	*Cystobasidium*	sp1	sp2
laryngis	*laryngis*	sp2	sp3
sp1		sp3	
sp2		*Papiliotrema*	*Papiliotrema*
sp3		*aspenensis*	*flavescens*
Cystofilobasidium	*Cystofilobasidium*	*flavescens*	*frias*
capitatum	*capitatum*	*frias*	*laurentii*
infirmominiatum	*infirmominiatum*	*laurentii*	*wisconsinensis*
macerans	*macerans*	*nemorosa*	
sp1		*wisconsinensis*	
Dioszegia	*Dioszegia*	sp1	
athyrii	*crocea*	sp2	
buhagiarii	*hungarica*	sp3	
butyracea		*Rhodotorula*	*Rhodotorula*
changbaiensis		*dairenensis*	*diobovata*
crocea		*diobovata*	*glutinis*
fristingensis		*glutinis*	*graminis*
hungarica		*mucilaginosa*	
rishiriensis		sp1	
takashimae		sp2	
xingshanensis		*Sporobolomyces*	*Sporobolomyces*
sp1		*japonicus* (P)	*roseus* (I&P)
sp2		*phaffii*	*ruberrimus* (P)
sp3		*roseus* (I&P)	*shibatanus*

Filobasidium	*Filobasidium*	*ruberrimus* (P)	
floriforme (P)[b]	*globisporum* (I&P)	*shibatanus* (I&P)	
globisporum (I&P)	*magnum* (I&P)	*symmetrica*	
magnum (I&P)	*oeirense*	*Taphrina*	
oeirense	*wieringae*	*inositophila*	
stepposum		*letifera*	
wieringae (I&P)		*sacchari*	
Metschnikowia		sp1	
pimensis		*Vishniacozyma*	*Vishniacozyma*
pulcherrima		*carnescens* (I&P)	*carnescens* (I&P)
sinensis		*dimennae*	*dimennae*
sp1		*foliicola* (I&P)	*foliicola*
sp2		*globispora*	*globispora*
		heimaeyensis	*heimaeyensis*
		tephrensis (I&P)	*tephrensis*
		victoriae (I&P)	*victoriae*
		sp1	sp1

[a]*Saccharomyces* was present but represented by ≤ 3 species.

[b](P) indicates species detection only by PacBio sequencing, (I&P) indicates species detection by both Illumina and PacBio sequencing. All other species were detected only by Illumina sequencing.

Yeast community composition based on Illumina sequencing was relatively consistent among fermentation replicates within and across years both before and after spontaneous fermentation (Figure 3.2). Based on Illumina sequencing, the most abundant yeast prior to fermentation was *Aureobasidium pullulans*, making up approximately 75% of all 2018 replicates and 50% of all 2019 replicates (Figure 3.2A). *Vishniacozyma carnescens* consistently accounted for approximately 20% of all must replicates and the genus *Filobasidium* was represented in all replicates at approximately 5% in 2018 and 25% in 2019. The remainder of all 2019 pre-fermentation replicates consisted of <5% each of the species *Curvibasidium cygneicollum*, *Cystofilobasidium captitatum*, *Symmetrospora coprosmae*, and *Udeniomyces pyricola*.

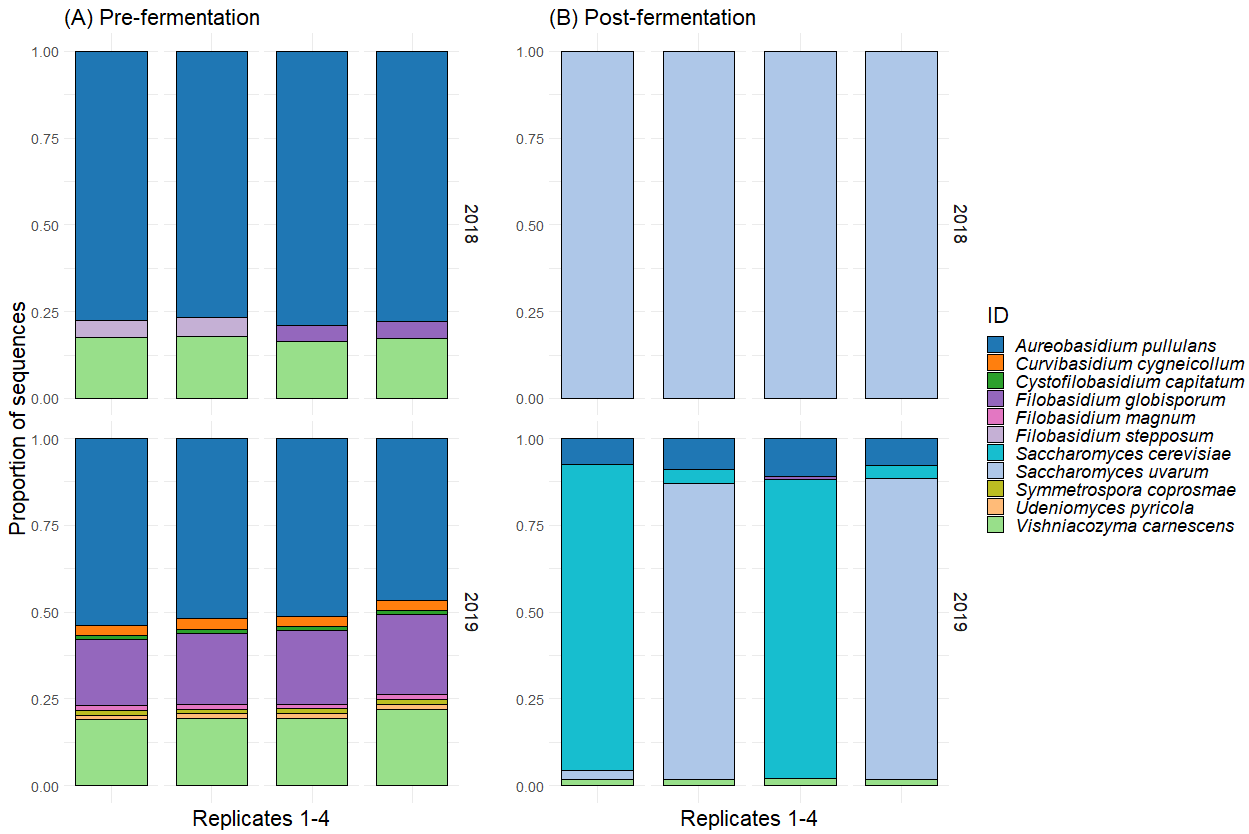

Figure 3.2. Yeast communities based on Illumina sequencing. Species making up <1% of each replicate are not shown. (A) Yeast sequence abundances in 2018 and 2019 pre-fermentation replicates. *S. uvarum* was present in all replicates at levels <0.1% and *S. cerevisiae* was detected in 2019 replicates 1, 2, and 4 at levels <0.01%. (B) Yeast sequence abundances in 2018 and 2019 post-fermentation replicates.

After fermentation, all 2018 replicates consisted of solely *S. uvarum*, excluding species making up <1% of each replicate (Figure 3.2B). Approximately 85% of all sequences in the 2019 post-fermentation replicates were identified as species of *Saccharomyces*, although two replicates were dominated by *S. cerevisiae* and two were dominated by *S. uvarum*. *A. pullulans* persisted in all 2019 replicates at levels of approximately 10%, as did *Vishniacozyma carnescens* at <5%. *Filobasidium globisporum* was also detected in a single post-fermentation replicate at <5%.

3.32 Yeast community composition before and after spontaneous fermentation based on PacBio sequencing

After removal of low-quality sequences, a total of 38 101 sequences were obtained by PacBio sequencing from the 2019 replicates taken before and after spontaneous fermentation. Replicate library sizes ranged from 3996 to 8499 sequences before fermentation and from 2768 to 4650 sequences after fermentation. When sequences were filtered for yeasts, 37 putative species were present, with 36 identified to at least genus and one to order. Four genera of the order Saccharomycetales were represented (*Candida*. *Hanseniaspora*. *Pichia*, and *Saccharomyces*), as were two additional ascomycete yeast genera (*Aureobasidium* and *Protomyces*). Basidiomycete yeasts were represented by 14 genera, including the red yeasts (e.g., *Sporobolomyces*).

Based on PacBio sequencing, a total of 33 yeast species were present prior to fermentation (Table 3.1), and 22 after spontaneous fermentation. The most diverse genera were *Filobasidium*, *Sporobolomyces* and *Vishniacozya*, each represented by four species before fermentation (Table 3.2). *Pichia kudriavzevii*, *S. cerevisiae* and *S. uvarum* each comprised at least 12% of at least one post-fermentation replicate but were not detected prior to fermentation. *Candida argentea* was also only detected post-fermentation but contributed only 2 sequences to a single replicate.

Yeast community composition was also relatively consistent among both pre- and post-fermentation replicates based on PacBio sequencing (Figure 3.3). Species composition of pre-fermentation replicates differed from the Illumina results due to the apparent increased detection of *Hanseniaspora uvarum* by PacBio. *H. uvarum* was the most abundantly detected species in all pre-fermentation replicates using the PacBio system, accounting for approximately 50% of all

sequences (Figure 3.3A). The relative abundances of the other species making up ≥1% of each replicate were comparable between sequencing systems, with *A. pullulans*, *F. globisporum* and *V. carnescens* in decreasing abundances, respectively.

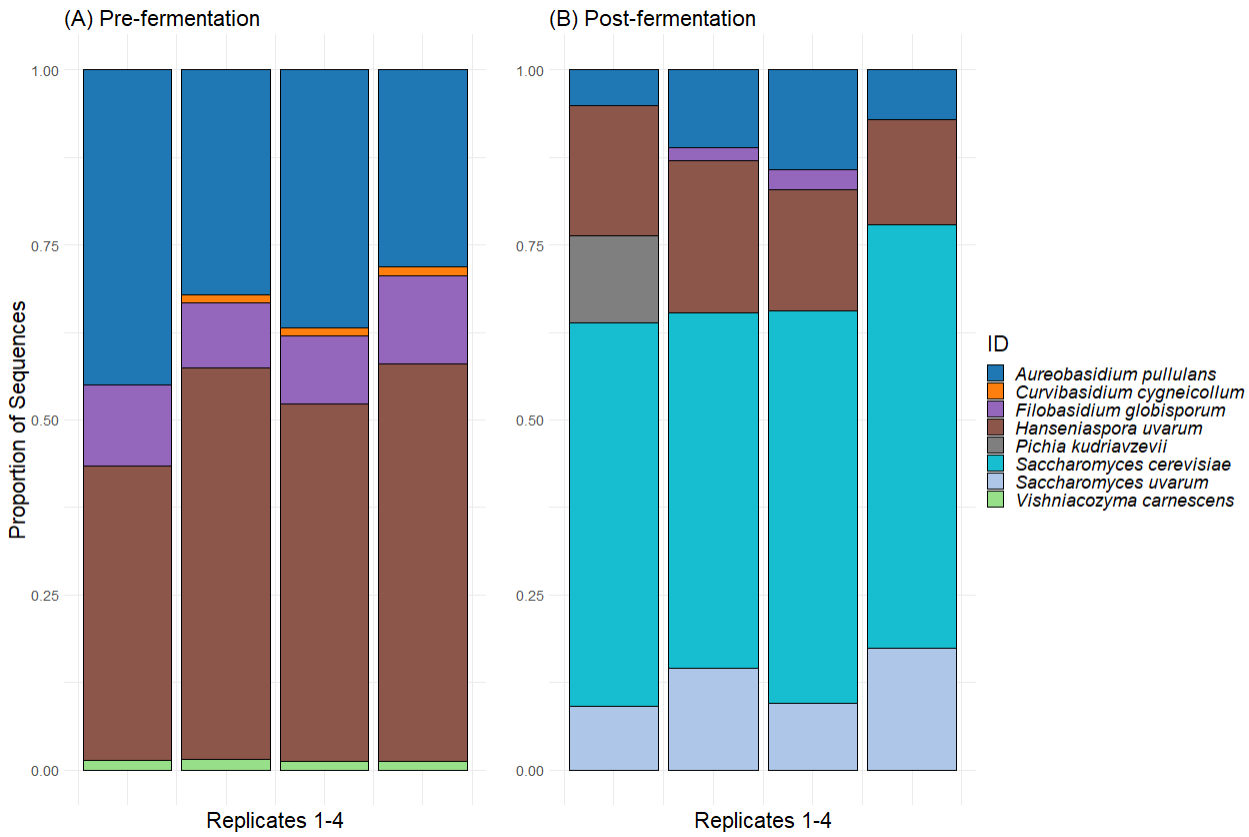

Figure 3.3. Yeast communities in 2019 based on PacBio sequencing. Species making up <1% of each replicate are not shown. (A) Pre-fermentation yeast sequence abundances. (B) Post-fermentation replicates.

All post-fermentation replicates analysed using the PacBio system were dominated by *S. cerevisiae*, making up approximately 50-60% of each replicate (Figure 3.3B). *S. uvarum* was also present in all replicates at lower abundances (approximately 10-20%). *A. pullulans* was present at 5-15% of sequences per replicate, and *F. globisporum* was present at <5% of two replicates, which was consistent with the Illumina sequencing results for both these species. The remainder

of the post-fermentation replicates, however, were comprised of *H. uvarum* at 15-20% consistently across replicates, and approximately 12% *Pichia kudriavzevii* in a single replicate. Neither of these species were detected by Illumina sequencing.

3.33 Fungal community based on Illumina and PacBio sequencing

The vineyard fungal community prior to fermentation, irrespective of replicates and in the context of all fungi, was composed of approximately 36% filamentous fungi (Figure 3.4A) based on Illumina sequencing. Species of the genus *Saccharomyces* were present, but only made up ~0.04% of sequences. After spontaneous fermentation however, the community became dominated by species of *Saccharomyces* (~92%) and filamentous fungi represented only ~2% of the post-fermentation community. Based on PacBio, filamentous fungi accounted for approximately 17% of the total vineyard fungal community before fermentation, decreasing to ~4% after fermentation (Figure 3.4B). The genus *Saccharomyces* was not detected by PacBio prior to fermentation, although we can assume it was present as the post-fermentation yeast community was ~64% *Saccharomyces*.

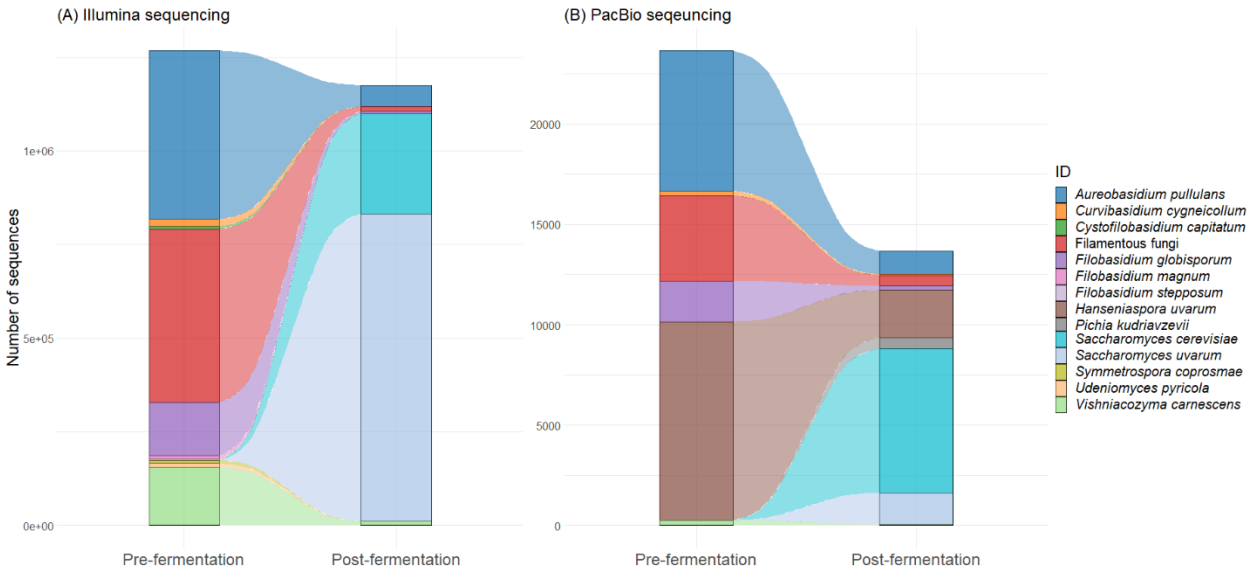

Figure 3.4. Community composition by number of sequences of yeasts and filamentous fungi pre- and post-fermentation. Species making up ≥1% of either the pre- or post-fermentation total communities (replicates and years pooled) are shown. (A) Illumina sequencing. (B) PacBio sequencing.

3.34 Statistical differences in vineyard yeast community compositions based on Illumina and PacBio sequencing

Yeast communities detected before and after fermentation were very distinct based on both Illumina (R^2=0.466, p=0.001) and PacBio (R^2=0.868, p=0.028) sequencing, apparently driven by a shift to a *Saccharomyces*-dominated community (Figure 3.5). Illumina sequencing also revealed yeast communities to be different between years (R^2=0.132, p=0.011), likely impacted by the increased abundance of *Filobasidium* from 2018 to 2019 (Figure 3.2), as well as a significant interaction effect of fermentation stage and year (R^2=0.120, p=0.014). There was also a significant decrease in average yeast species diversity (Illumina p=0.001; PacBio p=0.017) as well as in average richness (Illumina p=0.004; PacBio p=0.012) from pre- to post-fermentation (Table 3.3). The average dispersion of the between-year samples (2018-19) was

greater (0.29) than that for the between location samples (0.21) collected in 2021, but the difference was not statistically significant.

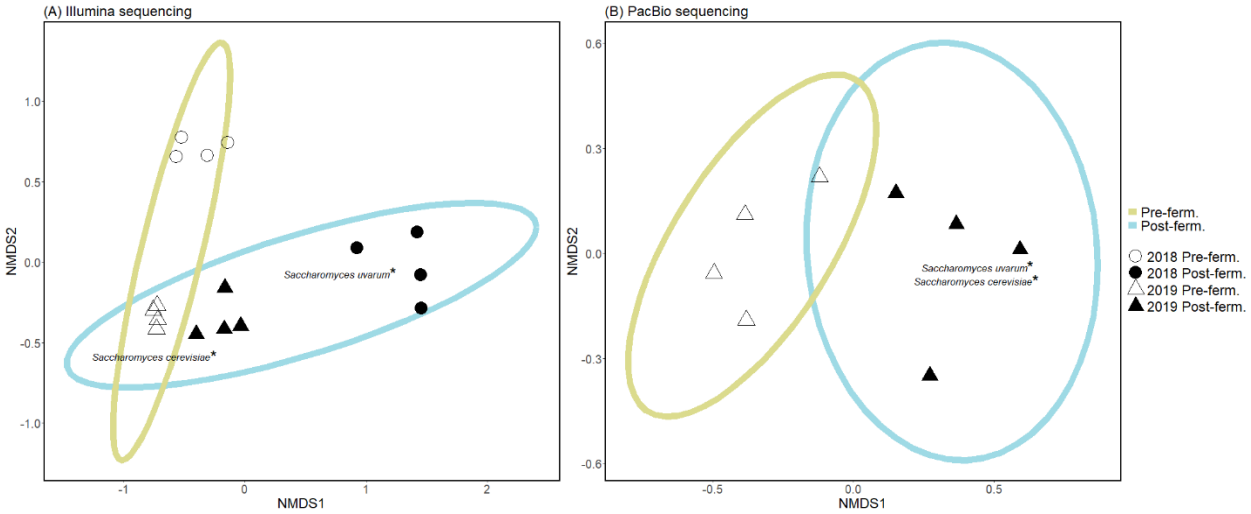

Figure 3.5. NMDS ordinations of pre-fermentation ("Pre-ferm.") and post-fermentation ("Post-ferm.") yeast communities. (A) Illumina sequencing. (B) PacBio sequencing.

Table 3.3. Average yeast diversity (Fisher's alpha ± SE) and richness (number of species ± SE) of 2018 and 2019 fermentation replicates before and after fermentation.

			Pre-fermentation	Post-fermentation
Illumina	2018	Diversity	5.3 ± 0.3	1.0 ± 0.1
		Richness	47 ± 2	12 ± 1
	2019	Diversity	8.5 ± 0.2	3.9 ± 0.2
		Richness	84 ± 3	42 ± 2
PacBio	2019	Diversity	2.9 ± 0.2	2.0 ± 0.2
		Richness	22 ± 2	15 ± 1

3.35 Chemical and sensory evaluation of wines

Prior to fermentation, pH, titratable acidity, and ethanol content of musts were comparable between years, although fructose and glucose levels in musts were higher in 2018 than 2019 (Table S3.2).

Tasting panel results from 2018 ranked post-fermentation replicate 3 as the most appealing and 1 as the least appealing. The average overall acceptability was 2.1/9, and the wines were noted to be earthy with a strong ethanol taste. Replicates 1 and 2 were described as bitter and sour, respectively. In 2019, replicate 4 was ranked as most appealing and replicate 3 as least appealing. The average overall acceptability was 3.96/9, and wines were noted to have a bitter taste. Replicate 3 also had a strong ethanol taste, while replicate 4 had sweet, fruity, and sour flavours. There was no consistent relationship between the ranking of the replicates and the post-fermentation measurements of pH, titratable acidity, volatile acidity, or ethanol content (Table S3.2). However, the 2019 replicates, which were rated as more acceptable overall in comparison to 2018, had slightly lower average volatile acidities, and higher average ethanol contents than the 2018 fermentations. The 2019 replicates also had higher average fructose levels and lower average glucose levels when compared to 2018, although the most highly ranked replicates from both years had 0 mg/ml fructose.

Regarding the relationships between wine acceptability and yeast species compositions, the better acceptability of the 2019 replicates was correlated with a higher overall diversity and proportion of basidiomycete yeasts in the 2019 fermentations as compared to 2018. Also, the ranking of the individual 2019 replicates was correlated with increased levels of *S. uvarum* as determined by both Illumina sequencing (the more appealing replicates were dominated by *S. uvarum* and the least appealing replicate was dominated by *S. cerevisiae*), and PacBio sequencing (the replicate with the lowest proportion of *S. uvarum* was the least appealing and the replicate with the highest proportion was the most appealing).

3.4 Discussion

We found that indigenous vineyard yeast communities changed drastically upon spontaneous fermentation, from a community dominated by non-fermentative yeasts, including a high diversity of basidiomycetes such as *Filobasidium* and *Vishniacozyma*, to a community of mainly *Saccharomyces* species, with the indigenous *S. uvarum* either dominating or co-dominating post-fermentation. While the pre-fermentation yeast community also differed between years, this variation can likely be attributed to yearly differences in growing season climate, e.g., the 2018 growing season was generally warmer and drier than 2019 (Liu et al. 2019; Environment and Climate Change Canada 2022). However, a detailed analysis incorporating climatic variables was not possible with the data available.

Yeast species comprise a substantial portion of the fungal community associated with vineyard grapes (Setati et al. 2012) and most vineyard studies report a diversity of basidiomycete yeasts, such as species of *Cryptococcus*, *Filobasidium*, *Papilioterma*, and *Rhodotorula* (Davenport 1976; Sabate et al. 2002). As identification methods have improved, and in accordance with taxonomic updates, the genera *Sporobolomyces* and *Vishniacozyma* are also increasingly cited (Wang et al. 2021b). However, basidiomycete yeasts have not generally been considered valuable contributors to wine fermentations and have therefore remained understudied compared to the fermentative ascomycete yeasts (Saccharomycetales). On the other hand, the ascomycete component of the vineyard yeast community is often dominated by the ubiquitous and abundant non-fermentative black yeast *A. pullulans*. or the common fermentative yeast *H. uvarum* (Bozoudi and Tsaltas 2018; Borren and Tian 2021).

Indeed, both our Illumina and PacBio sequencing data show a greater richness of basidiomycete yeast species relative to ascomycete in our pre-fermentation replicates. However,

while Illumina sequencing indicated that basidiomycete yeasts made up 46% of the pre-fermentation yeast community (based on sequence abundance), the PacBio system detected high proportions of *H. uvarum* in pre-fermentation replicates, resulting in a lower proportion (16%) of basidiomycete yeasts. As *H. uvarum* is detected in more than half of all studies that describe non-*Saccharomyces* yeast species associated with wine grapes (Drumonde-Neves et al. 2021), our PacBio results likely represent a closer approximation of the true community composition. Nevertheless, basidiomycete yeasts constitute a considerable proportion of our indigenous vineyard yeast community, regardless of the sequencing platform used.

The most abundant basidiomycete genus found in this study was *Filobasidium*, a frequently reported and often abundant member of vineyard yeast communities (Cureau et al. 2021b; Wang et al. 2021b). While *Filobasidium* is present in wine regions around the world (Merín et al. 2014; Nemcová et al. 2015; Ding et al. 2021), a recent analysis of its global distribution patterns suggests that the genus is more associated with cool climates like that of Nova Scotia (Drumonde-Neves et al. 2021). The next most abundant basidiomycete yeasts in our study were *V. carnescens* and *C. cygneicollum*. These species are both relatively newly described but their recent detection in several vineyards suggests that they are also common constituents (*V. carnescens*: Abdullabekova et al. 2020; Wang et al. 2021b) (*C. cygneicollum*: Vaudano et al. 2019; Li et al. 2021).

Although basidiomycete yeasts are non-fermentative, they may still impact wine quality. Species of *Filobasidium*, *Curvibasidium* and *Vishniacozyma* produce enzymes such as pectinases that may increase grape must yields and facilitate the extraction of pigments and tannins via enhanced breakdown of cell walls, as well as esterases that may contribute to wine flavour and aroma (Merín et al. 2014; Wang et al. 2021b). Further, *Filobasidium*, *Vishniacozyma*, and other

basidiomycete yeasts may exhibit biocontrol activity against grapevine pathogens (Wang et al. 2021b). Although our sensory analysis dataset is small and larger fermentation volumes would have provided greater confidence in our results, it may be possible that the higher overall appeal of our 2019 fermentation replicates compared to those from 2018 is linked to the higher proportions of these non-fermentative basidiomycete yeasts in 2019. Overall, the sensory and chemical components of our wines were not comparable to the commercially successful L'Acadie blanc wines produced by local wineries, which employ both inoculated and spontaneous fermentation methods. Common descriptors of L'Acadie blanc wines produced in the Annapolis Valley include medium- to full-bodied, dry, grassy or herbal, citrus, crisp apple, pear or honey, and stone fruit (Hayward et al. 2020; Moss et al. 2021).

The post-fermentation yeast community of our organic vineyard was either completely dominated by *S. uvarum*. or *S. uvarum* co-dominated with *S. cerevisiae*. *Saccharomyces uvarum* is a non-conventional fermentative yeast common to cool climate vineyards and low-temperature fermentations (Salvadó et al. 2011; Morgan et al. 2019). It has been found dominating wine fermentations in France (Demuyter et al. 2004), Italy (Tosi et al. 2009), Germany (Ultee et al. 2013) and British Columbia (Morgan et al. 2019; McCarthy et al. 2021). *Saccharomyces uvarum* produces less ethanol and acetic acid than *S. cerevisiae*, but more glycerol, 2-phenylethyl acetate, and ethyl 2-methyl butanoate, compounds associated with positive sensory experiences (Tosi et al. 2009; Maygar and Tóth 2011; Morgan et al. 2020). Again, acknowledging the limitations of our analyses, the higher sensory rankings among our 2019 wines might be explained by higher post-fermentation proportions of *S. uvarum*.

The abundance of *S. uvarum* in our Nova Scotia vineyard, together with results from surveys in British Columbia (Morgan et al. 2019; McCarthy et al. 2021) and Ontario (Nurgel et

al. 2004) suggest that *S. uvarum* is typical of Canadian vineyards and may often be the dominant fermentative yeast, despite the cooccurrence of *S. cerevisiae*. A Canadian isolate of *S. uvarum* from Niagara has even been characterized with respect to its fermentation of appassimento wines (Kelly et al. 2018; Inglis et al. 2020). *S. cerevisiae* was also common in our vineyard, but it is unclear if we detected indigenous or commercial strains. Although this question requires population level genetic analysis beyond the scope of the current study, Martiniuk et al. (2016) and Cheng et al. (2020) found commercial, potentially indigenous, and commercial-related *S. cerevisiae* strains coexisting in British Columbian vineyards.

Vineyard yeast communities have often been investigated using culture dependent methods, which have provided indispensable, but incomplete information. Next generation sequencing, on the other hand, is thought to provide a more accurate picture of yeast community composition (Belda et al. 2017). Studies directly comparing culture dependent and next generation sequencing approaches of vineyard yeasts predictably show improved species detection by the latter, although the dominant species detected are often similar between the two approaches (Dissanayake et al. 2018; Constantini et al. 2022).

Both the Illumina and PacBio systems used here target the ITS region of fungal rDNA, but use different procedures and primers sets to produce PCR products of different sizes, introducing unique biases into the amplification and taxonomic assignment. We also found the general species composition to be comparable between the Illumina and PacBio systems, except for the substantial discrepancy in the detection of *H. uvarum*, which was the most abundant pre-fermentation species based on the PacBio data (~49% of the yeast community) and remained abundant after fermentation. Conversely, although *H. uvarum* was present in all replicates based on Illumina sequencing, no more than 18 sequences were detected in any replicate.

It is unknown if all Illumina systems may have a bias against the detection of *Hanseniaspora*, and if so, what other conditions may be involved. While some studies using Illumina sequencing to assess grape or must samples have failed to detect or report the genus *Hanseniaspora* (Wei et al. 2018; Ding et al. 2021; Wang et al. 2021a; Constantini et al. 2022), numerous other Illumina based studies have reported it, sometimes in high abundances (Gao et al. 2019; Cureau et al. 2021b; Gómez-Albarrán et al. 2021; Sun et al. 2021; Wang et al. 2021b). It is possible that the choice of next generation sequencing primers, which varies among research groups, affects the detection of *Hanseniaspora*. Further investigation of the primer sets commonly used for next generation sequencing in a range of PCR conditions is warranted.

Improved knowledge of biases in taxonomic detection between next generation sequencing systems is required to inform subsequent claims linking yeast communities to wine attributes. While we have emphasized the impacts of basidiomycete yeasts in this study, had our focus been *Hanseniaspora*, failing to corroborate our Illumina results with PacBio sequencing would have led us to an incomplete representation of the total yeast community. A growing body of vineyard yeast research highlights marker species of a particular *terroir*, an approach that is useful for winemakers but sensitive to the biases of the sequencing system (Kamilari et al. 2021; Yan et al. 2022). We know of no other studies that compare next generation sequencing platforms for the characterization of vineyard yeast communities.

This is the first systematic evaluation of indigenous vineyard yeasts in Atlantic Canada. Our characterization of the yeast community of an organic vineyard in Nova Scotia should facilitate the use of indigenous yeasts in winemaking and create a baseline for further vineyard yeast research in the region. The abundance and diversity of basidiomycete yeasts in grape

musts, and the abundance and activity of *S. uvarum*, are both potential targets for non-conventional fermentations.

3.5 Supplementary materials

Table S3.1. NCBI GenBank accession numbers of *Saccharomyces* species in Figure 3.1, listed in descending order corresponding to the figure.

Species	GenBank Accession
S. arboricola	NR153296 (TYPE)
S. arboricola	KY104945
S. arboricola	EF580917
S. uvarum	MH595343
S. uvarum	MW980900
S. uvarum	NR153310 (TYPE)
S. eubayanus	KR871556
S. eubayanus	NR137586 (TYPE)
S. pastorianus	NR165985 (TYPE)
S. pastorianus	AY046151
S. eubayanus	MW710920
S. pastorianus	AF005715
S. kudriavzevii	NR111355 (TYPE)
S. kudriavzevii	FJ196779
S. kudriavzevii	AY046150
S. mikatae	NR111354 (TYPE)
S. mikatae	FJ196778
S. mikatae	AY046149
S. jurei	HG764813
S. species	HG764814
S. paradoxus	KP250840
S. paradoxus	AY046148
S. paradoxus	NR138272 (TYPE)
S. cerevisiae	NR111007 (TYPE)
S. cerevisiae	KC542799
S. cerevisiae	MK942688

Table S3.2. Chemical attributes of musts before and after fermentation, in 2018 and 2019.

	Pre-fermentation		Post-fermentation (average)	
	2018	2019	2018	2019
pH	3.21	3.24	3.22	3.21
% titratable acidity	0.88	0.86	0.81	0.82
% volatile acidity	-	-	0.035	0.011
% ethanol	0.02	0.36	8.41	10.25
Fructose (mg/ml)	98.0	61.9	0.02	0.51
Glucose (mg/ml)	87.2	54.2	0.09	1.12

Chapter 4: Basidiomycete yeasts associated with wine grapes and their applications in winemaking

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4.1 Introduction

Wine is the result of the juice released from crushed grapes being fermented by yeasts and bacteria. These microbes originate from the vineyard and surrounding environment and remain abundant on grapes after harvest to form a diverse community in the must. While the activity and influence of the highly fermentative yeast *Saccharomyces cerevisiae* has been extensively studied in winemaking, it is not the only yeast present during fermentation. Musts host a large variety of oxidative yeasts (capable only of aerobic respiration and not fermentation) including the ascomycete *Aureobasidium* and basidiomycete genera such as *Filobasidium*. *Rhodotorula*, *Sporobolomyces*, and *Vishniacozyma*, as well as semi-fermentative ascomycetes (with some combination of aerobic metabolism and limited capacity for alcoholic fermentation), such as the genera *Hanseniaspora*, *Candida*, *Metshchnikowia*, and *Pichia* (Jolly et al. 2014; Capozzi et al. 2015; Borren and Tian 2021). However, these groups generally do not persist to the completion of fermentation, as they are inhibited by increasing alcohol and decreasing oxygen levels and are outcompeted by fermentative ascomycete yeasts (highly efficient at alcoholic fermentation), which are generally more tolerant of low-oxygen conditions, such as species of *Lachancea*, *Saccharomyces* and *Torulaspora*.

During wine fermentation, all yeasts produce secondary metabolites that may influence the aroma and mouthfeel of the wine. Fermentations are multispecies systems regulated by many interacting factors, including the initial assemblage of yeast species and nutrient levels in the must, and subsequently the succession of available nutrients as species use and form products and interact with each other (Fleet 2003; Comitini et al. 2021). Depending on these variables, different yeast species influence the production of ethanol, glycerol, and volatile aromatic

compounds such as higher alcohols, acids, and esters (Jolly et al. 2014; Borren and Tian 2021; Comitini et al. 2021).

Despite the complexity of wine fermentation, most research has been focused on the biology of fermentative ascomycete yeasts, chiefly *S. cerevisiae* (Chambers & Pretorius 2010). Strains of *S. cerevisiae* exhibit high fermentation efficiency and dried commercial cultures are widely used to inoculate musts with predictable results. However, non-*Saccharomyces* yeasts produce a greater range of volatile compounds and higher levels of extracellular enzymes that influence wine aroma (Strauss et al. 2001; Capozzi et al. 2015). This is evident in spontaneous fermentations, which rely solely on the yeasts present on the grape skins, and in mixed inoculum fermentations (using multiple yeast species), both of which result in wines with more complex sensory attributes and a different range of aromas than fermentations inoculated with *S. cerevisiae* only (Egli et al. 1998; Varela et al. 2009; Ciani et al. 2010). To date, many fermentative ascomycete genera, including *Hanseniaspora* and *Metschnikowia*, are well studied regarding the production of aromatic compounds (Jolly et al. 2014; Comitini et al. 2017; Borren and Tian 2021).

However, basidiomycete yeasts are also present in wine fermentations and have often been overlooked despite their contributions to the characteristics of the wine (Rossouw & Bauer 2016; Wang et al. 2017). In some cases, basidiomycete yeasts are equally, or even more diverse and abundant than ascomycete yeasts in grape musts (Nemcová et al. 2015; Pinto et al. 2015; Section 2.3). Despite their ubiquity, the role of basidiomycete yeasts in winemaking is generally lacking in existing reviews that consider non-*Saccharomyces* yeasts in wine fermentation (Barata et al. 2012; Tempère et al. 2018; Lappa et al. 2020; Borren and Tian 2021; Comitini et al. 2021; Drumonde-Neves et al. 2021), and more broadly in biotechnology (Johnson 2013), although

Varela and Borneman (2017) briefly address applications of *Rhodotorula* in winemaking. Given the emerging attention paid to *Rhodotorula* and the promise of other non-*Saccharomyces* yeasts, it is reasonable to expect that basidiomycete yeasts could benefit wine production via mixed inoculation.

Furthermore, there is demand for wines that better express the terroir of a region or vineyard (Capitello et al. 2021; Angelini et al. 2023), which includes the influence of the indigenous yeast community, and therefore basidiomycete yeasts. Terroir, or the character of a wine as influenced by environmental variables including climate, soil, geomorphology, cultivation practices, and microflora, is a fundamental property that is important to both winemakers and consumers. Expression of indigenous yeasts can be achieved by vineyard management choices such as organic cultivation, which aims to maintain a more natural balance of microorganisms in the vineyard (Provost and Pedneault 2016), or winemaking choices such as spontaneous fermentation and the production of so-called "natural wines", made by spontaneous fermentation with minimal to no additives (Legeron 2020; Lu et al. 2020; Liang et al. 2023; Rossetti et al. 2023). Consumers will seek out and pay more for wine products that resonate with personal values to reduce negative environmental or health impacts (Cravero 2019; Tait et al. 2019; Fabbrizzi et al. 2021; Valenzuela et al. 2022) or reflect an interest in novel and diverse wines (Capitello et al. 2021; Angelini et al. 2023).

The role of basidiomycete yeasts in winemaking is relevant for their potential as inoculants as well as to understand their influence in spontaneous and "natural" fermentations, but is difficult to assess due to a lack of information and the fragmented nature of the existing information. To address these issues, I summarize the record of basidiomycete yeasts detected from wine grapes and musts and consolidate previous research that directly considers the role, or

supports potential application, of basidiomycete yeasts in winemaking. The objective of this review is to provide a valuable resource facilitating future research into the application of basidiomycete yeasts in winemaking.

4.2 Summary of basidiomycete yeasts reported on wine grapes and in musts

Literature characterizing the yeast community on wine grape surfaces or in wine grape musts was reviewed to produce a comprehensive summary of basidiomycete yeasts present in these environments, indiscriminate of variables such as geography, or the objectives of the study. The taxa included here reflect those that each author chose to report according to their own standards. Especially in high throughput sequencing studies, given the volume of sequence data, it is only practical to report abundant or important species – generally those making up some minimum proportion of sequences, or those determined to be significantly correlated with a variable of interest. The benefits and limitations of community characterization methods are discussed in section 4.6.

4.21 Culture based research

Although culture-based studies of wine grapes often fail to isolate, or at least report, basidiomycete yeasts, even in early studies, there is a clear record that a wide variety of basidiomycete yeast genera are present on grapes and in musts (Table 4.1). The basidiomycete yeast genera most often cited as associated with wine grapes are *Cryptococcus* and *Rhodotorula*, but when current taxonomy is assigned, *Papiliotrema*, *Filobasidium*, *Sporobolomyces*, *Naganishia*, *Curvibasidium*, and *Vishniacozyma* emerge as more commonly isolated than *Cryptococcus*, although *Rhodotorula* persists as the most commonly isolated basidiomycete

yeast genus from wine or must samples (Table 4.1). The phylogenetic relationships among these groups are outlined in Figure 4.1.

Table 4.1. Basidiomycete yeasts associated with wine grapes or musts reported from studies using culture dependent methods (current species names are used; Table A1).

Genus	Species	References
Anthracocystis	*penniseti*	Kachalkin et al. 2015; Abdullabekova et al. 2020
Buckleyzyma	*aurantiaca*	Raspor et al. 2006; Comitini and Ciani 2008
Bullera	*alba*	Renouf et al. 2005; Renouf et al. 2006; Renouf et al. 2007a; Čadež et al. 2010; Verginer et al. 2010; David et al. 2014; Brysch-Herzberg and Seidel 2015; Martins et al. 2022
	unica	Gayevskiy and Goddard 2012
Cryptococcus	*amylolentus*	Belda et al. 2016
	neoformans	Comitini and Ciani 2008
	sp(p).	Renouf et al. 2006; Čadež et al. 2010; Bourret et al. 2013; Milanović et al. 2013; Jara et al. 2016; Agarbati et al. 2019; Bougreau et al. 2019; Mateo et al. 2020
Curvibasidium	*cygneicollum*	Lederer et al. 2013; Vaudano et al. 2019
	nothofagi	Čadež et al. 2010; Gayevskiy and Goddard 2012; Setati et al. 2012; Lederer et al. 2013; Milanović et al. 2013; Bagheri et al. 2015; Nemcová et al. 2015; Escribano-Viana et al. 2018; Castrillo and Blanco 2022
	pallidicorallinum	Bourret et al. 2013; Brysch-Herzberg and Seidel 2015; Li et al. 2018
	rogersii	Bourret et al. 2013
Cystobasidium	*laryngis*	Prakitchaiwattana et al. 2004
	minutum	Yanagida et al. 1992; Nemcová et al. 2015
	pallidum	Bourret et al. 2013
	sloofiae	Setati et al. 2012
Cystofilobasidum	*capitatum*	Brysch-Herzberg and Seidel 2015
	infirmominiatum	Bourret et al. 2013
	macerans	Comitini and Ciani 2008; Gayevskiy and Goddard 2012; Bourret et al. 2013; Ultee et al. 2013; Brysch-Herzberg and Seidel 2015; Castrillo and Blanco 2022
Dioszegia	*hungarica*	Rosini et al. 1982; Raspor et al. 2006; Nemcová et al. 2015
Erythrobasidium	*hasegawianum*	Čadež et al. 2010
Filobasidium	*elegans*	Nemcová et al. 2015
	floriforme	Čadež et al. 2010; Díaz et al. 2013; Ultee et al. 2013; Brysch-Herzberg and Seidel 2015; Li et al. 2019; Feng et al. 2021
	globisporum	Brysch-Herzberg and Seidel 2015

	magnum	Sabate et al. 2002; Prakitchaiwattana et al. 2004; Čadež et al. 2010; Li et al. 2010; Verginer et al. 2010; Gayevskiy and Goddard 2012; Setati et al. 2012; Bourret et al. 2013; Díaz et al. 2013; Milanović et al. 2013; David et al. 2014; Kachalkin et al. 2015; Nemcová et al. 2015; Kántor et al. 2017; Li et al. 2018; Bougreau et al. 2019; Li et al. 2019; Abdullabekova et al. 2020; Kačániová et al. 2020
	oeirense	Prakitchaiwattana et al. 2004; Setati et al. 2012
	stepposum	Bourret et al. 2013; Castrillo et al. 2019; Castrillo and Blanco 2022
	uniguttulatum	Sabate et al. 2002
	wieringae	Čadež et al. 2010; Lederer et al. 2013; Milanović et al. 2013
	sp(p).	Bougreau et al. 2019
Hannaella	*luteola*	Bourret et al. 2013
	zeae	Čadež et al. 2010
Holtermanniella	*festucosa*	Gayevskiy and Goddard 2012; Bourret et al. 2013; Lederer et al. 2013
	takasimae	Čadež et al. 2010; Bourret et al. 2013; Brysch-Herzberg and Seidel 2015
Krasilnikovozyma	*huempii*	Brysch-Herzberg and Seidel 2015
Kwoniella	*dendrophila*	Setati et al. 2012;
	mangrovensis	Čadež et al. 2010
Meira	*geulakonigae*	Setati et al. 2012
Microstroma	*bacarum*	Rementeria et al. 2003; Renouf et al. 2007a; Čadež et al. 2010; Bourret et al. 2013; Nemcová et al. 2015
Mrakia	*cryoconiti*	Bourret et al. 2013
Naganishia	*adeliensis*	Čadež et al. 2010; Bourret et al. 2013; Mateo et al. 2020
	albida	Rosini et al. 1982; Longo et al. 1991; Yanagida et al. 1992; De La Torre et al. 1999; Jolly et al. 2003; Subden et al. 2003; Renouf et al. 2005; Raspor et al. 2006; Renouf et al. 2007a; Comitini and Ciani 2008; Zott et al. 2008; Koulougliotis and Eriotou 2016; Bougreau et al. 2019
	albidosimilis	Bourret et al. 2013
	bhutanensis	Bagheri et al. 2015
	diffluens	Kántor et al. 2017; Kačániová et al. 2020; Chalvantzi et al. 2021; Castrillo and Blanco 2022
	globosa	Setati et al. 2012; Bourret et al. 2013; Bougreau et al. 2019
	randhawae	Setati et al. 2012
	uzbekistanensis	Bourret et al. 2013; Mateo et al. 2020

Papiliotrema	*flavescens*	Renouf et al. 2005; Čadež et al. 2010; Li et al. 2010; Bezerra-Bussoli et al. 2013; Setati et al. 2012; Milanović et al. 2013; Brysch-Herzberg and Seidel 2015; Vigenti et al. 2015; Li et al. 2018; Li et al. 2019; Vaudano et al. 2019; Rantsiou et al. 2020; Costantini et al. 2022
	fusca	Renouf et al. 2007a; Čadež et al. 2010
	laurentii	Goto 1980; Yanagida et al. 1992; Sabate et al. 2002; Subden et al. 2003; Prakitchaiwattana et al. 2004; Renouf et al. 2005; Raspor et al. 2006; Renouf et al. 2007a; Bezerra-Bussoli et al. 2013; Setati et al. 2012; Bourret et al. 2013; Koulougliotis and Eriotou 2016; Castrillo et al. 2019; Chalvantzi et al. 2021; Castrillo and Blanco 2022
	nemorosa	Renouf et al. 2007a
	terrestris	Martins et al. 2014; Castrillo et al. 2019; Abdullabekova et al. 2020; Rantsiou et al. 2020; Castrillo and Blanco 2022
	sp(p).	Feng et al. 2021
Pseudomicrostroma	*phylloplanum*	Čadež et al. 2010
Pseudozyma	sp(p).	Bourret et al. 2013
Quambalaria	*cyanescens*	Mateo et al. 2020
Rhodosporidiobolus	*colostri*	Bourret et al. 2013
Rhodotorula	*babjevae*	Prakitchaiwattana et al. 2004; Renouf et al. 2005; Bourret et al. 2013; Milanović et al. 2013; Brysch-Herzberg and Seidel 2015; Escribiano-Viana et al. 2018; Li et al. 2018; Li et al. 2019; Vaudano et al. 2019
	diobovata	Setati et al. 2012; Bagheri et al. 2015
	glutinis	Mrak and McClung 1940; Davenport 1974; Rosini et al. 1982; Fleet et al. 1984; Yanagida et al. 1992; Regueiro et al. 1993; Rementeria et al. 2003; Subden et al. 2003; Renouf et al. 2005; Raspor et al. 2006; Renouf et al. 2007a; Čadež et al. 2010; Gayevskiy and Goddard 2012; Setati et al. 2012; Šuranská et al. 2012; Lederer et al. 2013; Milanović et al. 2013; Ultee et al. 2013; Martins et al. 2014; Bagheri et al. 2015; Kachalkin et al. 2015; Nemcová et al. 2015; Koulougliotis and Eriotou 2016; Kántor et al. 2017; Escribiano-Viana et al. 2018; Li et al. 2019; Abdullabekova et al. 2020; Kačániová et al. 2020; Martins et al. 2022
	graminis	Fleet et al. 1984; Renouf et al. 2005; Vigenti et al. 2015; Castrillo et al. 2019; Rantsiou et al. 2020; Castrillo and Blanco 2022; Costantini et al. 2022
	kratochvilovae	Renouf et al. 2007a

	mucilaginosa	Goto 1980; Longo et al. 1991; Povhe Jemec et al. 2001; Sabate et al. 2002; Renouf et al. 2005; Renouf et al. 2007a; Zott et al. 2008; Šuranská et al. 2012; Tello et al. 2012; Bourret et al. 2013; Díaz et al. 2013; Kachalkin et al. 2015; de Ponzzes-Gomes et al. 2014; Kántor et al. 2017; Mendes et al. 2017; Abdullabekova et al. 2020; Kačániová et al. 2020; Mateo et al. 2020; Liu et al. 2021; Castrillo and Blanco 2022
	toruloides	Šuranská et al. 2012; Belda et al. 2016
	sp(p).	Fleet et al. 1984; Parish and Carroll 1985; Sabate et al. 2002; Jolly et al. 2003; Subden et al. 2003; Combina et al. 2005b; Renouf et al. 2006; Sturm et al. 2006; Comitini and Ciani 2008; Bourret et al. 2013; Brysch-Herzberg and Seidel 2015; Jara et al. 2016; Agarbati et al. 2019; Castrillo et al. 2019
Saitozyma	*flava*	Rosini et al. 1982
Solicoccozyma	*aeria*	Bougreau et al. 2019
	terrea	Koulougliotis and Eriotou 2016
Sporisorium	sp(p).	Setati et al. 2012
Sporobolomyces	*carnicolor*	Renouf et al. 2007a
	coprosmae	Bourret et al. 2013
	japonicus	Davenport 1974
	longiusculus	Renouf, Claisse et al. 2007
	roseus	Davenport 1974; Longo et al. 1991; De La Torre et al. 1999; Subden et al. 2003; Renouf et al. 2005; Raspor et al. 2006; Verginer et al. 2010; Gayevskiy and Goddard 2012; Setati et al. 2012; Bourret et al. 2013; Lederer et al. 2013; David et al. 2014; Martins et al. 2022
	ruburrimus	Gayevskiy and Goddard 2012; Castrillo et al. 2019
	salmonicolor	Renouf et al. 2005; Renouf et al. 2007a; Šuranská et al. 2012
	shibatanus	Renouf et al. 2005; Čadež et al. 2010; Li et al. 2010; Verginer et al. 2010; Bezerra-Bussoli et al. 2013; Díaz et al. 2013; Martins et al. 2014; Brysch-Herzberg and Seidel 2015; Nemcová et al. 2015; Li et al. 2018; Li et al. 2019; Feng et al. 2021; Costantini et al. 2022
	sp(p).	Subden et al. 2003; Renouf et al. 2006; Jara et al. 2016
Symmetrospora	*oryzicola*	Renouf et al. 2007a; Gayevskiy and Goddard 2012
Tausonia	*pullulans*	Comitini and Ciani 2008
Ustilago	*maydis*	Čadež et al. 2010
	sp(p).	Setati et al. 2012;
Vanrija	*humicola*	Rementeria et al. 2003; Comitini and Ciani 2008

Vishniacozyma	*carnescens*	Li et al. 2010; Setati et al. 2012; Bourret et al. 2013; Lederer et al. 2013; Milanović et al. 2013; Martins et al. 2014; Bagheri et al. 2015; Castrillo et al. 2019; Castrillo and Blanco 2022; Costantini et al. 2022
	dimennae	Milanović et al. 2013
	foliicola	Renouf et al. 2007a; Brysch-Herzberg and Seidel. 2015
	heimaeyensis	Brysch-Herzberg and Seidel. 2015; Martins et al. 2022
	tephrensis	Bourret et al. 2013; Martins et al. 2014
	victoriae	Prakitchaiwattana et al. 2004; Gayevskiy and Goddard 2012; Bourret et al. 2013; Lederer et al. 2013; Milanović et al. 2013; Martins et al. 2014; Castrillo et al. 2019; Castrillo and Blanco 2022
None		Pataro et al. 2000; Torija et al. 2001; Mills et al. 2002; Raspor et al. 2002; Nurgel et al. 2005; Di Maro et al. 2007; Nisiotou and Nychas 2007; Urso et al. 2008; Barrajón et al. 2009; Chavan et al. 2009; Brežná et al. 2010; Li et al. 2011; Garofalo et al. 2016; Padilla et al. 2016; Čuš et al. 2017; Drumonde-Neves et al. 2017; Cioch-Skoneczny et al. 2018; Regecová et al. 2019; Çelebi Uzkuç et al. 2020; Belessi et al. 2022

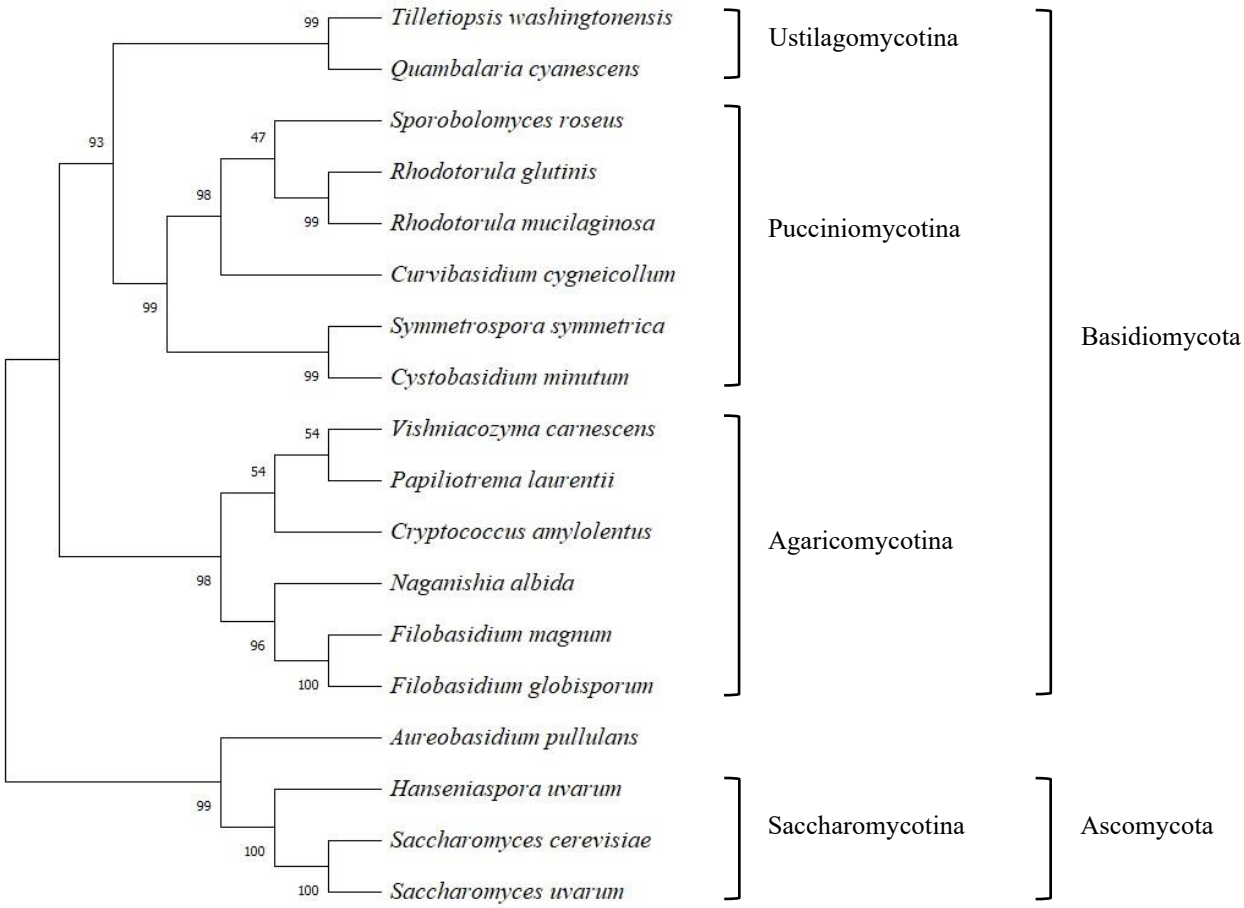

Figure 4.1. Maximum parsimony phylogenetic tree constructed using MEGA X version 10.2.4 (Kumar et al. 2018), using 26S sequences obtained from the NCBI GenBank database (Table S4.1), of (1) the basidiomycete yeast species most often isolated from wine grapes and musts, (2) *Tilletiopsis washingtonensis* and *Quambalaria cyanescens* to represent Ustilagomycotina, and (3) a lesser number of ascomycete yeast species commonly isolated from wine grapes and musts, as an outgroup. Bootstrap values from 500 replicates are shown next to the branches. Subphylum and phylum assignments are shown to the right.

4.22 Research using culture independent methods other than high throughput sequencing

Microbial community characterization methods that directly assess DNA within the sample material (e.g., must), offer an alternative to culture-based assessments of community composition. Prior to the availability of high throughput sequencing, methods such as PCR-DGGE and cloning were used. Brežna et al. (2010) and Ženišová et al. (2014) used fluorescence-

ITS PCR, cloning, and sequencing to assess yeast and fungal diversity associated with wine grapes and were able to identify *Rhodotorula glutinis* and *Filobasidium magnum*, although better species resolution was achieved by culture dependent means.

Some studies identified basidiomycete yeasts in culture but failed to detect the same taxa by PCR-DGGE (Prakitchaiwattana et al. 2004; David et al. 2014; Escribano-Viana et al. 2018), while others failed to detect basidiomycete yeasts by culturing and by PCR-DGGE (Mills et al. 2002; Di Maro et al. 2007; Nisiotou et al. 2007; Urso et al. 2008). While a few studies successfully identified basidiomycete yeasts by direct PCR-DGGE analysis, culturing remained more effective. For example, *Rhodotorula babjevae* and *Rhodotorula* sp. were identified by Renouf et al. (2005) using PCR-DGGE while corresponding culture work isolated 10 additional basidiomycete yeasts including *R. glutinis*, *Rhodotorula graminis* and *Rhodotorula mucilaginosa*. Similarly, Milanović et al. (2013) identified *Vishniacozyma carnescens*, *F. magnum*, *Vishniacozyma dimennae*, *R. glutinis*, and *Curvibasidium nothofagi*, which represented a smaller subset of the same species they cultured. *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* were also identified by Renouf et al. (2007b) but the authors did not provide corresponding culture work.

4.23 Research using high throughput sequencing

In general, high throughput sequencing results confirm the patterns established by culture dependent methods; *Rhodotorula*, *Filobasidium*, *Cryptococcus*. *Sporobolomyces*, *Vishniacozyma*, *Curvibasidium*, *Naganishia*, and *Papiliotrema* are the most reported genera from wine grapes and musts (Table 4.2). Some studies do not report any basidiomycete yeasts, at least among the most abundant taxa (Table 4.2), and many do not provide identification at the genus or species level for either some (Kecskeméti et al. 2016; Zhang et al. 2019) or all (Miura et al. 2017)

basidiomycete taxa. A conservative approach to assigning taxonomy is understandable, however, given that genus and species level identifications may be misleading due to the recent systematic updates of genera commonly cited from wine grapes and musts (Table A1).

Table 4.2. Basidiomycete yeasts associated with wine grapes or musts reported from studies using high throughput sequencing methods (current species names are used; Table A1).

Genus	Species	References
Buckleyzyma	*aurantiaca*	Wang et al. 2021b
	sp(p).	Zhu et al. 2021; Milanović et al. 2022; Tronchoni et al. 2022
Bullera	*alba*	Xu et al. 2020; Section 2.3
	sp(p).	Kioroglou et al. 2019; Xu et al. 2020; Cureau et al. 2021b
Cryptococcus	sp(p).	Bokulich et al. 2014; David et al. 2014; Taylor et al. 2014; Pinto et al. 2015; De Filippis et al. 2017; Morrison-Whittle et al. 2017; Sternes et al. 2017; Li et al. 2018; Wei et al. 2018; Gao et al. 2019; Kioroglou et al. 2019; Zhang et al. 2019; Lu et al. 2020; Xu et al. 2020; Costantini et al. 2022; Milanović et al. 2022; Tronchoni et al. 2022
Curvibasidium	*cygneicollum*	Setati et al. 2015; Dutra-Silva et al. 2021; Kamilari et al. 2021; Li et al. 2021; Liu et al. 2021; Section 2.3
	nothofagi	Setati et al. 2015; Bokulich et al. 2016; Sternes et al. 2017; Wang et al. 2021b
	sp(p).	Cureau et al. 2021b; Liu et al. 2021
Cystobasidium	*oligophagum*	Xu et al. 2020
	sloofiae	Setati et al. 2015
	sp(p).	Zhu et al. 2021; Martins et al. 2021; Milanović et al. 2022; Tronchoni et al. 2022
Cystofilobasidium	*infirmoniniatum*	Wang et al. 2021b
	macerans	Bokulich et al. 2016; Kamilari et al. 2021; Liu et al. 2021; Wang et al. 2021b; Section 2.3
	sp(p).	Sternes et al. 2017; Chen et al. 2020; Liu et al. 2021; Wang et al. 2021b; Zhu et al. 2021; Tronchoni et al. 2022
Dioszegia	sp(p).	David et al. 2014; Cureau et al. 2021b
Entyloma	*cosmi*	Liu et al. 2021
	sp(p).	Liu et al. 2021
Filobasidium	*chernovii*	Kamilari et al. 2021; Wang et al. 2021b; Section 2.3
	floriforme	Li et al. 2019
	globisporum	Section 2.3
	magnum	Rantsiou et al. 2020; Xu et al. 2020; Li et al. 2021; Wang et al. 2021b; Englezos et al. 2022; Section 2.3

	oeirense	Liu et al. 2020; Wang et al. 2021b
	stepposum	Rantsiou et al. 2020; Liu and Howell 2021; Liu et al. 2021; Wang et al. 2021b; Section 2.3
	uniguttulatum	Xu et al. 2020; Wang et al. 2021b
	wieringae	Wang et al. 2021b
	sp(p).	Mandakovic et al. 2020; Cureau et al. 2021a; Cureau et al. 2021b; Ding et al. 2021; Liu et al. 2021; Ma et al. 2021b; Martins et al. 2021; Wang et al. 2021a; Zhu et al. 2021; Costantini et al. 2022; Englezos et al. 2022; Milanović et al. 2022; Li et al. 2022; Tronchoni et al. 2022; Wei et al. 2022; Liang et al. 2023; Martiniuk et al. 2023; Rossetti et al. 2023; Gao et al. 2024
Genolevuria	sp(p).	Li et al. 2018; Zhu et al. 2021
Golubevia	sp(p).	Zhu et al. 2021
Hannaella	*sinensis*	Xu et al. 2020
	sp(p).	Cureau et al. 2021a; Cureau et al. 2021b; Ma et al. 2021b; Wei et al. 2022
Holtermanniella	*festucosa*	Wang et al. 2021b; Section 2.3
	takashimae	Wang et al. 2021b
	sp(p).	Mandakovic et al. 2020; Zhu et al. 2021; Tronchoni et al. 2022
Kondoa	sp(p).	Zhu et al. 2021
Krasilnikovozyma	*huempii*	Li et al. 2018
Kwoniella	sp(p).	Tronchoni et al. 2022
Malassezia	sp(p).	Mandakovic et al. 2020; Zhu et al. 2021; Wei et al. 2022
Microbotryum	*holostei*	Wang et al. 2021b
Mrakia	sp(p).	Zhu et al. 2021; Section 2.3
Naganishia	*adeliensis*	Wang et al. 2021b
	albida	Gao et al. 2019; Li et al. 2021
	albidosimilis	Li et al. 2018
	bhutanensis	Wang et al. 2021b
	globosa	Liu and Howell 2021; Wang et al. 2021b
	onofrii	Wang et al. 2021b
	uzbekistanensis	Wang et al. 2021b
	sp(p).	Chen et al. 2020; Ding et al. 2021; Ma et al. 2021b; Zhu et al. 2021; Li et al. 2022; Tronchoni et al. 2022; Wei et al. 2022; Gao et al. 2024

Papiliotrema	*aurea*	Xu et al. 2020; Section 2.3
	flavescens	Li et al. 2018; Section 2.3
	fusca	Xu et al. 2020
	laurentii	Li et al. 2018; Xu et al. 2020; Wang et al. 2021b
	pseudoalba	Xu et al. 2020
	terrestris	Li et al. 2021
	sp(p).	Cureau et al. 2021a; Cureau et al. 2021b; Zhu et al. 2021; Milanović et al. 2022; Li et al. 2022; Tronchoni et al. 2022; Gao et al. 2024
Piskurozyma	sp(p).	Tronchoni et al. 2022
Quambalaria	sp(p).	Milanović et al. 2022; Tronchoni et al. 2022
Rhodosporidiobolus	*colostri*	Wang et al. 2021b
	sp(p).	Mandakovic et al. 2020; Ma et al. 2021b; Zhu et al. 2021; Costantini et al. 2022; Tronchoni et al. 2022
Rhodotorula	*babjevae*	Setati et al. 2015; Xu et al. 2020; Liu and Howell 2021; Liu et al. 2021; Wang et al. 2021b; Section 2.3
	diobovata	Section 2.3
	glutinis	Bokulich et al. 2014; Setati et al. 2015; Gao et al. 2019; Li et al. 2021; Englezos et al. 2022; Section 2.3
	graminis	Sternes et al. 2017; Section 2.3
	kratochvilovae	Wang et al. 2021b
	mucilaginosa	Li et al. 2018; Liu et al. 2021; Wang et al. 2021b
	taiwanensis	Xu et al. 2020
	toruloides	Setati et al. 2015
	sp(p).	David et al. 2014; Pinto et al. 2015; Sternes et al. 2017; Li et al. 2018; Gao et al. 2019; Kioroglou et al. 2019; Zhang et al. 2019; Liu et al. 2020; Lu et al. 2020; Mandakovic et al. 2020; Li et al. 2021; Liu et al. 2021; Martins et al. 2021; Zhu et al. 2021; Costantini et al. 2022; Li et al. 2022; Milanović et al. 2022; Tronchoni et al. 2022; Martiniuk et al. 2023; Section 2.3; Gao et al. 2024
Saitozyma	*flava*	Xu et al. 2020
Solicoccozyma	*aeria*	Xu et al. 2020; Wang et al. 2021b
	terrea	Wang et al. 2021b
Sporobolomyces	*coprosmae*	Setati et al. 2015; Li et al. 2018; Xu et al. 2020

	japonicus	Section 2.3
	phaffii	Liu et al. 2021
	roseus	Setati et al. 2015; Li et al. 2018; Rantsiou et al. 2020; Kamilari et al. 2021; Liu et al. 2021; Wang et al. 2021b; Englezos et al. 2022
	ruberrimus	Xu et al. 2020; Wang et al. 2021b
	shibatanus	Setati et al. 2015; Englezos et al. 2022; Section 2.3
	sp(p).	David et al. 2014; Bokulich et al. 2016; Sternes et al. 2017; Kioroglou et al. 2019; Li et al. 2019; Xu et al. 2020; Liu et al. 2021; Martins et al. 2021; Wang et al. 2021a; Zhu et al. 2021; Costantini et al. 2022; Milanović et al. 2022; Tronchoni et al. 2022; Gao et al. 2024
Symmetrospora	*coprosmae*	Section 2.3
	oryzicola	Setati et al. 2015; Englezos et al. 2022
	symmetrica	Xu et al. 2020; Section 2.3
	sp(p).	Zhu et al. 2021; Englezos et al. 2022
Tausonia	*pullulans*	Wang et al. 2021b
	sp(p).	Zhu et al. 2021
Tilletiopsis	*washingtonensis*	Englezos et al. 2022; Section 2.3
	sp(p).	Zhu et al. 2021
Udeniomyces	*puniceus*	Kamilari et al. 2021; Wang et al. 2021b
	pyricola	Section 2.3
	sp(p).	Chen et al. 2020; Ma et al. 2021b; Steenwerth et al. 2021; Zhu et al. 2021; Tronchoni et al. 2022; Martiniuk et al. 2023
Ustilago	*bullata*	Wang et al. 2021b
	sp(p).	Wang et al. 2021b; Zhu et al. 2021; Costantini et al. 2022
Ustilentyloma	*graminis*	Wang et al. 2021b
Vishniacozyma	*carnescens*	Li et al. 2018; Rantsiou et al. 2020; Liu and Howell 2021; Liu et al. 2021; Wang et al. 2021b; Englezos et al. 2022; Section 2.3
	dimennae	Wang et al. 2021b
	heimaeyensis	Xu et al. 2020
	taibaiensis	Li et al. 2018
	tephrensis	Wang et al. 2021b; Section 2.3

	victoriae	Castañeda et al. 2018; Li et al. 2019; Liu et al. 2020; Rantsiou et al. 2020; Xu et al. 2020; Liu et al. 2021; Wang et al. 2021b; Englezos et al. 2022; Perpetuini et al. 2022
	sp(p).	Chen et al. 2020; Liu et al. 2021; Ma et al. 2021b; Martins et al. 2021; Zhu et al. 2021; Milanović et al. 2022; Tronchoni et al. 2022; Wei et al. 2022; Liang et al. 2023; Martiniuk et al. 2023; Section 2.3
None		Wang et al. 2015a; Portillo and Mas 2016; Stefanini et al. 2016; Morgan et al. 2019; Gómez-Albarrán et al. 2021; McCarthy et al. 2021

4.3 Basidiomycete yeasts in winemaking

While basidiomycete yeasts are rarely directly applied in commercial winemaking, what is known of their activities during fermentation and the purported effects on wine aromatics demonstrates their potential. Briefly, the main categories of aromatic compounds that may be influenced by basidiomycete yeasts are (1) alcohols, which may contribute positively but will be perceived as harsh and unpleasant at elevated levels, and may mask other pleasant aromas, (2) acids, which contribute positively to tartness, aroma, and mouthfeel at appropriate levels, but are otherwise sour and unpleasant, (3) esters, which primarily contribute pleasant fruity and floral aromas but may be overwhelming in high concentrations, (4) terpenoid compounds, which generally have pleasant citrus, herbal, woody, or floral aromas, and less commonly, (5) aldehydes and (6) ketones, with a range of nutty, herbal, fruity, and rich aromas, (7) phenolic compounds, which may carry distinctive and pleasant aromas but are often distasteful above relatively low thresholds, as well as the generally negative effects of (8) volatile sulfur substances, and (9) pyrazines. Aromatic properties of compounds are given in Table 4.3. Phenolic compounds also moderate the volatility of other aromatic compounds and influence the colour of wine (Muñoz-González et al. 2014; He et al. 2023).

Table 4.3. Aromatic compounds named in this review and corresponding associated aromas.[a]

Compound	Aromas and attributes
Acids	
Acetic acid	Vinegar, sour, fatty; Mouthfeel: warm
Propionic acid	Rancid
Butyric acid	Cheesy, rancid
Isobutyric acid	Cheesy, buttery, rancid
Isovaleric acid	Cheesy, sweaty, rancid, putrid
Hexanoic acid	Vinegar, sour, cheesy, fatty, sweaty, rancid
Octanoic acid	Soapy, faintly fruity, sickly sweet, cheesy, buttery, oily, fatty, rancid
Nonanoic acid	Cheesy, waxy
Decanoic acid	Citrus, sickly sweet, fatty, rancid
Tetradecanoic acid	Oily, waxy
Alcohols	
Benzyl alcohol	Almond, fatty
Phenethyl alcohol	Rose, pollen, floral, sweet
Propanol	Alcohol (stupefying), ripe fruit
2-Methyl-1-butanol	Onion, wine, burnt
3-Methyl-1-butanol	Malt, whiskey, burnt
Isobutyl alcohol	Alcohol, nail polish, faintly sweet, wine
Isoamyl alcohol	Nail polish, ripe fruit, marzipan, cheesy, malt, whisky, burnt
1-Hexanol	Grassy, herbal, green pepper, sweet
1-Heptanol	Herbal, sweet
1-Octanol	Herbal, orange, citrus, rose
1-Nonanol	Mushroom, herbal, fruity, sweet
Aldehydes	
Benzaldehyde	Cherry, sweet, almond, nutty, bitter
2-Methylbutanal	Almond, cocoa, malt
Hexanal	Grassy, herbal, tea leaf, apple, fatty
Hexen-2-al	Herbal, fruity
Dodecanal	Orange

Esters	
Acetates	
Ethyl acetate	Glue, nail polish, varnish, pineapple, fruity, sweet, balsamic
Ethyl phenylacetate	Honey, sweet, beeswax
Isoamyl acetate	Banana, pear, fruity, sweet
2-Phenylethyl acetate	Apple, cherry, pear, fruity, rose, floral, honey, sweet, tobacco
Ethyl esters	
Ethyl isobutyrate	Strawberry
Ethyl hexanoate	Green apple, banana, strawberry, fruity, anise, violet, floral, brandy, wine
Ethyl heptanoate	Pineapple, fruit, brandy, wine
Ethyl octanoate	Apricot, banana, pear, pineapple, fruity, floral, sweet, fatty
Ethyl nonanoate	Banana, grape, fruity, rose, floral, waxy
Ethyl decanoate	Fruity, rose, floral, waxy, fatty
Ethyl dodecanoate	Fruity, floral, sweet, creamy
Ethyl tetradecanoate	Soapy, waxy
Ethyl hexadecanoate	Fruity, sweet, fatty, waxy, rancid
Ethyl lactate	Raspberry, milky
Other esters	
Methyl benzoate	Floral, strawberry, fruity, honey
Diethyl succinate	Fruity, wine; Mouthfeel: full
Ketones	
Acetoin	Buttery, fatty
5-Ethyl-6-methyl-3E-hepten-2-one	Herbal, oily
2-Octanone	Herbal, fruity
Phenols	
Phenol	Tar, sickly sweet
4-Vinylphenol	Pharmaceutical
Pyrazines	
2,5-Dimethylpyrazine	Cocoa, roasted nuts, roast beef
3-Ethyl-2,5-dimethylpyrazine	Potato, earthy, roasty
Terpenoid compounds	
Terpenes	
Citronellol	Lemon, citrus

Farnesol	Herbal, floral
Geraniol	Geranium, rose
Linalool	Woody, fruity, citrus blossom, rose, floral, spicy, Muscat
Nerol	Citrus blossom, rose, floral
Nerolidol	Woody, orange, fruity, floral
α-Terpineol	Woody, anise, floral, sweet
Norisoprenoids	
β-Damascenone	Bark, cooked apple, peach, plum, fruity, rose, floral, honey, sweet
Safranal	Saffron
TDN[b]	Floral, gasoline
Vitispirane	Eucalyptus, woody, fruity, flowery, earthy
Sulfur compounds	
Methionol	Cauliflower, cooked potato
Benzothiazole	Rubber, cabbage
Others	
Ethanol	Alcohol; Mouthfeel: hot, pungent
Glycerol	Mildly sweet; Mouthfeel: full, oily

[a]Aromas and attributes are as described in the literature cited in this review; additional sources were consulted as needed (Lambrechts and Pretorius 2000; Aznar et al. 2001; Silva Ferreira et al. 2003; Campo et al. 2005; Chung et al. 2005; Swiegers et al. 2005; Li et al. 2008; Verginer et al. 2010; Wang et al. 2017; Niu et al. 2019; Ruiz et al. 2019; Ma et al. 2021b; Rigou et al. 2021; Wang et al. 2023a; Wu et al. 2023)

[b]1,1,5-trimethyl-1,2-dihydronaphthalene

Broadly, aroma may be divided into components termed “varietal”, “fermentation”, and “aging”. Varietal aroma is dependent on cultivar-specific precursor compounds and is ultimately composed mainly of terpenes, methoxypyrazine, and thiols (sulfur compounds), while fermentation aroma is primarily influenced by alcohols, esters, aldehydes, and acids produced by yeast and bacteria metabolism, and aging aroma develops during storage (He et al. 2023). Yeasts, including basidiomycete yeasts, that are active during wine fermentation contribute to aroma by using (removing) constituent compounds from the must, producing solvents (e.g., alcohols and acetate esters) that help to extract aromatic compounds, secreting enzymes that facilitate the production of varietal aroma compounds, synthesizing fermentation aroma compounds directly (Fleet 2003). These activities may also facilitate fermentation by impacting the metabolism of other microorganisms (e.g., by providing reagent compounds), including fermentative yeasts like *S. cereivisae* (Belda et al. 2016; Tolosa and Prieto 2019). Finally, basidiomycete yeasts may influence the overall “body” of a wine, which includes effects of the concentrations of sugar and ethanol, as well as the mouthfeel (Wang et al. 2023a).

Basidiomycete yeasts, however, cannot complete fermentations on their own and so their use should be in conjunction with an inoculation method that incorporates fermentative yeast(s), for example, mixed inoculum or a method such as *pied de cuve* inoculation which uses must that has begun to ferment (Morgan et al. 2019). Alternatively, steps could be taken to increase their occurrence in spontaneous fermentations (e.g., reducing damage to grapes at harvest, prior to crushing and pressing), but the results of this approach would likely be inconsistent and less noticeable. Regardless, the activities of basidiomycete yeasts during a wine fermentation must be considered only in the context of the parameters of that fermentation (e.g., grape cultivar, indigenous yeast community, inoculant(s) and additive(s) used, overall fermentation procedure).

SO_2 addition early in fermentation warrants particular consideration, in view of its aim to reduce unwanted spoilage microorganisms, including non-*Saccharomyces* yeasts (Giacosa et al. 2019). SO_2 does not universally wipe out the indigenous yeast population, but its use does alter the community composition (Raspor et al. 2002; Lange et al. 2014; Morgan et al. 2019; Windholtz et al. 2021a) and may reduce the influence of non-*Saccharomyces* yeasts on wine aroma (Sumby et al. 2021). The SO_2 resistance of individual basidiomycete yeasts during wine fermentation is not known, and therefore they should be used in fermentations without SO_2, or screened for SO_2 resistance if intended for use in fermentations utilizing SO_2.

The complex nature of wine aroma is such that increased or decreased production of any compound cannot be judged as inherently good or bad independently from a particular fermentation. A trait that is negative in one scenario, such as the high production of acetic acid in mixed inoculation of *Papiliotrema flavescens* and *S. cerevisiae*, may be modified by changing a fermentation parameter such as grape cultivar (Rossouw and Bauer 2016). In this light, the following examples in which basidiomycete yeasts impact aroma formation in wine are presented to illustrate their potential, rather than as instructions for use.

4.31 Glycerol production

Glycerol is a substantial component of wine and although it is generally considered to increase both sweetness and viscosity, numerous studies indicate that glycerol actually has negligible effects on viscosity and its impact is mainly on sweetness (Noble and Bursick et al. 1984; Yanniotis et al. 2007; Goold et al. 2017). Nevertheless, increased glycerol production is often favoured in winemaking, and basidiomycete yeasts, including *P. flavescens*, *Vishniacozyma victoriae*, *Symmetrospora oryzicola*, and *Tremella globispora* may produce high levels of glycerol during wine fermentation, in concert with other desirable compounds (Rossouw and

Bauer 2016; Englezos et al. 2022). For example, a high-glycerol producing strain of *P. flavescens* was also associated with increased levels of isobutyl alcohol, isoamyl alcohol, decanoic acid, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, ethyl lactate, and acetoin, and decreased levels of multiple acids, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate, depending on grape cultivar, in sequential inoculation with *S. cerevisiae*, compared to a *S. cerevisiae*-only control (Rossouw and Bauer 2016). Furthermore, panelists were able to distinguish wines inoculated with *P. flavescens*, to which they attributed "oaky", "floral" and "earthy" descriptors.

Glycerol metabolism may also enable yeast growth in later stages of fermentation by initially protecting cells from the high osmotic stress associated with high sugar concentrations, maintaining cell redox balance, and providing precursor molecules for the synthesis of phospholipids in cell membranes (Swiegers et al. 2005). Therefore, a high glycerol producing basidiomycete yeasts like *P. flavescens* may have a greater influence on wine aroma by surviving longer in fermentations. There is also evidence that mixed inoculation with *S. cerevisiae* can reduce the effect of increased acetic acid formation in glycerol producing yeast strains (Swiegers et al. 2005; Rossouw and Bauer 2016).

Higher levels of glycerol may also support a glycerol metabolism in some yeasts as glucose and nitrogen are depleted (Kot et al. 2016; Klein et al. 2017). For example, some strains of *R. glutinis* and *S. shibatanus* can use glycerol as a carbon source, and their growth in individual culture suggests that glycerol concentration could impact their production of lipids during wine fermentation, which may in turn enable the production of aromatic compounds or provide nutrients to other yeasts upon their death (Kot et al. 2016; Kot et al. 2021). *S. cerevisiae* and other fermentative ascomycetes may also utilize glycerol as glucose becomes scare near the

end of fermentation, although the ability varies among strains and has not been explored in the context of wine fermentation (Klein et al. 2017).

4.32 Ethanol production

Although some consumers seek out the "full-bodied, fruit-forward" wines produced by sweeter grapes with higher °Brix (1 g sucrose/100 g solution), others prefer "lighter" wines that lack the higher alcohol content that often accompanies these fruitier wines (Goold et al. 2017). The growth of the wine industries in warmer climates as well as the warming of established wine regions, combined with a desire to harvest fully ripened grapes that best express fruity and varietal aromas, is creating a demand for fermentation methods that maintain these positive aspects but restrict ethanol levels (Swiegers et al. 2005; Gonzalez et al. 2021). Non-*Saccharomyces* yeasts generally produce less ethanol than *Saccharomyces* yeasts, and combined inoculations, primarily using ascomycete yeasts with some fermentative capacity, have been explored to reduce ethanol in wine fermentations (Contreras et al. 2014; Quirós et al. 2014).

A higher proportion of basidiomycete yeasts should correspond with a reduction in ethanol production, given their non-fermentative metabolism, but the mechanisms by which these yeasts moderate ethanol levels are not well understood. Species of *Udeniomyces* and *Rhodotorula* (including *R. mucilaginosa* and *R. glutinis*), as well as *F. magnum* and *P. flavescens*, may either increase or decrease ethanol concentration, depending on fermentation conditions (Rossouw and Bauer 2016; Chen et al. 2020; Englezos et al. 2022; Li et al. 2021; 2022). As expected, a reduction in ethanol is generally correlated with a higher concentration of final residual sugars and reduced fermentation rate (Sternes et al. 2017; Englezos et al. 2022; Li et al. 2021). I am unaware of any research that explicitly tests basidiomycete yeasts to reduce ethanol in wine (beyond inclusion in preliminary isolate screening, e.g., Contreras et al. (2014)),

although other authors periodically propose the use of ascomycete yeast strains with a strictly aerobic metabolism for this purpose (Gonzalez et al. 2013; Mateo and Maicas 2016). More recent studies have aimed to better facilitate ethanol reduction using these yeasts by using aerated fermentation conditions while simultaneously mitigating the negative effects of aeration (Jolly et al. 2022).

4.33 Correlations between basidiomycete yeasts and fermentation aromatics

In most cases in which the abundance of a basidiomycete yeast is correlated with increased or decreased production of an aromatic compound associated with fermentation aroma, the chemical pathway (e.g., whether the yeast produces an intermediary compound or directly synthesizes the final compound in question) is unknown. However, examples of these correlations indicate that under the appropriate fermentation conditions, it is likely that most, if not all, basidiomycete yeasts can contribute positively to winemaking.

Although high concentrations of acids are undesirable, sufficient acidity is also an essential aspect of wine. Moderating acidity is therefore a significant component of winemaking which may be influenced by basidiomycete yeasts. *S. shibatanus*, *F. magnum*, *P. flavescens*, and *V. victoriae* have been associated with the production of acetic acid (Verginer et al. 2010; Rossouw and Bauer 2016; Englezos et al. 2022; Wu et al. 2023), which makes up most of the volatile acid component of wines but carries a vinegar aroma above concentrations of 0.7-1.1 g/L (Swiegers et al. 2005). The concentrations of a wide range of volatile fatty acids, including propionic, butyric, isobutyric, isovaleric, hexanoic, octanoic, nonanoic, decanoic, and tetradecanoic acids may also be affected by basidiomycete yeasts including *R. mucilaginosa*, *R. glutinis*, *S. oryzicola*, and species of the genera *Filobasidium*, *Naganishia*, *Hannaella*, *Udeniomyces*, and *Malassezia*, as determined by analyses of aromatic compounds formed during

mixed inoculum fermentations (Calabretti et al. 2011; Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a) and correlated with species abundances during fermentations (Chen et al. 2020; Englezos et al. 2022; Wei et al. 2022; Gao et al. 2024). Basidiomycete yeasts that produce volatile fatty acids that exceed thresholds may be especially undesirable, given their negative aromas, and as the growth of *S. cerevisiae* may be inhibited by high concentrations (Fleet 2003).

Basidiomycete yeasts are also commonly correlated with the production of a variety of higher alcohols, which generally contribute positive aromas, provided levels remain within acceptable thresholds (<300 mg/L) (Wang et al. 2023b). For example, nutty and fatty aromas may be heightened by the increased levels of benzyl alcohol associated with *Vishniacozyma* and *Udeniomyces* (Ma et al. 2021b; Englezos et al. 2022), while *R. mucilaginosa* and *S. roseus* may impart rose-like floral and sweet aromas via the increased production of phenethyl alcohol (Verginer et al. 2010; Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a). Levels of propanol, isobutyl alcohol, and isoamyl alcohol, which may all carry distinctive alcohol aromas, but in low levels, impart sweet fruitiness, have been linked to many basidiomycete yeasts including *R. mucilaginosa* (Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a), *R. glutinis* (Englezos et al. 2022), *S. roseus* (Verginer et al. 2010) and *P. flavescens* (Rossouw and Bauer 2016; Wang et al. 2023b). On the other hand, 2- and 3-methyl-1-butanol are often associated with more negative aromas and are produced by *S. roseus* in culture (Verginer et al. 2010), although it is unclear how production during fermentation may differ or impact the overall character of the wine. Chemical variants of hexanol, heptanol, octanol, and nonanol may impart a variety of positive or negative herbal, floral, citrus, or even mushroom-like aromas to wine, and again, depending on interactions with other factors, most basidiomycete yeasts (e.g., *Rhodotorula*,

Rhodosporidiobolus, *Hannaella*, *Papiliotrema*, *Vishniacozyma*) will have some impact on levels of these alcohols (Ma et al. 2021b; Wei et al. 2022; Gao et al. 2024).

Aldehydes and ketones also contribute aromas to wine that help make up its distinctive character, although some, such as acetoin, are generally considered as negative, or at least to give rise to negative compounds such as diacetyl (Jolly et al. 2014; Comitini et al. 2021). Basidiomycete yeasts have been linked to increased production of a variety of aldehydes, such as *Filobasidium* with the sweet, nutty, or cherry aromas of benzaldehyde, *Hannaella* with the orange citrus aroma of dodecanal, and herbal or fruity aromas of hexen-2-al, and *Udeniomyces* with increased levels of all three of these aldehydes, as well as with hexanal, which may impart grassy, herbal, or apple aromas (Ma et al. 2021b). *Sporobolomyces roseus* was also noted to produce 2-methylbutanal in culture, which may increase cocoa, malty, or nutty aromas (Verginer et al. 2010). However, negative correlations may also occur, such as between *Vishniacozyma* and the closely related *Hanaella* and benzaldehyde (Ma et al. 2021b). Fewer correlations between basidiomycete yeasts and ketones are known, but the associations of *F. magnum* with 5-ethyl-6-methyl-3E-hepten-2-one (Wu et al. 2023), *Filobasidium*, *Hanaella*, and *Udeniomyces* with 2-octanone (Ma et al. 2021b), and *P. flavescens* with acetoin (Rossouw and Bauer 2016) suggest that additional research is likely to reveal additional correlations.

A more well researched group of compounds that contributes to the aroma of wine is the esters. A vast diversity of esters is known from wines, but some of the most investigated groups are the acetates and ethyl esters, due to their relative ubiquity. The fruity, floral, and sweet aromas of ethyl acetate, ethyl phenylacetate, isoamyl acetate, and 2-phenylethyl acetate are associated with *R. mucilaginosa* (Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a), *S. shibatanus* and *Symmetrospora* (Verginer et al. 2010; Englezos et al. 2022), *Filobasidium* and

Hannaella (Wei et al. 2022), *P. flavescens* (Rossouw and Bauer 2016), and *Udeniomyces* (Chen et al. 2020). Ethyl esters are also widely associated with basidiomycete yeasts, known from production in culture (Verginer et al. 2010), via the detection of increased levels during mixed inoculum fermentations (Rossouw and Bauer 2016; Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a; Wu et al. 2023) and from correlations of increased levels with population abundances during fermentations (Englezos et al. 2022; Gao et al. 2024). Given that basidiomycete yeasts seem to be universally capable of ester production in wine fermentations, and the vast array of esters that are known to contribute distinguishing aromas to different wines, basidiomycete yeasts may be a largely untapped reservoir for unexpected and unique aroma formation in wine via the production of esters.

However, the use of basidiomycete yeasts, like the use of any other microorganisms in wine fermentations, carries the risk of an overproduction of undesirable compounds. In addition to levels of those previously listed rising above acceptable thresholds, increased concentrations of pyrazines, phenols, and sulfur compounds generally carry negative consequences, although they may contribute aromas distinctive to some wines, in low concentrations. Phenolic off-flavours may result from an abundance of *Rhodotorula* or *Papiliotrema* (Shinohara et al. 2000), while *F. magnum* has been linked to increased production of 2-methoxy-4-vinylphenol, which conveys an unpleasant pharmaceutical aroma (Wu et al. 2023). *S. roseus* and *R. glutinis* may increase levels of the sulfur compound 1,3-benzothiazole, giving aromas of rubber or cabbage, and *S. roseus* may also increase 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine, which impart roasted aromas that may be nutty or chocolatey or may rather be unpleasantly reminiscent of meat or vegetables (Verginer et al. 2010; Englezos et al. 2022). *V. carnescens* and *Malassezia*

are also associated with increases of sulfur compounds such as methionol, which imparts aromas of cabbage or potato (Englezos et al. 2022; Wei et al. 2022).

4.34 Enzymatic activities: facilitation of fermentation and influence on varietal aroma

High enzymatic activity during wine fermentation may increase the production of precursor compounds that support the metabolism of other yeasts (Belda et al. 2016), and of compounds with pleasant fruity or floral aromas (Fia et al. 2014). For example, β-glucosidase is active in the production of varietal aromatic compounds by partially breaking down a variety of non-volatile glycosides and oligosaccharides to produce volatile terpenoid compounds, in addition to glucose, which may be utilized by ascomycete yeasts like *S. cerevisiae* (Swiegers et al. 2005; Maicas and Mateo 2015; Belda et al. 2016). Basidiomycete yeasts of the genus *Rhodotorula*, including species *R. glutinis*, *R. mucilaginosa*, and *Rhodotorula toruloides* (Belda et al. 2016; Wang et al. 2017), and others including *Sporobolomyces shibatanus* (Baffi et al. 2011; Baffi et al. 2013) and *Cryptococcus amylolentus* (Belda et al. 2016) are known to produce β-glucosidase in wine fermentations, although enzyme activities are variable among strains (Ge et al. 2023).

Mixed inoculum fermentations using *S. cerevisiae* together with *R. mucilaginosa* or *S. shibatanus* with high β-glucosidase activity demonstrate the possible benefits of β-glucosidase-producing yeasts to wine aroma, although the interacting effects of grape cultivar and inoculation parameters such as yeast strain, concentration, and timing are not well understood (Wang et al. 2017; Vilela 2020). In some cases, a pattern emerges in which the addition of *R. mucilaginosa* results in increased levels of hexanoic, octanoic, and decanoic acids and the corresponding esters ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl hexadecanoate, along with increases of benzyl alcohol, phenethyl alcohol, isoamyl alcohol, 1-hexanol, ethyl acetate, 2-phenethyl

acetate, ethyl isobutyrate, diethyl succinate, and vitispirane (Wang et al. 2017; Ma et al. 2021a). The resulting wines have more intense fruit and floral aromas, although they may also possess a faint animal aroma and higher concentrations of undesirable sulfur compounds.

These negative effects can seemingly be mitigated, however, by adjusting inoculation parameters, as Wang et al. (2023a) also found increased acids following mixed inoculation with *R. mucilaginosa*, but they remained below acceptable thresholds and so contributed to an overall balanced aroma in the wine and increased the complexity and richness of the mouthfeel. The levels of a similar variety of volatile compounds (phenethyl alcohol, isobutyl alcohol, phenylethyl acetate, ethyl octanoate, and ethyl 9-decenoate) were also elevated, and the wines received high scores in attributes of green fruit, tropical fruit, aroma intensity, and floral notes (Wang et al. 2023a).

The second pattern of effects resulting from inoculation with β-glucosidase-producing strains of *R. mucilaginosa* or *S. shibatanus* is characterized by an increase in terpenoid compounds, which is expected as glucosidases convert non-aromatic terpenoid precursors to their aromatic form (Swiegers et al. 2005; Belda et al. 2017). Higher concentrations of a greater variety of terpenoids, including nerol, β-damascenone, geraniol, citronellol, linalool, and terpineol, as well as phenethyl acetate, were produced following sequential inoculations with lower ratios of *R. mucilaginosa* to *S. cerevisiae* (Ma et al. 2021a), simultaneous inoculation of *R. mucilaginosa* and *S. cerevisiae* with different grape cultivars (Calabretti et al. 2011; Wang et al. 2023a), and when using *S. shibatanus* (Baffi et al. 2011; Baffi et al. 2013). Resulting wines had increased astringent and acidic properties (Calabretti et al. 2011) but also had stronger fruity and floral aromas and high scores in general impression and intensity of flavour (Calabretti et al. 2012; Ma et al. 2021a).

α-L-arabinofuranosidase, which is produced by strains of *Rhodotorula toruloides* (Martínez et al. 2006), also facilitates β-glucosidase activity by cleaving the 1,6-glycosidic linkage in the first step of monoterpene hydrolysis, allowing β-glucosidase to then liberate monoterpenols (Gunata et al. 1988). *Udeniomyces*, *P. flavescens*, *Tilletiopsis washingtonensis* and *T. globispora* have also been linked to higher production of terpenes such as citronellol, farnesol, geraniol and nerolidol (Rossouw and Bauer 2016; Ma et al. 2021b; Englezos et al. 2022; Wang et al. 2023b), and the genus *Filobasidium*, including *F. magnum*, may moderate levels of the norisoprenoids (an alternate class of terpenoid compounds that similarly contribute fruity or floral aromas) safranal and 1,1,5-trimethyl-1,2-dihydronaphthalene (TDN) (Wu et al. 2023; Gao et al. 2024), suggesting that these groups may also have desirable β-glucosidase or α-L-arabinofuranosidase activity.

Other enzymes, such as pectinases, are beneficial during early stages of winemaking to improve juice extraction, as well as in later stages for improved clarification, extraction of phenols and pigments, and release of aromatic compounds (Louw et al. 2006; Fleet 2008). Pectinases, along with xylanases and α-L-arabinofuranosidase, play roles in the degradation of plant cells walls, aiding in the hydrolysis of pectin, xylan, and cellulose, by which precursor compounds may be made available for fermentation and aroma development (Belda et al. 2016; Tolosa and Prieto 2019). Commercial preparations of enzymes like pectinases are often added to wine fermentations but may have undesirable side activities, creating interest in pectinolytic yeasts that may be used as inoculants (Alimardani-Theuil et al. 2011).

Basidiomycete yeasts including *Rhodotorula* (Vaughn et al. 1969; Merín et al. 2015), *Naganishia* (Federici 1985; Merín et al. 2015), and *Piskurozyma capsuligena* (Merín et al. 2014) may produce pectinases. Strains of *P. capsuligena* and *Rhodotorula dairenensis* isolated from

wineries produce pectinases under appropriate wine fermentation conditions, and strains of both species have been characterized as having additional favourable cellulase and xylanase activities (Merín et al. 2014; Merín et al. 2015). *P. capsuligena* is also known to produce α-amylase and glucoamylase (De Mot and Verachtert 1985) which may be utilized for similar beneficial effects in grape wine fermentation, considering the applications of α-amylase to increase juice extraction, pre-fermentation sugar content, and final clarity and phenolic content of banana wine (Cheirsilp and Umsakul 2008) and Chinese yellow wine (Bian et al. 2022), and glucoamylase to improve rice wine fermentations (Ueda et al. 1991; Yang et al. 2013).

Few studies examine protease or esterase production by basidiomycete yeasts in the context of wine production. However, strains of *Rhodotorula* may produce proteases (Chomsri 2008) as well as acetyl xylan esterase (Lee et al. 1987), which functions in concert with xylanases to complete xylan degradation (Tolosa and Prieto 2019). In general, esterase activity must be balanced by ester synthesis during fermentation to allow a net increase in ester accumulation, considering the positive impact of esters on wine aroma (Swiegers et al. 2005). However, esterases are also active in the formation of acetate esters, which contribute pleasant wine aromas, and esterase activity alters the overall balance of different esters (Wang et al. 2023b). Further research might capitalize on the purported higher occurrence of esterases associated with basidiomycete rather than ascomycete yeasts (Buzzini and Martini 2002). Proteolytic activity can decrease haze-causing protein content in wine and generate usable nitrogen for other microorganisms (i.e., by degrading polypeptides and proteins to amino acids and peptide residues) that may also be used to synthesize aromatic compounds (Chomsri 2008; Tolosa and Prieto 2019). Notably, *S. cerevisiae* and other fermentative yeasts require sufficient nitrogen to complete fermentation (Maicas and Mateo 2015; Tolosa and Prieto 2019). Some

strains of *Sporobolomyces* produce proteases, but their activity has not been explored in wine fermentations (Kot et al. 2021).

Strains of *F. magnum*, closely related to *N. albida* and *P. capsuligena*, may have high lipoxygenase activity, which increases the degradation of astringent higher fatty acid esters and promotes the production of terpenoids such as β-damascenone and safranal (Wu et al. 2023). While associations between *F. magnum* and positive aroma compounds may in some cases be absent or negative (Englezos et al. 2022; Ma et al. 2023), effects are likely dependent not only on strain, but also on interactions with other microorganisms. Although I am unaware of any research using *F. magnum* in mixed inoculum wine fermentations, Wu et al. (2023) found increased levels of acetic acid, 2-phenylethyl ester, methyl benzoate, safranal, 5-ethyl-6-methyl-3E-hepten-2-one, and 2-methoxy-4-vinylphenol only in co-culture with *Bacillus kochi* (chosen for its protease and α-amylase activity) during the aging (fermentation) of tobacco.

4.35 Yeast interactions

Individual microorganisms do not influence the characteristics of a wine independently from the rest of the microbial community. Interactions among species influence the dynamic nature of the yeast community during fermentation, and therefore also influence the production of aromatic compounds. An overabundance of basidiomycete yeasts early in the fermentation may limit the subsequent and necessary growth of fermentative yeasts like *S. cerevisiae*, by depleting essential nutrients or producing toxic compounds, although the death of non-*Saccharomyces* yeasts during the early stages of fermentation also provides nutrients to support the growth of *S. cerevisiae* (Fleet 2003).

Basidiomycete yeasts are again less well researched than their ascomycete counterparts considering yeast-yeast interactions during wine fermentations, but the same relationships that

occur among ascomycete yeasts may be presumed to exist in some capacity for basidiomycete yeasts. Interactions may be positive, such as the overproduction of acetaldehyde by a hybrid strain of *Saccharomyces* in mixed inoculum fermentations, promoting the growth and fermentation rate of a *S. cerevisiae* strain when compared to monoculture fermentations (Cheraiti et al. 2005). Oluwa (2020) propose that metabolites produced by *P. flavescens* may serve a similar function in fermentation to support the later growth of other yeasts. Conversely, some yeasts may deplete nitrogen or other nutrient sources before *S. cerevisiae* becomes competitive, altering the progression of fermentation and formation of aromatic compounds (Bordet et al. 2020; Zilelidou and Nisiotou 2021). For example, the consumption of nitrogen sources during fermentations that experienced sequential inoculation using *Metschnikowia pulcherrima* (followed by inoculation with *S. cerevisiae*) was connected to the expression of varietal thiols, fatty acids, and ethyl esters (Seguinot et al. 2020), and competition for nitrogen and nutrients between different yeasts and *S. cerevisiae* was similarly linked to the production of higher alcohols and esters by Rollero et al. (2018).

These effects are due to the successive nature by which aromatic compounds are formed as fermentation unfolds, which depends on the assemblage of microorganisms. Higher alcohols are derived from amino acids and are in turn used along with additional amino acids and ethanol as precursors in acetate ester formation, while ethyl ester synthesis requires medium-chain fatty acids such as hexanoic or octanoic acid (Wang et al. 2023b). Therefore, basidiomycete yeasts that produce higher alcohols or fatty acids may influence wine aroma either by the specific aromatic effects of those compounds, or by facilitating the production of aromatic esters by other yeasts. As fermentation proceeds, the changing proportions and types of carbon, nitrogen, and

other compounds determine the final composition of the wine (Carrau et al. 2008; Jiang et al. 2019; Wang et al. 2023b).

Basidiomycete yeasts such as *Papiliotrema laurentii* that may be present in wine musts and during fermentations may also produce toxins that inhibit the growth of other yeasts (Yurkov and Golubev 2013). Although "killer toxins", or proteins that are lethal to other sensitive yeast strains, are generally associated with ascomycete yeasts (killer yeasts) in the context of winemaking (Mannazzu et al. 2019), strains of *P. capsuligena* secrete a killer toxin active against species of *Cryptococcus* and *Filobasidium* (Keszthelyi et al. 2006), which may alter the overall yeast community during fermentation. Physical contact between yeasts may also be antagonistic, as demonstrated by reduced cell viability of *S. cerevisiae* in fermentations inoculated with *Lachancea thermotolerans* allowing for cell-cell contact, vs. fermentations in which *L. thermotolerans* was restricted by a dialysis membrane, and the resulting wines had different concentrations of higher alcohols, fatty acids, and esters (Petitgonnet et al. 2019).

Some next-generation sequencing studies of wine grapes and fermenting musts include correlation analyses among species that capitalize on their large, quantitative datasets. For example, correlations of fungal communities during spontaneous wine fermentations showed that larger populations of *Papiliotrema*, *Vishniacozyma* and *Filobasidium* were negatively correlated with abundance of *S. cerevisiae* (Wei et al. 2022; Liang et al. 2023), while *Vishniacozyma* and *Filobasidium* exhibited a co-occurrence effect with *Pichia* and *Hanseniaspora* (Ma et al. 2021b). Similar negative correlations were modelled between each *Vishniacozyma heimaeyensis* and *S. roseus* with *Pichia* spp. based on metabolic activity (Martins et al. 2022). Abundances of common vineyard constituents *Metschnikowia* and *Naganishia* were negatively correlated with each other on grape surfaces (Zhu et al. 2021), and during spontaneous fermentation, *Naganishia*

and *Hannaella* were negatively correlated with *Pichia* (Ma et al. 2021b). More broadly, *Vishniacozyma* and *Udeniomyces* were negatively correlated with higher abundances of most other fungal genera on grapes, while *Rhodotorula* was mostly positively correlated with other fungal genera (Zhu et al. 2021). Martins et al. (2022) further suggest a possible strong association between *R. glutinis* and *B. alba*.

4.36 Practical challenges to the use of basidiomycete yeasts in winemaking

Basidiomycete yeasts identified from grape musts and wine fermentations based on high throughput sequencing studies may not exist in culture, and therefore, despite any correlations with the concentration of aromatic compounds or improved perception of the wine, it may not be possible to isolate these yeasts and use them deliberately to inoculate fermentations. Compared to *S. cerevisiae* and other fermentative ascomycete yeasts, basidiomycete yeasts may require different isolation, proliferation, and maintenance protocols. Species of interest may require preliminary testing to determine the culture ideal conditions, although the literature concerning basidiomycete yeasts in industrial and medical contexts may provide a useful resource (Saha et al. 2009; Homolka 2014; Kot et al. 2016; Elfeky et al. 2020). Basidiomycete yeasts also need to be evaluated for their effects on fermentations in concert with one or more fermentative ascomycete yeasts, which adds a layer of complexity to the results. However, experimentation using mixed inoculum in wine fermentations is already common in the context non-*Saccharomyces* ascomycete yeasts, which also possess a wide range of variation among strains (Vilela 2020).

It is also important to note that some basidiomycete yeasts associated with wine grapes are opportunistic human pathogens (e.g., *P. laurentii* and *Rhodotorula* spp.), raising concerns for their isolation and application. However, infections are rare and occur almost exclusively in

immunocompromised individuals (Banerjee et al. 2013; Ioannou et al. 2019), and ascomycete yeasts routinely used in winemaking, including *Saccharomyces*, may also cause infection (Enache-Angoulvant and Hennequin 2005). A routine laboratory safety protocol should prevent incident (Baron and Miller 2008), but further consideration of this topic would be prudent, given the novelty of using basidiomycete yeasts in winemaking.

For example, some species may require addition to fermentations as fresh (not freeze-dried) cultures, which may carry increased risks of contact between the active yeast and winery workers. Some basidiomycete yeasts, such as the genus *Malassezia*, may ultimately be best avoided due to the number of species known to cause infection and the apparently marginal benefits to winemaking (Theelen et al. 2018; Englezos et al. 2022; Wei et al. 2022). Regarding some other genera, such as *Cryptococcus*, species pathogenic to humans (e.g., *Cryptococcus neoformans*) are exclusive of species known from winemaking (Montoya et al. 2021; Table 4.1; Table 4.2). Indeed, *Cryptococcus* species associated with vineyards are increasingly being reassigned to other genera (Table 4.4). Given that basidiomycete yeasts do not survive past the end of fermentation and are already present in most, if not all, wine fermentations, the risk to consumers seems negligible.

Table 4.4. Current taxonomy of species reported from wine grapes and musts formerly assigned to *Cryptococcus*.

Invalid *Cryptococcus* taxonomy	**Current taxonomy**
C. adeliensis	*Naganishia adeliensis*
C. albidosimilis	*Naganishia albidosimilis*
C. albidus	*Naganishia albida*
C. aureus	*Papiliotrema aurea*
C. bhutanensis	*Naganishia bhutanensis*
C. carnescens	*Vishnizcozyma carnescens*
C. diffluens	*Naganishia diffluens*
C. dimennae	*Vishniacozyma dimennae*
C. flavescens	*Papiliotrema flavescens*
C. flavus	*Saitozyma flava*
C. foliicola	*Vishniacozyma foliicola*
C. heimaeyensis	*Vishniacozyma heimaeyensis*
C. huempii	*Krasilnikovozyma huempii*
C. humicol(a)/(us)	*Vanrija humicola*
C. hungaricus	*Dioszegia hungarica*
C. laurentii	*Papiliotrema laurentii*
C. luteola	*Hannaella luteola*
C. macerans	*Cystofilobasidium macerans*
C. magnus	*Filobasidium magnum*
C. nemorosus	*Papiliotrema nemorosa*
C. oeriensis	*Filobasidium oeirense*
C. randhaw(ae)/(ai)/(ii)	*Naganishia randhawae*
C. saitoi	*Naganishia globosa*
C. stepposum	*Filobasidium stepposum*
C. taibaiensis	*Vishniacozyma taibaiensis*
C. tephrensis	*Vishniacozyma tephrensis*
C. terre(a)/(us)	*Solicoccozyma terrea*
C. terrestris	*Papiliotrema terrestris*
C. uzbekistanensis	*Naganishia uzbekistanensis*
C. victoriae	*Vishniacozyma victoriae*
C. wieringae	*Filobasidium wieringae*
C. zeae	*Hanaella zeae*

4.4 Factors influencing vineyard yeast communities: environment and analyses

Although a comprehensive evaluation of the literature considering wine grape and must yeast communities was conducted, direct comparisons among community analyses are difficult – a challenge that is repeatedly noted in the context of wine grape yeast communities (Barata et al.

2012; Sumby et al. 2021). The words of Fleet (2003) that “There are many unanswered questions as to why certain yeast species predominate on wine grapes, and others are absent.” were echoed nearly 20 years later, and only three years prior to this review, by Sumby et al. (2021) expressing that “Thus, the study of the grapevine fungal microbiome has many unanswered questions and further careful investigation […] is warranted”.

In this light, I encourage the reader to be cautious in assigning causality to the presence or abundance of taxonomic groups, as there are many interacting factors that affect yeast community composition, including basidiomycete yeasts (Table 4.5). These include geography, climate, cultivation practices, grape cultivar, and grape ripeness and damage, which act together to create the unique and dynamic terroir of a vineyard, or even areas of micro-terroir within a vineyard (Pretorius et al. 1999; Barata et al. 2012; Sumby et al. 2021).

Basidiomycete yeast species abundances on grapes, in grape musts, and during fermentation also depend on the presence and proportions of one another and on other microbes. For example, yeasts interact with filamentous fungi on grapes, as each produces metabolites that may influence the growth of the other (Fleet 2003). Several basidiomycete yeasts, including *P. laurentii*, *V. victoriae*, and *Rhodotorula* spp., may be antagonistic to mycotoxigenic filamentous fungi in the vineyard via the formation of biofilms (aggregations of adhesive cells) on the fruit surface, mycoparasitism, the secretion of defensive enzymes, or by inducing host resistance (Oztekin et al. 2023). Their presence, whether naturally occurring or enhanced as part of a biocontrol strategy, alters the fungal community composition.

Table 4.5. Sampling variables that impact basidiomycete yeast community composition.[a]

Variables	Examples
Geography of vineyard	Latitude and longitude, slope degree and direction, soil type, elevation, surrounding environment.
Climate	Temperature, precipitation, humidity, wind.
Vineyard cultivation practices	Cultivation system (e.g., organic, conventional), cover crop usage, types of pesticides and fertilizers and timing of application(s).
Sampling regime	Number and size of samples, physical and temporal distance between samples.
Grape cultivar	Cabernet Sauvignon, Chardonnay, Merlot, Riesling.
Condition of grapes	Ripeness, degree of damage, time of harvest.
Substrate type	Grape skins, fresh pressed must, cold settled must, fermenting wine (further: stage of fermentation, aseptic vs. standard winery equipment).
Fermentation practices	Quantity, timing, and types of additives (e.g., species and strains of yeast and bacterial inoculant(s), sulfites, preservatives)

[a]Informed by Pretorius et al. (1999), Barata et al. (2012), Bordet et al. (2020), and Sumby et al. (2021).

The actual yeast community, as influenced by these environmental factors, is represented with varying accuracy and precision by community characterization methods. Researchers target specific functional or taxonomic groups of yeasts, either deliberately or inadvertently, by variation in methods for isolation, sequencing, identification, and analysis, and incorporate cutoffs below which yeasts are not detected or are not reported. Finally, changes to yeast taxonomy also complicate comparisons among studies and several common genera have undergone extensive systematic revisions since they were first reported as wine grape associates, with further revisions no doubt to come. For example, the genus *Cryptococcus* is extensively cited from grapes, musts, and fermentations, but many members have since been reclassified to various other genera (Table 4.4).

4.41 Geography and climate

Some basidiomycete yeast species have been associated with climate or location, despite confounding factors such as the increased use of pesticides in years with higher rainfall (Barata

et al. 2012). For example, the genera *Filobasidium* and *Naganisha* (Filobasidiaceae), including *F. magnum* and *N. albida*, were most abundant in dryer, cooler, sunnier regions in China (Li et al. 2021; Li et al. 2022). *N. albida* has also been associated with a cooler, maritime region of Spain (Longo et al. 1991), and cooler regions in Japan (Yanagida et al. 1992). In agreement, a global meta-analysis of vineyard yeasts suggested that *Filobasidium* and *Dioszegia* prefer cooler climate vineyards (Drumonde-Neves et al. 2021). However, this may not always hold true at finer scales, as *Filobasidium* was most abundant in a Croatian coastal vineyard with relatively high maximum temperatures (Milanović et al. 2022).

The relative abundance of *Rhodotorula* has also been linked to geographical regions (Kioroglou et al. 2019), in some cases seeming to prefer cooler, sunnier, and/or more humid environments (Yanagida et al. 1992; Lederer et al. 2013; Li et al. 2021; Li et al. 2022) and higher elevation (Li et al. 2021; Milanović et al. 2022), although in other research these effects are not present (Longo et al. 1991; Jolly et al. 2003). Other genera linked to climate variables include *Curvibasidium cygneicollum* correlating with low levels of sun and high elevation (Li et al. 2021), *Papiliotrema* associating with temperature (although inconsistencies suggest interacting effects with other variables) (Yanagida et al. 1992; Li et al. 2022), *Udeniomyces* being more abundant in a region with higher precipitation (Steenwerth et al. 2021), and *Cystobasidium* and *Sporobolomyces* correlating with higher temperatures and precipitation (Milanović et al. 2022). Furthermore, Gao et al. (2019) linked basidiomycete yeast groups at higher taxonomic assignments with geographic areas that differed in number of frost-free days, dryness, altitude, and average temperature.

Multiple studies have correlated basidiomycete yeast species abundances, including of the genera *Cryptococcus*, *Cystobasidium*, *Piskurozyma*, *Rhodotorula*, *Papiliotrema*,

Sporobolomyces, *Udeniomyces*, and *Vishniacozyma* with location at the scale of region (Longo et al. 1991; Taylor et al. 2014; Pinto et al. 2015; Tronchoni et al. 2022), sub-region or individual vineyard (Raspor et al. 2006; Gayevskiy and Goddard 2012; Liu et al. 2021; Wang et al. 2021b; Milanović et al. 2022; Martiniuk et al. 2023), or even location within a single vineyard (Setati et al. 2012; Vaudano et al. 2019). For example, Liu et al. (2021) found differential abundances of *R. babjevae*, *S. roseus*, *Filobasidium oeirense*, *Filobasidium stepposum*, and *Cystofilobasidium macerans* in three nearby vineyards within a single region. Considered as a whole, research that has connected basidiomycete yeast abundances to geographic or climatic variables strongly suggests that basidiomycete yeasts in vineyards vary according to these variables, but that effects are highly moderated by other factors.

4.42 Cultivation practices

Yeast community composition, including that of basidiomycete yeasts, differs among vineyards employing different cultivation practices (Comitini and Ciani 2008; Setati et al. 2012; Milanović et al. 2013; Martins et al. 2014; Setati et al. 2015; Castañeda et al. 2018; Castrillo et al. 2019), although there are few consistencies in species level effects, likely due in part to the variation in fungicide regimes within management practices. For example, while organic and biodynamic vineyards may use only copper- or sulfur-based fungicides, conventional vineyards may also use these products, in addition to synthetic fungicides, and may choose to apply them more often and in higher concentrations than some organic vineyards. Therefore, it is unlikely that fungal species susceptible to copper or sulfur fungicides will consistently be associated with any single cultivation practice.

Indeed, depending on species, higher proportions of *Rhodotorula* have been linked to each of biodynamic (Setati et al. 2012), organic (Xu et al. 2020; Castrillo and Blanco 2022),

integrated (Setati et al. 2012; Bagheri et al. 2015), and conventional cultivation practices (Milanović et al. 2013; Martins et al. 2014; Xu et al. 2020; Castrillo and Blanco 2022). Associations with cultivation practices are similarly mixed for *Filobasidium*, *Naganishia*, *Papiliotrema* and *Vishniacozyma* (Comitini and Ciani 2008; Setati et al. 2012; Milanović et al. 2013; Rantsiou et al. 2020; Xu et al. 2020; Castrillo and Blanco 2022; Perpetuini et al. 2022). However, research to date most often demonstrates that *Sporobolomyces* is positively associated with conventionally managed vineyards (Martins et al. 2014; Kecskeméti et al. 2016; Rantsiou et al. 2020; Section 2.3), although the opposite result was found by Xu et al. (2020), perhaps reflecting differential concentrations of copper in the vineyards (Martins et al. 2014; Martins et al. 2022).

4.43 Grape cultivar

Given the large number of wine grape cultivars, many comparisons of grape yeast communities among cultivars exist as sole examples of studies that included any particular cultivar. Furthermore, common basidiomycete yeasts, such as *Rhodotorula* and *Sporobolomyces*, are often present in similar abundances among cultivars, (Li et al. 2010; Brysch-Herzberg and Seidel 2015), while cultivars that host differing abundances of rarer yeasts cannot confidently be assigned as causal. Although some authors suggest preferences for red vs. white cultivars (Raspor et al. 2006; Li et al. 2010), the larger body of evidence is inconsistent and insufficient to support claims either way.

However, there is consensus that *Filobasidium* and the closely related *Naganisha* are more abundant in Cabernet Sauvignon musts than those of other cultivars (Cureau et al. 2021b; Li et al. 2021; Tronchoni et al. 2022). The Tremellomycetes, including *Dioszegia*, *C. cygneicollum* and *Papiliotrema* (Cureau et al. 2021b; Tronchoni et al. 2022) and the

Microbotryomycetes, including *Rhodotorula* (Tronchoni et al. 2022) may also be more abundant in Cabernet Sauvignon (Bokulich et al. 2014). However, associations with cultivar likely interact with geographical factors, demonstrated by higher abundances of *P. flavescens* on Cabernet Sauvignon in only two of four regions sampled, although neither temperature nor rainfall clearly explained the differences (Li et al. 2010). *Cystobasidium* may also have a greater association with Cabernet Sauvignon compared to other cultivars, although the authors note that all species that differed between cultivars were found in low abundances (Tronchoni et al. 2022).

Filobasidium elegans, *F. magnum*. *C. nothofagi*, and *Dioszegia hungarica* were also associated with Green Veltliner (Nemcová et al. 2015), indicating possible physiological similarities with Cabernet Sauvignon. The genus *Curvibasidium* has likewise been associated with Zinfandel (Cureau et al. 2021b) and Pinot noir (Liu et al. 2021). The genera *Cryptococcus*, *Cystofilobasidium*, *Entyloma*, *Papiliotrema*, and species *Cystobasidium minutum*, *Microstroma bacarum*, *N. albida*, *R. glutinis*, *R. mucilaginosa*, *Sporobolomyces phaffii*, and *S. roseus* may also form associations with various cultivars including Chardonnay, Leon Millot, Pinor noir, Sauvignon blanc, Syrah, and Zinfandel (Yanagida et al. 1992; Raspor et al. 2006; Lederer et al. 2013; Zhang et al. 2019; Cureau et al. 2021b; Liu et al. 2021). However, the strengths of these associations were dependent on the year of sampling (Nemcová et al. 2015) and region (Yanagida et al. 1992), highlighting the fact that cultivar effects interact with several other variables including climate.

Cultivar preferences may be due to variation in skin thickness, bunch tightness, pH, sugar level, and nutrient availability, which may be influenced in turn by yearly variation in climate (Cioch-Skoneczny et al. 2018; Lederer et al. 2013). For example, higher relative abundances of *Sporobolomyces* and *Cystobasidium* were negatively correlated with glucose concentration in

Maraština musts (Milanović et al. 2022), indicating that these genera may generally be more abundant in grape cultivars that are less sweet at the time of harvest.

4.44 Ripeness and damage to grapes

Regardless of cultivar, grape ripeness and degree of damage affects the yeast community composition, although taxa-specific effects vary from study to study. Differences are likely due in part to biochemical and physiological differences in grapes that are intrinsic to ripening, and differences in visitation by insects as the plants transition from flowering to fruiting and then as the grapes ripen (Zhu et al. 2021). Basidiomycete yeasts are generally thought to be more abundant on sound berries that offer fewer available nutrients, while ripe berries release juices, even if they remain visually intact, that better support ascomycete yeasts (Barata et al. 2012). Indeed, in many cases, basidiomycete yeasts, including species of *Cystobasidium*. *Udeniomyces*, *Papiliotrema*, *Vishniacozyma*, *Naganishia*, *Rhodotorula*, *Microbotryum*, and *Sporobolomyces*, are more abundant at earlier stages of ripening (Renouf et al. 2007b; Mateo et al. 2020; Liu and Howell 2021; Wang et al. 2021b; Costantini et al. 2022), or reach peak abundance at an intermediate stage prior to harvest, as has been observed for *Sporobolomyces*, *Cryptococcus*, *Filobasidium*, *Tilletiopsis*, and *Golubevia* (Zhu et al. 2019), and *Rhodotorula* spp. (Renouf et al. 2005). Some basidiomycete yeast species have been directly linked to berry damage, such as the greater abundance of *S. shibatanus* found on intact rather than damaged grapes (Nemcová et al. 2015).

Conversely, other studies have found the relative abundance of basidiomycete yeasts to increase from earlier stages of ripening to harvest, generally led by increased populations of *Vishniacozyma* (Zhu et al. 2021; Liu and Howell 2021; Wang et al. 2021b), *Papiliotrema* (Renouf et al. 2005), *Filobasidium* (Ding et al. 2021; Liu and Howell 2021; Wang et al. 2021b)

and/or *Naganishia* (Renouf et al. 2005; Ding et al. 2021), although results may be vineyard dependent.

4.45 Stage of fermentation

The nature of wine fermentation is such that sugars, nutrients, and oxygen are abundant at the start, supporting a high diversity of microorganisms, only to be depleted and replaced by secondary metabolites, creating an environment that is less hospitable to most species (Fleet 2003). Indeed, while basidiomycete yeasts range widely in their proportion of the fungal or yeast community present on grapes and in wine musts, most species usually do not persist to the end of fermentation. In many instances, for a wide variety of genera including *Rhodotorula*, *Cryptococcus*, *Filobasidium*, *Vishniacozyma*, *Curvibasidium*, *Papiliotrema*, *Naganishia*, *Sporobolomyces*, *Dioszegia*, *Cystofilobasidium* and *Udeniomyces*, they cease to be detected by early to mid stages when populations are monitored throughout fermentation (Fleet et al. 1984; Combina et al. 2005b; Renouf et al. 2007a; Milanović et al. 2013; Ultee et al. 2013; Bagheri et al. 2015; Nemcová et al. 2015; Pinto et al. 2015; Sternes et al. 2017; Li et al. 2018; Bougreau et al. 2019; Chen et al. 2020; Liu et al. 2020; Lu et al. 2020; Xu et al. 2020; Kamilari et al. 2021; Li et al. 2021; Englezos et al. 2022; Martiniuk et al. 2023).

However, basidiomycete yeasts may persist longer or even increase during middle stages of fermentation. This has most often been reported for *Filobasidium*, including species *F. magnum* and *Filobasidium chernovii* (David et al. 2014; Chen et al. 2020; Xu et al. 2020; Kamilari et al. 2021) but it may also be the case for others, including *S. roseus* and *Bullera alba* (David et al. 2014), *Cryptococcus* (De Filippis et al. 2017), *Symmetrospora*, *Naganishia*. and *Piskurozyma* (Chen et al. 2020), and *Udeniomyces puniceus*, *C. macerans*, and *C. cygneicollum* (Kamilari et al. 2021). This effect may occur when a species has some tolerance to fermentation

conditions, such as reduced oxygen and increased ethanol, and can utilize compounds produced earlier in the fermentation, but cannot survive in late-fermentation conditions (Fleet 2003). Indeed, Martins et al. (2022) demonstrated that *R. glutinis*, *B. alba*, *S. roseus* and *V. heimaeyensis* may exhibit tolerance to osmotic stress and maintain active growth in concentrations of up to 10% ethanol.

Considering these traits, basidiomycete yeasts may also maintain a relatively high, or at least a consistent, abundance until the end of fermentation, as has been demonstrated by *Cystofilobasidium* (Bokulich et al. 2016; Chen et al. 2020), *Curvibasidium pallidicorallinum* (Li et al. 2018), *P. flavescens* (Li et al. 2018; Li et al. 2021), *Rhodotorula* spp. (Díaz et al. 2013; Li et al. 2018; Li et al. 2021; Liu et al. 2021), *Vishniacozyma* spp. (Chen et al. 2020; Liu et al. 2020), and *Filobasidium* and *Sporobolomyces* (Wang et al. 2021a). An unclassified Sporidiobolaceae, most likely *Rhodotorula* or *Sporobolomyces*, also persisted to the completion of fermentation and even increased in abundance in some treatments (Cureau et al. 2021a). However, tolerance to pH, temperature, ethanol concentration, SO_2 concentration, and osmotic pressure during fermentation is strain dependent (Ge et al. 2023).

4.46 Variation in community characterization methods

Many studies use identification methods based on the isolation of pure cultures and employ various culturing and colony selection methods, which impact the assemblage of yeasts isolated (Beuchat 1992). The observed diversity of basidiomycete yeasts on grapes and in grape musts may be reduced by limited types of isolation media, a limited number or variety of cultures selected for identification, or authors choosing to identify or report only isolates adhering to certain properties, such as species of "oenological relevance" (Granchi et al. 1999) or those with fermentative enzymatic activities (Fernández et al. 2000). Regarding media types,

Wallerstein laboratory (WL), yeast extract peptone dextrose (YEPD or YPD), and lysine nutrient media (LM) are commonly used in grape community research, and although they may support the growth of basidiomycete yeasts, they were developed for fermentative ascomycete yeasts (Morris and Eddy 1957; Hall 1971). Once cultures have been isolated, further variation occurs due to the identification methods used, which may be based on physiology, morphology, PCR-RFLP, Sanger sequencing, or other techniques (Barata et al. 2012). Although now seldom used independently from other methods, preliminary screening of basidiomycete yeast cultures by physical traits may be problematic as different species display different ranges of phenotypic plasticity, phenotypic variation overlaps among closely related species, and yeasts generally have fewer taxonomically distinguishing traits than filamentous fungi (Cao et al. 2021). Other physiological tests may pose similar challenges; for example, mating tests are unsuitable to delineate species of basidiomycete yeasts that may hybridize, such as *Cryptococcus* and *Malassezia* (Wu et al. 2015; Boekhout et al. 2021).

Culture independent methods such as PCR-DGGE have also be used, generally together with culture-based methods, although detection of basidiomycete yeasts was often poor (see section 4.22), and their use is declining with the increasing availability of high throughput sequencing. Nevertheless, these methods may produce yet another assortment of identified yeast species. High throughput sequencing provides the most accurate representation of microbial communities, including of wine grape yeasts (Belda et al. 2017). However, differences in sequencing platform (Kumar et al. 2019) and data analysis pipeline (rarefying, OTU picking, taxonomic assignment) still impact the reported community composition (McKnight et al. 2019; Halwachs et al. 2017; Lücking et al. 2020). Variation in DNA extraction methods, choice of barcoding gene region(s) and of primer pair, even for the same gene, may also have substantial

impacts on the detection different taxa, regardless of the sequencing method (Op De Beeck et al. 2014; Stielow et al. 2015). For example, some common ITS region primers preferentially amplify ascomycete fungi while others are biased toward basidiomycetes (Bellemain et al. 2010), although newer primers designed for high throughput sequencing, if available, may mitigate these biases (Toju et al. 2012; Bokulich and Mills 2013) and improve identification of yeasts (Usyk et al. 2017)

Studies that use more than one method to detect and identify yeasts demonstrate variation in basidiomycete yeast community composition results. For example, Rantsiou et al. (2020) isolated *P. flavescens*, *Papiliotrema terrestris* and *R. graminis* from musts by culturing, but none of these species were detected at an incidence >0.2% by high throughput sequencing from the same samples, suggesting that their abundance in culture may be due to successful competition. Rather, *F. magnum*, *F. stepposum*, *S. roseus*, *V. carnescens*, and *V. victoriae* were detected in higher abundances by high throughput sequencing (Rantsiou et al. 2020). Li et al. (2019), using a culture-based approach, detected *Filobasidium floriforme* during early fermentation only, while high throughput sequencing of the same samples detected *F. floriforme* throughout the fermentation.

It is difficult to determine if this type of effect may be due to the detection of non-living "relic" DNA by high throughput sequencing (Carini et al. 2016), or if the culture-based methods were simply not sensitive enough to detect *F. floriforme* at low abundances, given that Stefanini et al. (2016) demonstrated that the relic yeast DNA does not accumulate during wine fermentations. In practice, many factors surely interact in complex relationships to influence the detected yeast community. This is well demonstrated by the different communities on wine grapes that Costantini et al. (2022) described using culture dependent and high throughput

techniques, which featured different proportions of basidiomycete yeasts. Although the differences are broadly attributed to the different methods, there were undoubtedly additional effects resulting from different DNA extraction protocols, targeting different gene regions depending on method, and using different databases for sequence identification (Costantini et al. 2022).

4.5 Conclusion

There is an extensive diversity of basidiomycete yeasts reported from wine grapes and musts. According to current classifications, *Rhodotorula* is likely the most common basidiomycete yeast resident of these environments, followed by *Filobasidium*, *Papiliotrema*, *Naganishia*, *Sporobolomyces*, and *Vishniacozyma*. Given that most wine grape and wine must associated species of *Cryptococcus* have been reclassified to other genera, *Cryptococcus* should no longer be considered one of the most common constituents of these environments. Overall, there is much variation in the assemblage of basidiomycete yeasts on wine grapes and in wine musts due to the many interacting factors that influence their presence, proportions, detection, and identification.

The research to date demonstrates several mechanisms by which basidiomycete yeasts may impact wine fermentations. Like other non-*Saccharomyces* yeasts, basidiomycete yeasts often produce high levels of enzymes during wine fermentations that can provide reagents for the metabolism of fermentative yeasts, as well as having a more direct influence the formation of varietal aroma (Belda et al. 2016). Basidiomycete yeasts may also produce, or moderate the production of, glycerol, ethanol, acids, higher alcohols, acetaldehydes, ketones, and esters, as well as a variety of undesirable aromatic compounds like sulfur compounds. However, the final

concentrations of these compounds depend on the total microbial community and the complex interactions that occur throughout fermentation. Basidiomycete yeasts undoubtedly influence this community by depleting nutrients, producing precursory compounds that facilitate fermentation, producing toxins or otherwise competing with other yeasts, and yet their roles remain understudied.

High throughput sequencing community analyses should be used to call attention to basidiomycete yeasts that may be targeted for isolation in culture. For example, the effects of *Udeniomyces* during wine fermentations are only known from correlations of species abundances, as determined by high throughput sequencing, with aromatic compound concentrations (Chen et al. 2020; Ma et al. 2021b; Englezos et al. 2022). While it is promising that *Udeniomyces* may persist at least until middle stages of fermentation (Kamilari et al. 2021) and shows potential to increase production of terpenes and moderate concentrations of aldehydes, acids, higher alcohols, and esters (Chen et al. 2020; Ma et al. 2021b; Englezos et al. 2022), more targeted studies are needed to better understand its role during fermentation. Many other basidiomycete yeasts known from wine grapes and musts are similarly lacking in characterization.

More research is warranted to explore methods of isolation, culture, and preservation of grape associated basidiomycete yeast strains. Promising strains should be characterized for production of enzymes and aromatic compounds relevant to winemaking and tested in mixed inoculum fermentations with a fermentative ascomycete yeast such as *S. cerevisiae*. Care should be taken to vary parameters such as grape cultivar, concentration of inoculants and timing of inoculation, and use of additives like SO_2. A better understanding of basidiomycete yeast activity

during wine fermentation will not only inform the direct application of basidiomycete yeasts for winemaking but will also improve our knowledge of wine as a product of microbial interactions.

4.6 Supplementary materials

Table S4.1. NCBI GenBank accession numbers of species in Figure 4.1.

Species	GenBank Accession
Sporobolomyces roseus	KC433882
Rhodotorula mucilaginosa	FR853164
Rhodotorula glutinis	AM748550
Curvibasidium cygneicollum	AF189931
Symmetrospora symmetrica	AB279627
Cystobasidium minutum	FJ527203
Cryptococcus amylolentus	KC006847
Papiliotrema laurentii	AJ876597
Vishniacozyma carnescens	FR853151
Naganishia albida	FR853169
Filobasidium magnum	FJ527156
Filobasidium globisporum	DQ377680
Tilletiopsis washingtonensis	AJ749823
Quambalaria cyanescens	AM262976
Aureobasidium pullulans	JQ678685
Hanseniaspora uvarum	KF263960
Saccharomyces cerevisiae	KJ660850
Saccharomyces uvarum	MH595098

Chapter 5: Conclusion and knowledge transfer

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Chapter 5 preface

Components

The “Knowledge Transfer and Application of Results” component of this thesis fulfils a requirement of the PhD in Applied Science program at Saint Mary’s University to describe the next steps that can be taken towards knowledge transfer. Although variation is expected depending on the nature of research, an outline of specific steps is required. These steps may include “articulation of next steps toward application of the research results” and “consultation with recipients of knowledge transfer”.

The figures included in the reports provided to the vineyards (section 5.21) have not been assigned numbers as thesis figures because they are provided within the context of the reports rather than as thesis data. A report was not prepared for the Agriculture and Agri-Food Canada (AAFC) Kentville Research Centre vineyard (V5).

5.1 Yeast communities on Nova Scotia wine grapes

5.11 Community composition

The yeasts of freshly pressed grapes were analysed by high throughput sequencing to provide the first comprehensive characterization of this community in Nova Scotia vineyards of the Annapolis Valley region. L'Acadie blanc grapes were chosen for this investigation due to their importance in the Nova Scotian appellation wine "Tidal Bay" and their widespread growth and use throughout Nova Scotia, and to contribute to the as-yet small body of work that considers the microbiome of cold and disease tolerant hybrid *Vitis* cultivars. The yeast community composition associated with wine grapes from the Annapolis Valley, although unique in its details, was broadly unsurprising: the most abundant groups were (1) the black yeast *Aureobasidium pullulans*, which is well known from vineyard (and many other) environments (Bozoudi and Tsaltas 2018), (2) the Sporidiobolales, which collectively encompasses the basidiomycete yeasts that are most commonly isolated from wine grapes and musts, such as *Rhodotorula glutinis* and *Sporobolomyces shibatanus*, and (3) the genus *Filobasidium*, another highly cited basidiomycete yeast in vineyard and wine research.

The Annapolis Valley wine grape ecosystem was, however, may be distinctive in its high proportion of basidiomycete yeasts compared to fermentative ascomycete yeasts. Although generally, basidiomycetes are more strongly associated with grapes, especially at earlier stages of ripening, than musts (Fleet 2003; Barata et al. 2012), ascomycete yeasts such as species of *Hanseniaspora* and *Metschniakozyma* are often still the dominant group in crushed grape samples (Jolly et al. 2014; Capozzi et al. 2015; Borren and Tian 2021). Thus, while the sample substrate likely played a role in the high proportion of basidiomycete yeasts on Nova Scotian grapes, the geography and climate of the region are also probable contributing factors. Further

research that considers differences in climatic variables between the Nova Scotia region and other wine regions for correlation with yeast community composition is warranted. Additional basidiomycete yeasts that were abundant in my samples were *Vishniacozyma carnescens*, *Symmetrospora* spp., and *Tilletiopsis washingtonensis*; all are known from wine grapes and musts.

At a finer scale, the eight vineyards sampled differed significantly in yeast community composition, showing a stronger effect of vineyard compared to year of sampling. This result is relevant to the concept of microbial terroir, and demonstrates that even relatively small differences in geography, climate, and vineyard management methods may impact the yeast community present on wine grapes. Comparable differences in yeast communities have been found among vineyards within the same region in other parts of the world, including North America (Cureau et al. 2021b; Wang et al. 2021b), South America (Miura et al. 2017), Europe (Stefanini et al. 2016; Milanović et al. 2022), and Australia (Liu et al. 2021). Vineyard managers and winemakers may exploit a unique yeast community composition in marketing their wines, and further research may enable the use of indigenous yeasts in more targeted winemaking applications.

5.12 Effects of organic vs. conventional cultivation practices

Given the public and consumer interest in methods of food production that are more environmentally friendly, as well as food products themselves that contain fewer additives (Cravero 2019; Tait et al. 2019; Fabbrizzi et al. 2021; Valenzuela et al. 2022), the yeast communities were also compared between three organically cultivated vineyards and five conventionally cultivated vineyards. Indeed, cultivation practice was found to be the strongest predictor of yeast community composition in freshly crushed grapes from the Annapolis Valley.

Organic vineyards were characterized by the genus *Symmetrospora* and by three *Filobasidium* species, excluding *Filobasidium magnum*, while conventional vineyards were distinguished by higher proportions of Sporidiobolales and *F. magnum*. The absence of Sporidiobolales in the organic vineyards also seemingly allowed for higher proportions of *V. carnescens* and *T. washingtonensis*, although these differences were not statistically significant. Based on previous research, it was expected that species diversity and species richness would be higher in organic vineyards (Tello et al. 2012; Setati et al. 2012; Martins et al. 2014; Bagheri et al. 2015; Setati et al. 2015), but this was not the case in the vineyards sampled, indicating that a comparable number of species may be supported in comparable evenness, regardless of cultivation practices, despite the species in question being different.

It remains difficult, however, to link the presence or abundance of any taxonomic group to cultivation practice more broadly, let alone to specific aspects of that practice such as which fungicides are used. While it is consistent that yeast community composition is affected by cultivation practice, the community will ultimately be a product of the interactions among cultivation practice, geography, climate, grape cultivar, and microbial interactions. However, the results presented in this thesis confirm that differences in yeast community composition occur in basidiomycete-dominated communities and provide a novel and relevant community composition information for the region and cultivar sampled.

5.13 Influence of basidiomycete yeasts

The high abundance of basidiomycete yeasts in freshly crushed grapes from Annapolis Valley vineyards prompted a review of the application of basidiomycete yeasts in winemaking. The body of work concerning this topic is much smaller than that of investigations considering fermentative ascomycete yeasts but demonstrates that a wide diversity of basidiomycete yeasts is

known from wine grapes and wine musts, and they have the potential to impact the aroma and body of wine. Furthermore, their presence and abundance in wine fermentations may be manipulated to improve outcomes or produce a wider range of products. Basidiomycete yeasts may be of particular interest for the secretion of enzymes that facilitate the release of varietal aromatic compounds such as terpenes, as well as the production of many compounds essential to the distinctive aroma of different wines. For example, *Rhodotorula mucilaginosa* can produce β-glucosidase, leading to increased levels of pleasant terpenoid compounds, and produce acetate esters associated with fruity, floral, and sweet aromas (Wang et al. 2017; Ma et al. 2021a; Wang et al. 2023a).

5.14 Changes in yeast communities after fermentation

Despite the exciting potential of basidiomycete yeasts, winemaking is dependent on fermentation completed by ascomycete yeasts, most often *Saccharomyces cerevisiae*, or occasionally, other species of Saccharomycetes. Researchers have established an unusual prevalence of *Saccharomyces uvarum*-dominated fermentations in British Columbia, and multiple strains have been favourably characterized for use in wine fermentation (Morgan et al. 2019; Morgan et al. 2020; McCarthy et al. 2021; Lyons et al. 2021). Following an analysis of yeast communities after spontaneous fermentation of organic grapes, the present thesis presents the first confirmation that *S. uvarum* is also present and active, along with *S. cerevisiae*, in fermentations of Nova Scotian grapes. *S. uvarum* represents new opportunities for manipulation of fermentations to produce wines with different aroma profiles than when using *S. cerevisiae* (Varela et al. 2016; Knight et al. 2018). The discovery *S. uvarum* in Nova Scotia also lends support to the concept of a broader Canadian terroir that is especially characteristic of spontaneous fermentations.

5.15 Commentary on the impact of sequencing platform

In the comparison of the organic vineyard samples pre- and post-fermentation there were two main consistent effects of sequencing platform on yeast community composition: differential detection of *Hanseniaspora uvarum* and *Saccharomyces* spp. Prior to fermentation, approximately 50% of the yeast community was comprised of *H. uvarum* according to the PacBio sequencing results, while this species was not present in any pre-fermentation samples at a proportion >1% of the community when analysed by Illumina. This effect was repeated post-fermentation, with *H. uvarum* persisting at approximately 15% in all replicates as determined by PacBio and absent according to Illumina. Furthermore, although both platforms detected both *S. cerevisiae* and *S. uvarum* after fermentation was complete, the proportions were not the same. Using Illumina, either one species or the other dominated each replicate, while in the PacBio data, *S. cerevisiae* made up approximately 50%, and *S. uvarum* approximately 10% of each replicate. This is the first study to compare the Illumina MiSeq and PacBio sequencing platforms for the analysis of vineyard yeast community data, and it raises concerns regarding the validity of high throughput sequencing data, or at least the data obtained using some high throughput sequencing primers, in this application.

Although not presented in this thesis, PacBio data were also obtained from other vineyards sampled in 2019 (V1A & V2-6) and the 2020 samples from V6. This dataset will be publicly released no later than May 1, 2025, in the NCBI SRA database with the BioProject accession number PRJNA860361. Preliminary analysis shows that although *H. uvarum* was not abundant in any pre-fermentation replicates of any other vineyards (discrepancy in pre-fermentation abundance was displayed only in the V7 data), the same discrepancy in the abundance of *H. uvarum* was present in post-fermentation replicates of most vineyards (*H.*

uvarum was not abundant according to either sequencing platform in the V2 data). Different proportions of *S. cerevisiae* and *S. uvarum* were also found in the post-fermentation replicates of most vineyards upon preliminary comparisons of the PacBio and Illumina data (*Saccharomyces* was not abundant in the V1A post-fermentation data of either platform). These results emphasize the need for more comprehensive comparisons of yeast species abundance data using different sequencing platform.

5.2 Knowledge transfer and application of results

5.21 Reports provided to vineyards

The text that follows immediately will be provided to each vineyard that participated in this research. It summarizes the research of this thesis in a way that aims to be brief, straightforward, and most relevant to vineyard managers and winemakers, and proposes how this knowledge might be practically used. Each vineyard was also provided with the appropriate figures, as follow the text, to show the fungal and yeast communities of their vineyard(s).

[Vineyard Name]

Final Report from the “Indigenous Yeasts” project (2018-2023): Fungal and Yeast Communities in the Vineyard

Provided by Adèle Bunbury-Blanchette (SMU), Lihua Fan (AAFC Kentville), and Gavin Kernaghan (MSVU).

Grapes were collected from the [name] vineyard at harvest in [year(s)] and were crushed at the AAFC Kentville facility. All fungi present in the samples were identified by DNA sequencing, and additional filtering took place to assess yeasts. Eight vineyards in the Annapolis Valley participated in this study from 2018-2021, with three years of sampling for most vineyards. Considering all vineyards, the regional fungal and yeast communities are described as follows:

Grape musts in 2018-2020 were dominated by the yeast-like fungus *Aureobasidium pullulans*, which is abundant in many natural and agricultural environments, including in vineyards and on wine grapes. This species was included as a yeast in the figures provided. Otherwise, most samples, from 2018-2020 as well as in 2021, were dominated by basidiomycete yeasts in the genera *Filobasidium*, *Rhodotorula*, *Sporobolomyces*, *Vishniacozyma*, *Symmetrospora*, and *Tilletiopsis*.

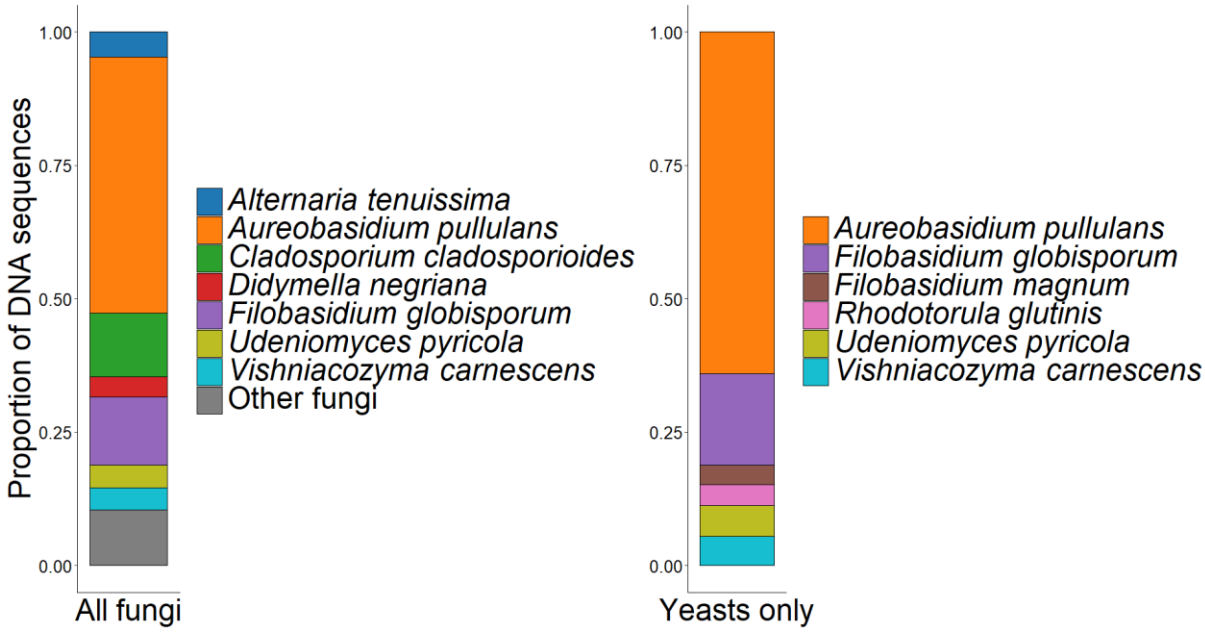
Basidiomycete yeasts are non-fermentative yeasts generally more strongly associated with the vineyard environment and the surfaces of grapes, rather than with fermentation. However, basidiomycete yeast species are of increasing interest in winemaking research, as they do contribute to aroma formation, and some may survive to later stages of fermentation. For example, *Rhodotorula* may produce high levels of β-glucosidase, enabling the production of

desirable aromatic terpenoid compounds. Like the more familiar ascomycete yeast *Saccharomyces*, basidiomycete yeasts may be isolated in culture and added to wine fermentations, although basidiomycete yeasts must be added as a co-inoculant, as they cannot complete fermentation on their own.

The following page contains figures specific to the fungal and yeast communities of [name] vineyard. Data from 2018-2020 originated from a single bulk sample of grapes per year, while data from 2021 are the result of three replicate samples from different points in the vineyard. Due to the different sampling regimes, we considered the 2018-2020 dataset and the 2021 dataset separately in the following figures. Vineyard site and cultivation practice (organic vs. conventional management) were both strong predictors of the yeast community composition. This means that [name] vineyard had a unique yeast "fingerprint" distinct from all other vineyards sampled for this project.

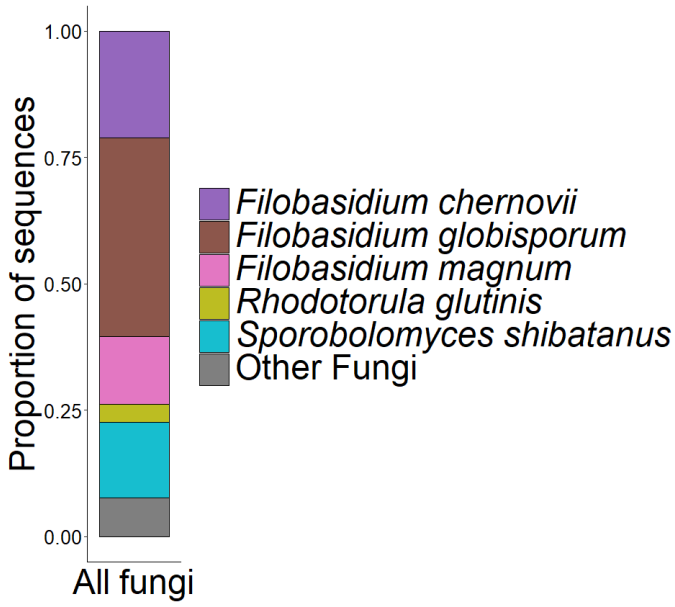
V1A

2019



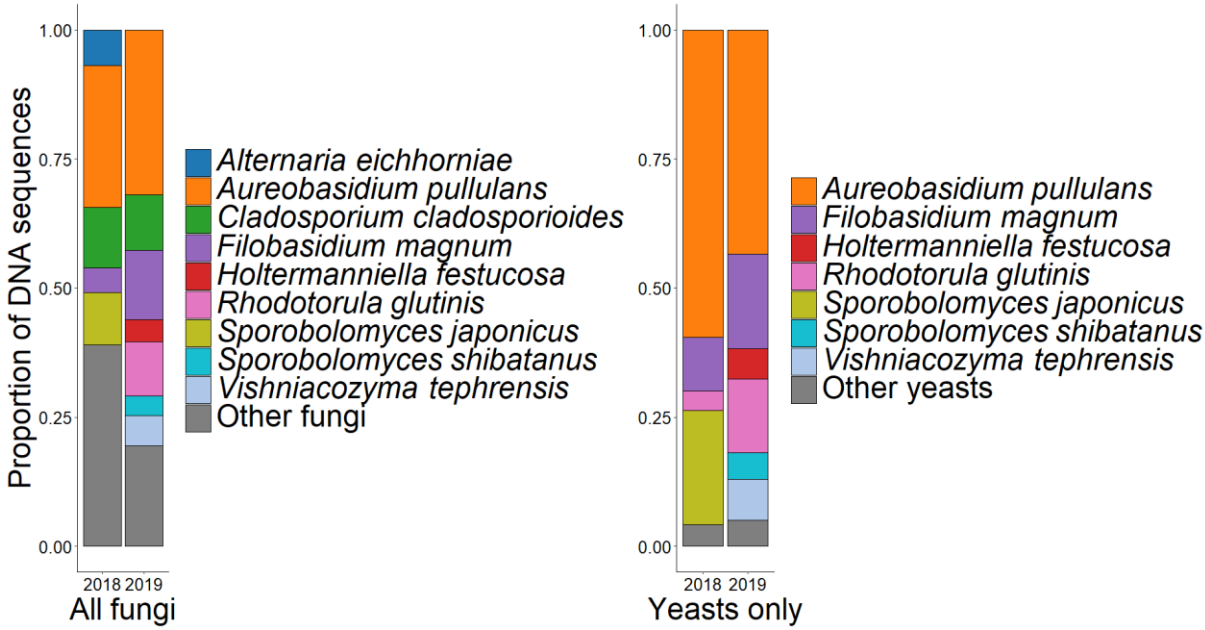
V1B

2021. The "Yeasts only" community was equivalent to the "All fungi" community.

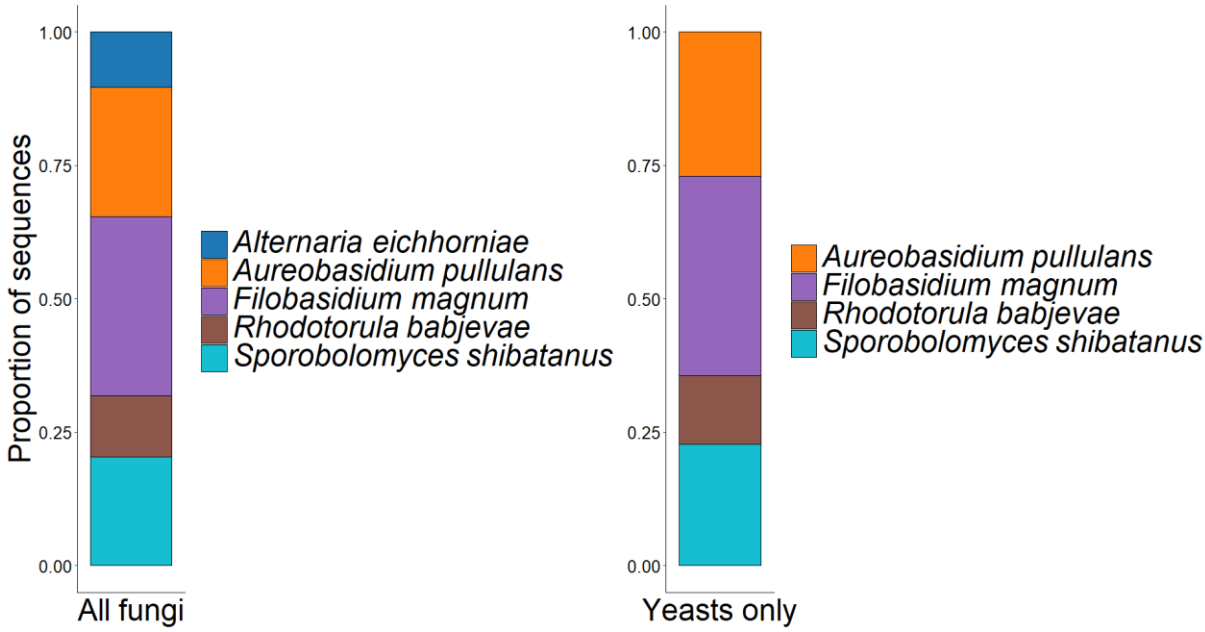


V2

2018 & 2019

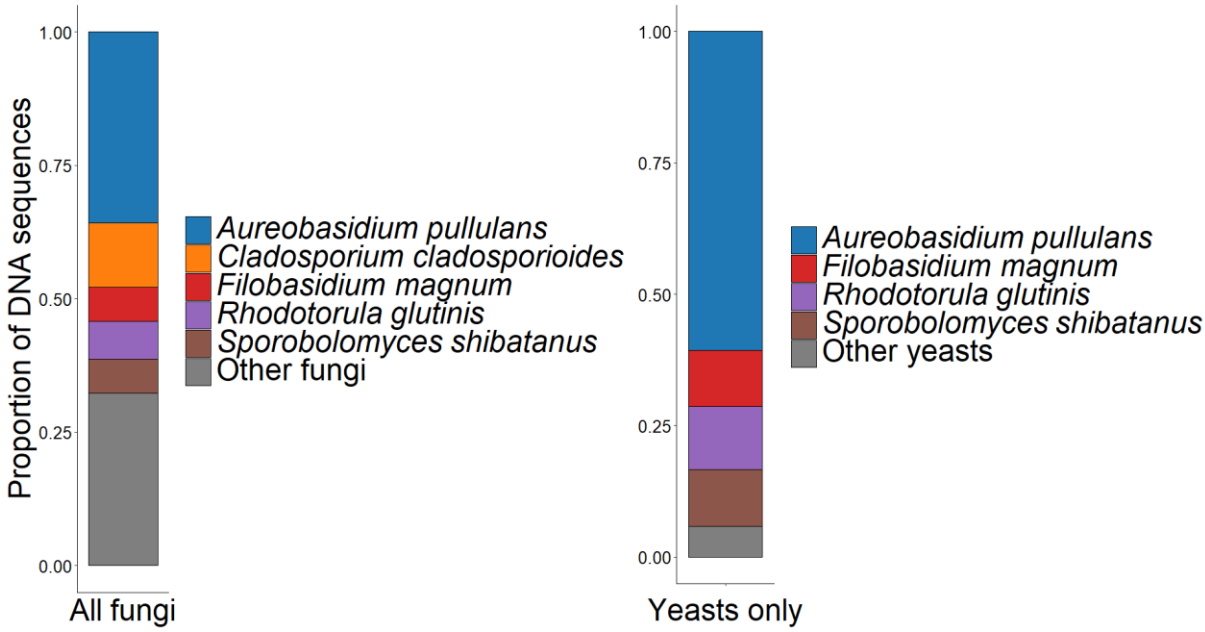


2021

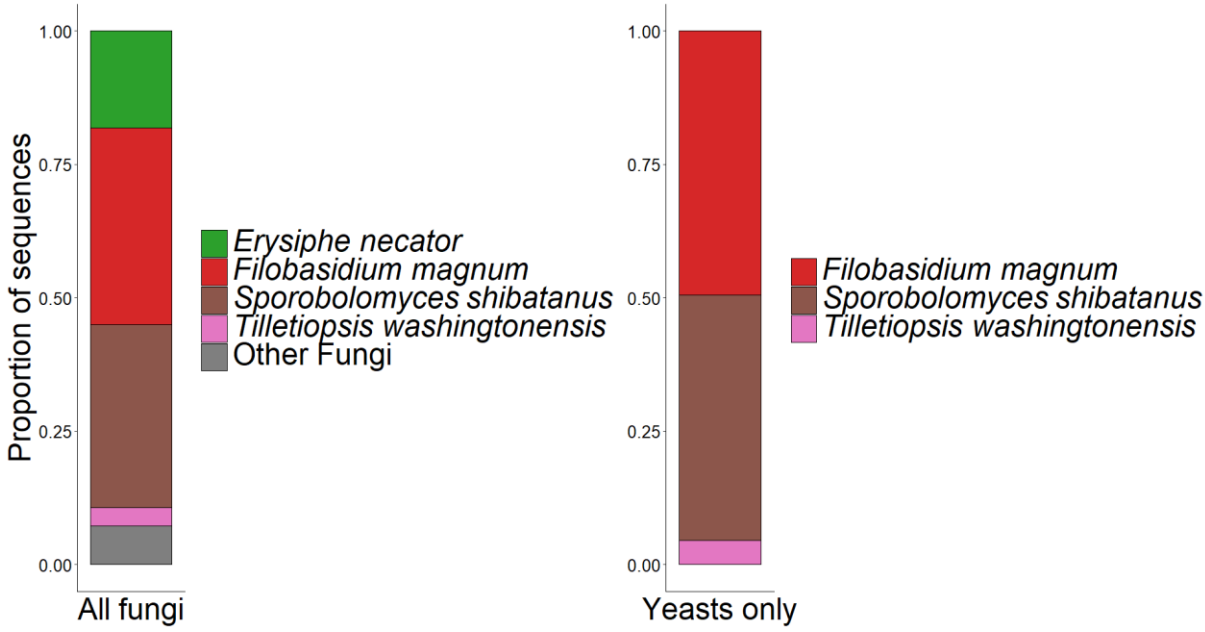


V3

2019

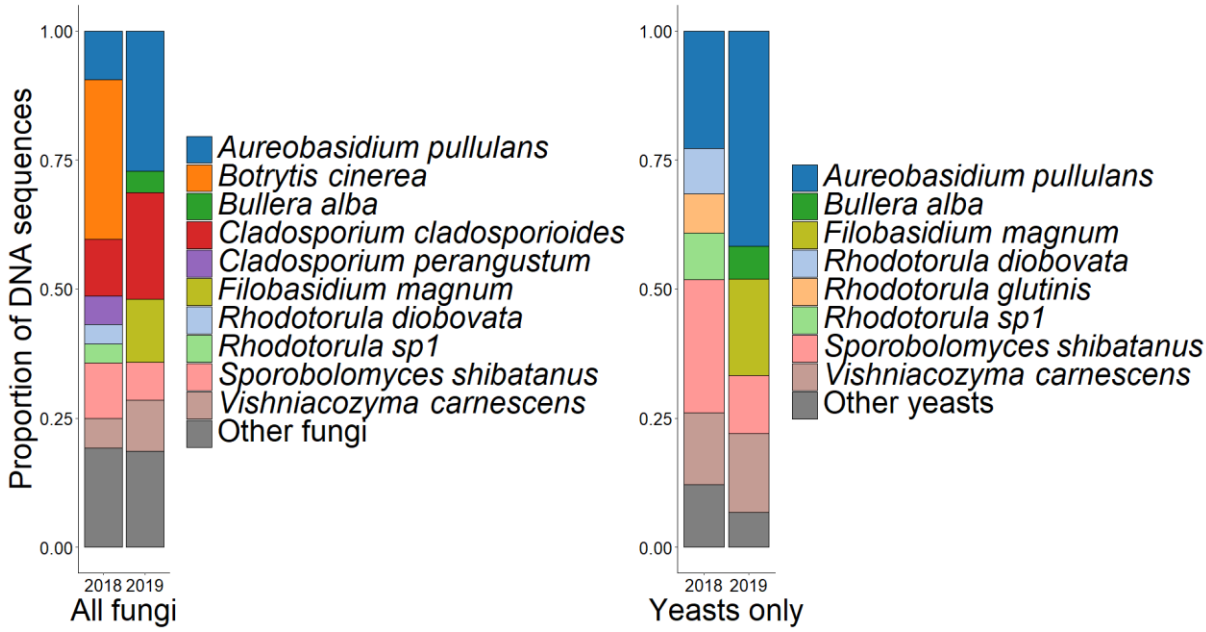

Proportion of DNA sequences
1.00
0.75
0.50
0.25
0.00
All fungi
Aureobasidium pullulans
Cladosporium cladosporioides
Filobasidium magnum
Rhodotorula glutinis
Sporobolomyces shibatanus
Other fungi
Yeasts only
Aureobasidium pullulans
Filobasidium magnum
Rhodotorula glutinis
Sporobolomyces shibatanus
Other yeasts

2021

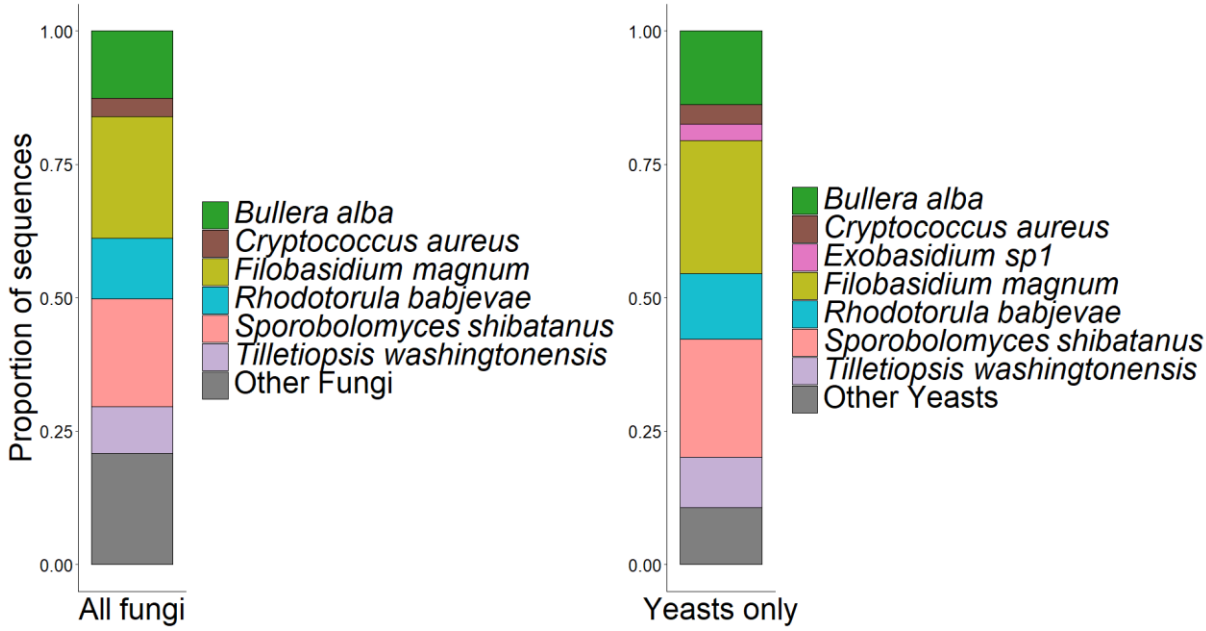

Proportion of sequences
1.00
0.75
0.50
0.25
0.00
All fungi
Erysiphe necator
Filobasidium magnum
Sporobolomyces shibatanus
Tilletiopsis washingtonensis
Other Fungi
Yeasts only
Filobasidium magnum
Sporobolomyces shibatanus
Tilletiopsis washingtonensis

V4

2018 & 2019

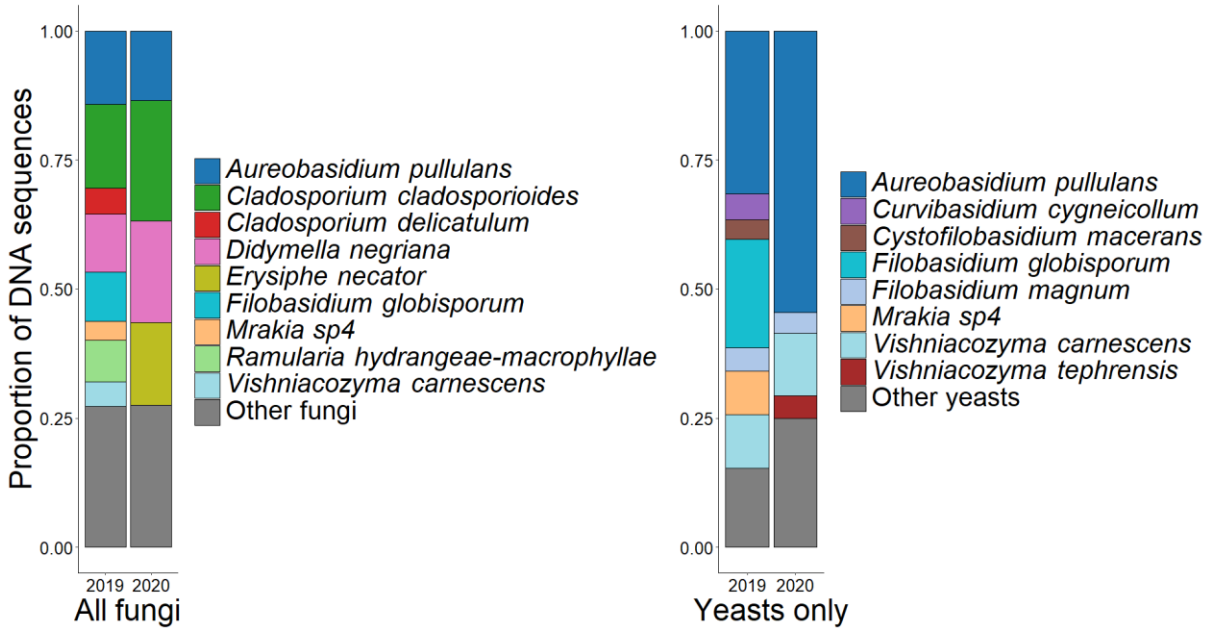


2021

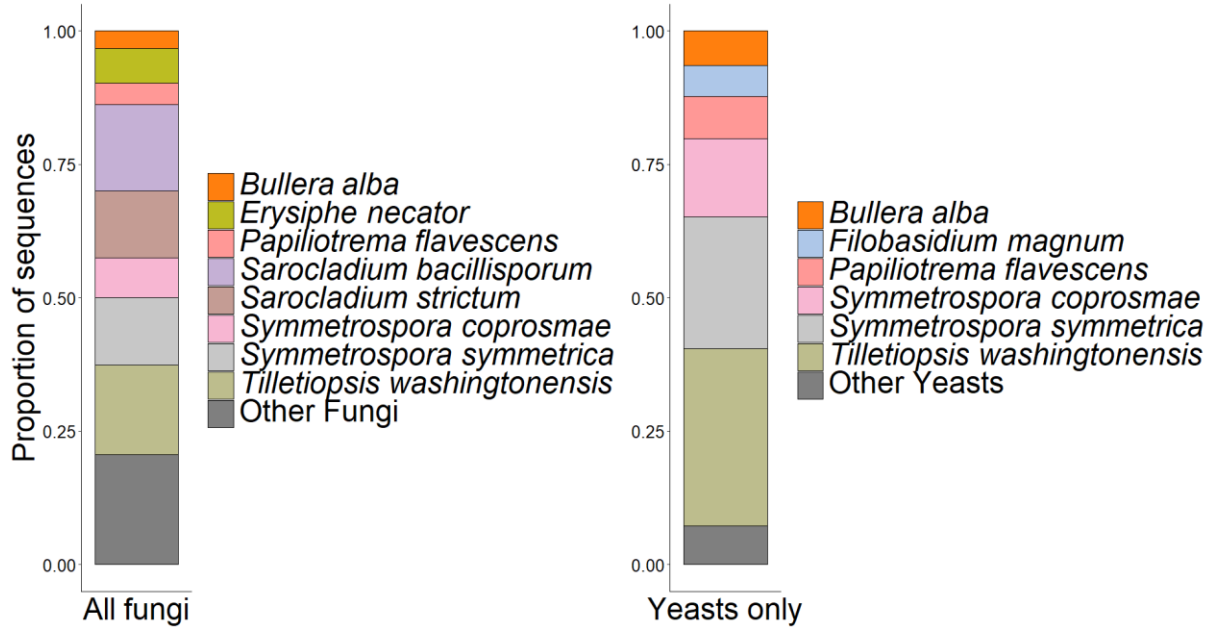


V6

2019 & 2020

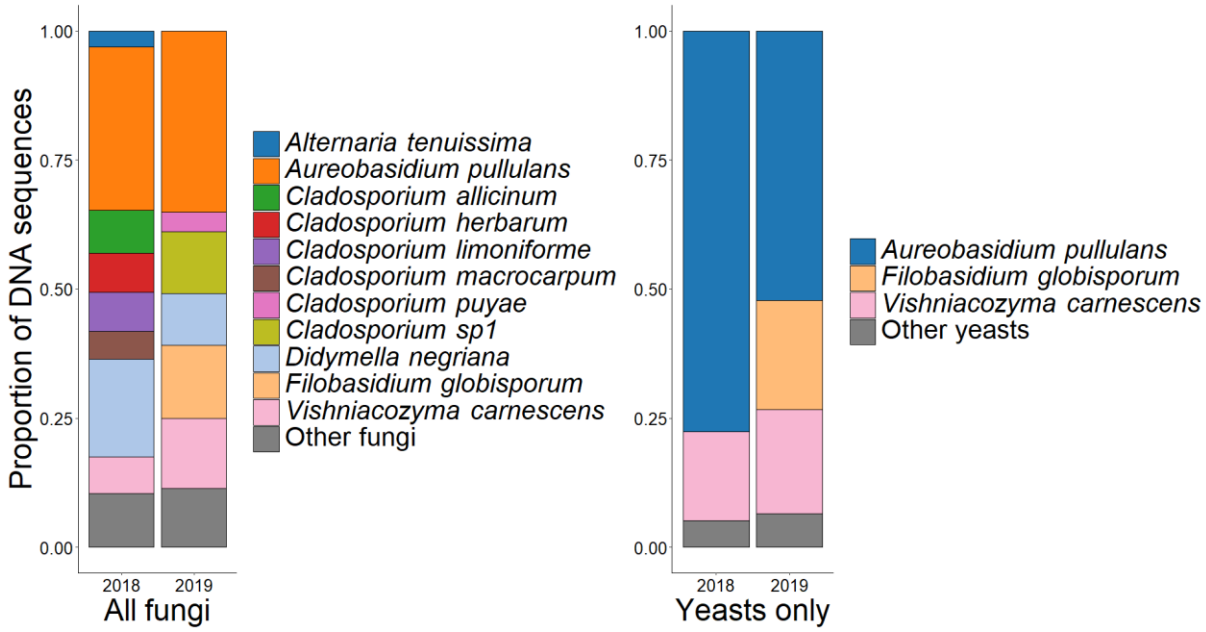


2021

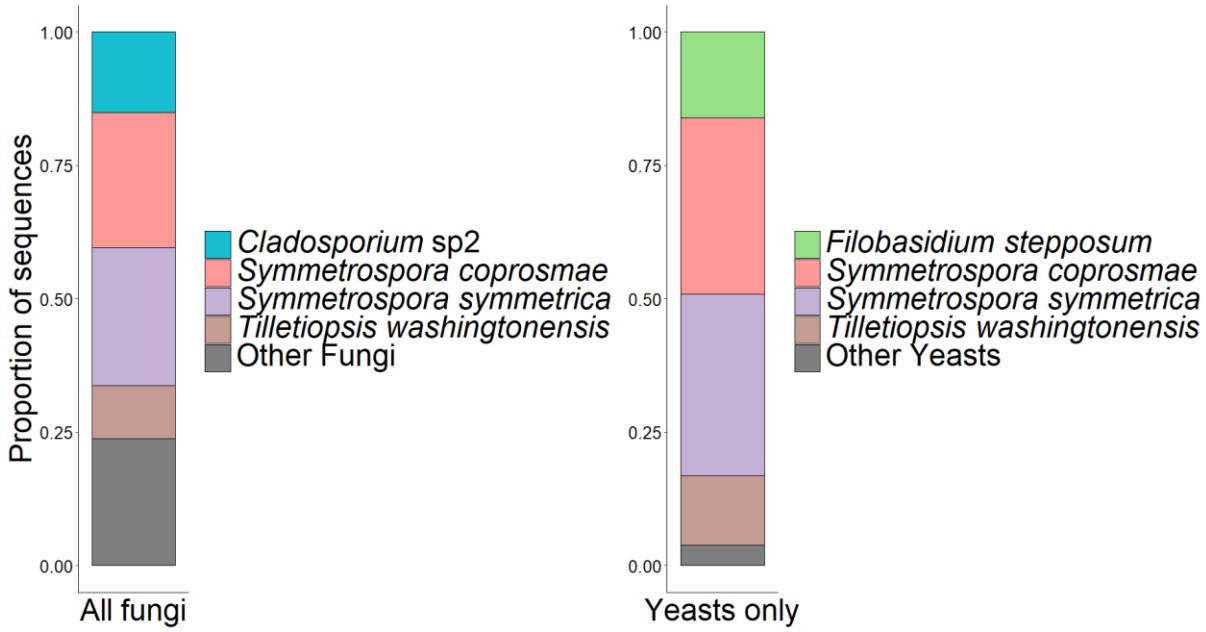


V7

2018 & 2019

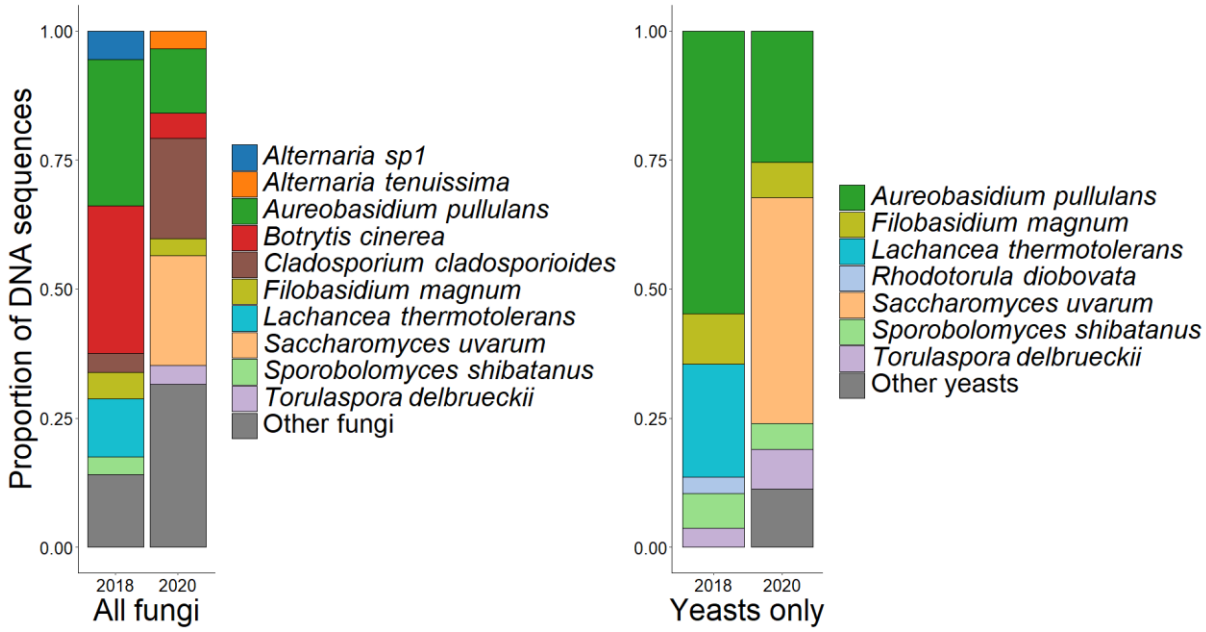


2021

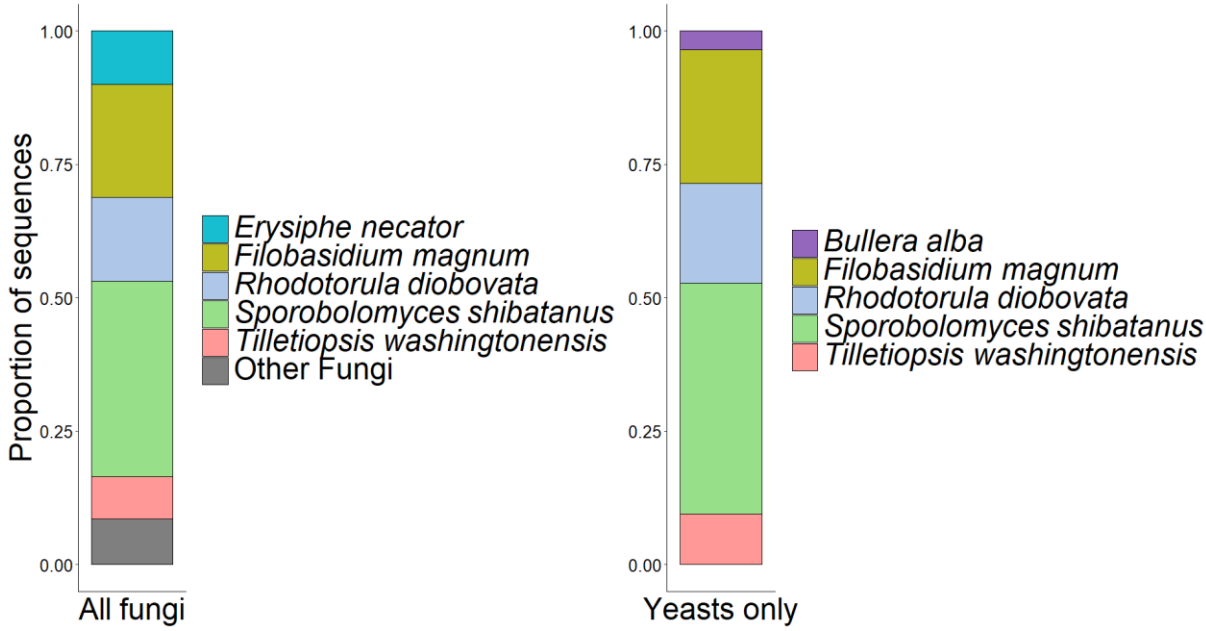


V8

2018 & 2020



2021



5.22 List of additional knowledge transfer events

The work of this thesis was also shared with each of the participating vineyards in the form of a 2-page mid-project update in April of 2021 which summarized the methods and results to date at that time. Publication of Chapter 2 and Chapter 3 have made relevant information available to industry professionals who did not participate in the research, as well as to fellow researchers who may work with industries in other parts of Canada, or worldwide. Chapter 4, although not yet submitted for publication, is intended to contribute a novel application of the results by providing the context and means by which basidiomycete yeasts known from wine grapes and wine musts may be used in winemaking. Finally, aspects of this thesis were disseminated at several academic conferences. References for the publications of Chapter 2 and Chapter 3, and all conference presentations are as follows, in descending chronological order:

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2024. Yeast communities of a North American hybrid wine grape differ between organic and conventional vineyards. J Appl Microbiol. lxae092. doi:10.1093/jambio/lxae092.

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2024. Nova Scotia wine grape yeast communities: composition and potential for winemaking. Presented at: Kentville Research and Development Centre 2024 Seminar Series; Mar 20; Agriculture and Agri-Food Canada, Kentville (NS).

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2023. Yeast community composition in Nova Scotia vineyards: from broad patterns to case study. Presented at: Joint Meeting of the Canadian Society for Ecology and Evolution and the Canadian Botanical Association; Jun 11-14; Winnipeg (MB).

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2023. Yeast communities on grapes differ according to organic vs. conventional vineyard management. Presented at: 4th Annual CanFunNet Fungal Biology Conference; May 31-Jun 2; virtual.

Bunbury-Blanchette AL, Fan L, English MM, Kernaghan G. 2023. Yeast communities before and after spontaneous fermentation of wine grapes: a case study from Nova Scotia. Can J Microbiol. 69(1):32-43. doi:10.1139/cjm-2022-0179.

Bunbury-Blanchette AL, Fan L, English MM, Kernaghan G. 2022. Yeast communities from a Nova Scotia vineyard before and after spontaneous fermentation: a case study. Presented at: 3rd Annual CanFunNet Fungal Biology Conference; Jun 1-3; virtual.

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2021. Yeast communities in Nova Scotia vineyards. Poster presented at: Botany 2021 Virtual! The Annual Conference of these Premier Scientific Societies: Botanical Society of America, American Society of Plant Taxonomists, Society of Herbarium Curators, Mycological Society of America, International Association for Plant Taxonomy, American Fern Society; Jul 19-23; virtual.

Bunbury-Blanchette AL, Fan L, Kernaghan G. 2021. Characterizing yeasts in Nova Scotia vineyards. Presented at: Joint CanFunNet and Great Lakes Mycology Conference; May 26-28; virtual.

5.3 Closing statement

This thesis characterizes the yeast communities present in fresh wine grape musts from vineyards located in the Annapolis Valley Region of Nova Scotia, Canada, and reviews the roles of basidiomycete yeasts in winemaking. It aims to be of use to vineyard managers and winemakers, and to serve as a basis for further research in these areas. Wines are the product of a microbial community succession in a limited substrate, and as such, the production of wine is the result of a complex network of interactions that begins in the vineyard and continues throughout fermentation. This thesis addresses several aspects of this dynamic system to contribute to a greater understanding of its entirety.

References

Abdullabekova DA, Magomedova ES, Aliverdieva DA, Kachalkin AV. 2020. Yeast communities of vineyards in Dagestan: ecological, taxonomic, and genetic characteristics. Biol Bull Russ Acad Sci. 47(4):344-351. doi:10.1134/S1062359020030024.

Agarbati A, Canonico L, Ciani M, Comitini F. 2019. The impact of fungicide treatments on yeast biota of Verdicchio and Montepulciano grape varieties. PLOS ONE. 14(6):e0217385. doi:10.1371/journal.pone.0217385.

Alcohol and Tobacco Tax and Trade Bureau. 2023. Established American Viticultural Areas. US Department of the Treasury; [accessed 2024 Feb 13]. https://www.ttb.gov/wine/established-avas.

Alexandre H. 2020. Wine yeast terroir: separating the wheat from the chaff – for an open debate. Microorganisms. 8(5):787. doi:10.3390/microorganisms8050787.

Ali S, Wright AH, Tanney JB, Renaud JB, Sumarah MW. Fungal endophytes: discovering what lies within some of Canada's oldest and most resilient grapevines. J Fungi. 10(2):105. doi:10.3390/jof10020105.

Alimardani-Theuil P, Gainvors-Claisse A, Duchiron F. 2011. Yeasts: an attractive source of pectinases – from gene expression to potential applications: a review. Process Biochem. 46(8):1525-1537. doi:10.1016/j.procbio.2011.05.010.

Allen MC, Nielsen AL, Peterson DL, Lockwood JL. 2021 Terrestrial eDNA survey outperforms conventional approach for detecting an invasive pest insect within an agricultural ecosystem. Environ DNA. 3(6):1102-1112. doi:10.1002/edn3.231.

Alonso González AP, Parga-Dans E, Fuentes Fernández R. 2022. Certification of natural wine: policy controversies and future prospects. Front Sustain Food Syst. 6:875427. doi:10.3389/fsufs.2022.875427.

Alsammar H, Delneri D. 2020. An update on the diversity, ecology and biogeography of the *Saccharomyces* genus. FEMS Yeast Res. 20(3):foaa013. doi:10.1093/femsyr/foaa013.

Amato M, Ballco P, López-Galán B, De Magistris T, Verneau F. 2017. Exploring consumers' perception and willingness to pay for "Non-Added Sulphite" wines through experimental auctions: a case study in Italy and Spain. Wine Econ Policy. 6(2):146-154. doi:10.1016/j.wep.2017.10.002.

Andrews S, Lindenbaum P, Howard B, Ewels P. 2017. FastQC [software]. www.bioinformatics.babraham.ac.uk/projects/.

Angelini F, Castellani M, Ventrucci M. 2023. Cumulative information on quality and willingness to pay: a study on wine evaluation. J Foodserv. doi:10.1080/15378020.2023.2259315.

Aplin JJ, Edwards CG. 2021. Impacts of non-*Saccharomyces* species and aeration on sequential inoculation with *Saccharomyces cerevisiae* to produce lower alcohol Merlot wines from Washington state. J Sci Food Agric. 101(4):1715-1719. doi:10.1002/jsfa.10769.

Aplin JJ, White KP, Edwards CG. 2019. Growth and metabolism of non-*Saccharomyces* yeasts isolated from Washington state vineyards in media and high sugar grape musts. Food Microbiol. 77:158-165. doi:10.1016/j.fm.2018.09.004.

Araujo MV, Costa da Silva MA, Callegaro de Menezes D, Bruch KL. 2017. The perspective of organic wine in Brazil – trends, demands and production. BIO Web Conf. 9:03011. doi:10.1051/bioconf/20170903011.

Ashton RH. 2014. “Nothing good ever came from New Jersey”: expectations and the sensory perception of wines. J Wine Econ. 9(3):304-319. doi:10.1017/jwe.2014.28.

Badotti F, de Oliveira FS, Garcia CF, Vaz ABM, Fonseca PLC, Nahum LA, Oliveira G, Góes-Neto A. 2017. Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). BMC Microbiol. 17(1):42. doi:10.1186/s12866-017-0958-x.

Baffi MA, Martin N, Tobal TM, Ferrarezi AL, Lago JHG, Boscolo M, Gomes E, Da-Silva R. 2013. Purification and characterization of an ethanol-tolerant β-glucosidase from *Sporidiobolus pararoseus* and its potential for hydrolysis of wine aroma precursors. Appl Biochem Biotechnol. 171(7):1681-1691. doi:10.1007/s12010-013-0471-0.

Baffi MA, Tobal T, Henrique J, Lago G, Leite RSR, Boscolo M, Gomes E, Da-Silva R. 2011. A novel β-glucosidase from *Sporidiobolus pararoseus*: characterization and application in winemaking. J Food Sci. 76(7):C997-C1002. doi:10.1111/j.1750-3841.2011.02293.x.

Bagheri B, Bauer FF, Setati ME. 2015. The diversity and dynamics of indigenous yeast communities in grape must from vineyards employing different agronomic practices and their influence on wine fermentation. S Afr J Enol Vitic. 36(2):243-251.

Baker TC, Myrick AJ, Wolfin MS, Wang Y. 2021. Visual responses of flight-dispersing spotted lanternflies, *Lycorma delicatula* toward a tall vertical silhouette in a vineyard. J Insect Behav. 34(1):49-60. doi:10.1007/s10905-021-09766-0.

Banerjee P, Haider M, Trehan V, Mishra B, Thakur A, Dogra V, Loomba P. 2013. *Cryptococcus laurentii Fungemia*. Indian J Med Microbiol. 31(1):75-77. doi:10.4103/0255-0857.108731.

Barata A, Malfeito-Ferreira M, Loureiro V. 2012. The microbial ecology of wine grape berries. Int J Food Microbiol. 153(3):243-259. doi:10.1016/j.ijfoodmicro.2011.11.025.

Baron EJ, Miller JM. 2008. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. Diagn. 60(3):241-246. doi:10.1016/j.diagmicrobio.2007.09.016.

Barrajón N, Arévalo-Villena M, Rodríguez-Aragón LJ, Briones A. 2009. Ecological study of wine yeast in inoculated vats from La Mancha region. Food Control. 20(9):778-783. doi:10.1016/j.foodcont.2008.10.002.

Bavaresco L, Squeri C. 2022. Outlook on disease resistant grapevine varieties. BIO Web Conf. 44:06001. doi:10.1051/bioconf/20224406001.

Bazzicalupo AL, Bálint M, Schmitt I. 2013. Comparison of ITS1 and ITS2 rDNA in 454 sequencing of hyperdiverse fungal communities. Fungal Ecol. 6(1):102-109. doi:10.1016/j.funeco.2012.09.003.

Beggs JD. 1978. Transformation of yeast by a replicating hybrid plasmid. Nature. 275:104-109. doi:10.1038/275104a0.

Belda I, Ruiz J, Alastruey-Izquire A, Navascués E, Marquina D, Santos A. 2016. Unraveling the enzymatic basis of wine “flavorome”: a phylo-functional study of wine related yeast species. Front Microbiol. 7:12. doi:10.3389/fmicb.2016.00012.

Belda I, Zarraonaindia I, Perisin M, Palacios A, Acedo A. 2017. From vineyard soil to wine fermentation: microbiome approximation to explain the “terroir” concept. Front Microbiol. 8:821. doi:10.3389/fmicb.2017.00821.

Belessi CE, Chalvantzi I, Marmaras I, Nisiotou A. 2022. The effect of vine variety and vintage on wine yeast community structure of grapes and ferments. J Appl Microbiol. 132(5):3672-3684. doi:10.1111/jam.15471.

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. 2010. ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. BMC Microbiol. 10(1):189. doi:10.1186/1471-2180-10-189.

Bellon JR, Yang F, Day MP, Inglis DL, Chambers PJ. 2015. Designing and creating *Saccharomyces* interspecific hybrids for improved, industry relevant, phenotypes. Appl Microbiol Biotechnol. 99(2):8597-8609. doi:10.1007/s00253-015-6737-4.

Bely M, Stoeckle P, Masneuf-Pomarede I, Dubourdieu D. 2008. Impact of mixed *Torulaspora delbrueckii–Saccharomyces cerevisiae* culture on high-sugar fermentation. Int J Food Microbiol. 122(3):312-320. doi:10.1016/j.ijfoodmicro.2007.12.023.

Benjamin Bridge. 2024. Gaspereau Valley (NS): Benjamin Bridge; [accessed 2024 Feb 16]. https://www.benjaminbridge.com/.

Beule L, Karlovsky P. 2020. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): application to microbial communities. PeerJ. 8:e9593. doi:10.7717/peerj.9593.

Bezerra-Bussoli C, Baffi MA, Gomes E, Da-Silva R. 2013. Yeast diversity isolated from grape musts during spontaneous fermentation from a Brazilian winery. Curr Microbiol. 67(3):356-361. doi:10.1007/s00284-013-0375-9.

Bian S, Zhang R, Liu Q, Guan Z, Jin Z, Zhu K, Jiao A. 2022. Effects of the addition of thermostable α-amylase on the physicochemical and antioxidant properties of extrusion-pretreated *Apios fortunei* used for yellow wine fermentation. LWT. 154:112845. doi:10.1016/j.lwt.2021.112845.

Bisson LF, Karpel JE. 2010. Genetics of yeast impacting wine quality. Annu Rev Food Sci Technol. 1(1):139-162. doi:10.1146/annurev.food.080708.100734.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H. 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Mol Ecol Resour. 13(2):218-224. doi:10.1111/1755-0998.12065.

Bleidorn C. 2017. Sequencing technologies. In: Bleidorn C, editor. Phylogenomics: An Introduction. Switzerland: Springer International Publishing; p. 43-60.

Boekhout T, Aime MC, Begerow D, Gabaldón T, Heitman J, Kemler M, Khayhan K, Lachance MA, Louis EJ, Sun S, et al. 2021. The evolving species concepts used for yeasts: from phenotypes and genomes to speciation networks. Fungal Divers. 109(1):27-55. doi:10.1007/s13225-021-00475-9.

Boekhout T, Fonseca Á, Sampaio JP, Bandoni RJ, Fell JW, Kwon-Chung KJ. 2011. Discussion of teleomorphic and anamorphic basidiomycetous yeasts. In: Kurtzman CP, Fell JW, Boekhout T, editors. Yeasts: A Taxonomic Study. London (UK): Elsevier; p. 1339-1374.

Bokulich NA, Collins TS, Masarweh C, Allen G, Heymann H, Ebeler SE, Mills DA. 2016. Associations among wine grape microbiome, metabolome, and fermentation behaviour

suggest microbial contribution to regional wine characteristics. Mbio. 7(3):e00631-16. doi:0.1128/mBio.00631-16.
Bokulich NA, Mills DA. 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. Appl Environ Microbiol. 79(8):2519-2526. doi:10.1128/AEM.03870-12.
Bokulich NA, Thorngate JH, Richardson PM, Mills DA. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc Natl Acad Sci USA. 111(1):e139-e148. doi:10.1073/pnas.1317377110.
Boncinelli F, Dominici A, Gerini F, Marone E. 2019. Consumers wine preferences according to purchase occasion: personal consumption and gift-giving. Food Qual Prefer. 71:270-278. doi:10.1016/j.foodqual.2018.07.013
Bordet F, Joran A, Klein G, Roullier-Gall C, Alexandre H. 2020. Yeast-yeast interactions: mechanisms, methodologies and impact on composition. Microorganisms. 8(4):600. doi:10.3390/microorganisms8040600.
Börlin M, Venet P, Claisse O, Salin F, Legras JL, Masneuf-Pomarede. 2016. Cellar-associated *Saccharomyces cerevisiae* population structure revealed high-level diversity and perennial persistence at Sauternes wine estates. Appl Environ Microbiol. 82(10):2909-2918. doi:10.1128/AEM.03627-15.
Borneman AR, Pretorius IS. 2015. Genomic insights into the *Saccharomyces sensu stricto* complex. Genetics. 199(2):281-291. doi:10.1534/genetics.114.173633.
Borren E, Tian B. 2021. The important contribution of non-*Saccharomyces* yeasts to the aroma complexity of wine: a review. Foods. 10(1):13. doi:10.3390/foods10010013.
Bougreau M, Ascencio K, Bugarel M, Nightingale K, Longeragan G. 2019. Yeast species isolated from Texas High Plains vineyards and dynamics during spontaneous fermentations of Tempranillo grapes. PLOS ONE. 14(5):e0216246. doi:10.1371/journal.pone.0216246.
Bourret TB, Grove GG, Vandemark GJ, Henick-Kling T, Glawe DA. 2013. Diversity and molecular determination of wild yeasts in a central Washington State vineyard. N Am Fungi. 8(15):1-32. doi:10.2509/naf2013.008.015.
Boynton PJ, Kowallik V, Landermann D, Stukenbrock EH. 2019. Quantifying the efficiency and biases of forest *Saccharomyces* sampling strategies. Yeast. 36(11):657-668. doi:10.1002/yea.3435.
Bozoudi D, Tsaltas D. 2018. The multiple and versatile roles of *Aureobasidium pullulans* in the vitivinicultural sector. Fermentation. 4(4):85. doi:10.3390/fermentation4040085.
Bradshaw TL, Hazelrigg AL, Berkett LP. 2018. Characteristics of the cold-climate winegrape industry in Vermont, USA. Acta Hortic. 1205:469-476. doi:10.17660/ActaHortic.2018.1205.57.
Brežná B, Ženišová K, Chovanová K, Cheboňová V, Kraková L, Kuchta T, Pangallo D. 2010. Evaluation of fungal and yeast diversity in Slovakian wine-related microbial communities. Antonie van Leeuwenhoek. 98(4):519-529. doi:10.1007/s10482-010-9469-6.

Brilli L, Buscioni G, Moriondo M, Bindi M, Vincenzini M. 2014. Influence of interannual meteorological variability on yeast content and composition in Sangiovese grapes. Am J Enol Vitic. 65:375-380. doi:10.5344/ajev.2014.13116.

Brysch-Herzberg M, Seidel M. 2015. Yeast diversity on grapes in two German wine growing regions. Int J Food Microbiol. 214:137-144. doi:10.1016/j.ijfoodmicro.2015.07.034.

Bunbury-Blanchette AL. 2022 Jul 20. Data from: Yeast communities from Nova Scotia vineyards before and after spontaneous fermentation [dataset]. NCBI Sequence Read Archive BioProject PRJNA860361. https://ncbi.nlm.nih.gov/bioproject/860361.

Bunbury-Blanchette AL. 2023 Oct 16. Data from: Yeast communities from Nova Scotia vineyards before and after spontaneous fermentation, V2 [dataset]. Mendeley Data. doi:10.17632/jy959cnzps.2.

Bunbury-Blanchette AL, Fan L, English MM, Kernaghan G. 2023. Yeast communities before and after spontaneous fermentation of wine grapes: a case study from Nova Scotia. Can J Microbiol. 69(1):32-43. doi:10.1139/cjm-2022-0179.

Burns KN, Kluepfel DA, Strauss SL, Bokulich NA, Cantu D, Steenwerth KL. 2015. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by geographic features. Soil Biol Biochem. 91:232-247. doi:10.1016/j.soilbio.2015.09.002.

Čadež N, Zupan J, Raspor P. 2010. The effect of fungicides on yeast communities associated with grape berries. FEMS Yeast Res. 10(5):619-630. doi:10.1111/j.1567-1364.2010.00635.x.

Calabretti A, La Cara F, Sorrentino A, Di Stasio M, Santomauro F, Rastrelli L, Gabrielli L, Limone F, Volpe MG. 2012. Characterization of volatile fraction of typical Irpinian wines fermented with a new starter yeast. World J Microbiol Biotechnol. 28(4):1433-1442. doi:10.1007/s11274-011-0943-8.

Calabretti A, Volpe MG, Sorrentino A, Ionata E, Santomauro F, La Cara F. 2011. Aglianico and Fiano wines obtained with an autochthonous non-*Saccharomyces* yeast. Ann Microbiol. 61(1):131-136. doi:10.1007/s13213-010-0150-0.

Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11(12):2639-2643. doi:10.1038/ismej.2017.119.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 13(7):581-583. doi:10.1038/nmeth.3869.

Cameron BI, Ketter BS, Karakis S. 2012. An emerging wine region in Nova Scotia, Canada: terroir trials and tribulations. Proceedings of the AGU Fall Meeting; San Fransisco (CA); 1-3 Dec 2012.

Cameron ES, Schmidt PJ, Tremblay BJM, Emelko MB, Müller KM. 2021. To rarefy or not to rarefy: enhancing diversity analysis of microbial communities through next-generation sequencing and rarefying repeatedly. bioRxiv. doi:10.1101/2020.09.09.290049

Campo E, Saenz-Navajas MP, Cacho J, Ferreira V. 2012. Consumer rejection threshold of ethyl phenylacetate and phenylacetic acid, compounds responsible for the sweet-like off odour in wines made from sour rotten grapes. Aust J Grape Wine Res. 18(3):280-286. doi:10.1111/j.1755-0238.2012.00198.x.

Capece A, Romaniello R, Siesto G, Romano P. 2012. Diversity of *Saccharomyces cerevisiae* yeasts associated to spontaneously fermenting grapes from an Italian "heroic vine-growing area". Food Microbiol. 31(2):159-166. doi:10.1016/j.fm.2012.03.010.

Capitello R, Agnoli L, Charters S, Begallo D. 2021. Labelling environmental and terroir attributes: young Italian consumers' wine preferences. J Clean Prod. 304:126991. doi:10.1016/j.jclepro.2021.126991.

Capozzi V, Garofalo C, Chiriatti MA, Grieco F, Spano G. 2015. Microbial terroir and food innovation: the case of yeast biodiversity in wine. Microbiol Res. 181:75-83. doi:10.1016/j.micres.2015.10.005.

Carrau FM, Medina K, Farina L, Boido E, Henschke PA, Dellacassa E. 2008. Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: effects of yeast assimilable nitrogen on two model strains. FEMS Yeast Res. 8(7):1196-1207. doi:10.1111/j.1567-1364.2008.00412.x

Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N. 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbiol. 2(3):1-6. doi:10.1038/nmicrobiol.2016.242.

Castañeda LE, Miura T, Sánchez R, Barbosa O. 2018. Effects of agriculture management on phyllosphere fungal diversity in vineyards and the association with adjacent native forests. PeerJ. 6:e5715. doi:10.7717/peerj.5715.

Castellini A, Mauracher C, Troiano S. 2017. An overview of the biodynamic wine sector. In J Wine Res. 9:1-11. doi:10.2147/IJWR.S69126.

Castrillo D, Blanco P. 2022. Influence of vintage, geographical location and agricultural management on yeast populations in Galician grape musts (NW Spain). Oeno One. 56(4):65-79. doi:10.20870/oeno-one.2022.56.4.4898.

Castrillo D, Rabuñal E, Neira N, Blanco P. 2019. Yeast diversity on grapes from Galicia, NW Spain: biogeographical patterns and the influence of the farming system. Oeno One. 53(3):573-587. doi:10.20870/oeno-one.2019.53.3.2379.

Çelebi Uzkuç NM, Şişli B, Ay M, Togay SÖ, Karagül Yüceer Y, Bayhan A, Kırca Toklucu A. 2020. Effects of spontaneous fermentation on Karalahna and Cabernet Sauvingnon young red wines: volatile compounds, sensory profiles and identification of autochthonous yeasts. Eur Food Res Technol. 246(1):81-92. doi:10.1007/s00217-019-03395-w.

Chalvantzi I, Banilas G, Tassou C, Nisiotou A. 2021. Biogeographical regionalization of wine yeast communities in Greece and environmental drivers of species distribution at a local scale. Front Microbiol. 12:705001. doi:10.3389/fmicb.2021.705001.

Chamberlain G, Husnik J, Subden RE. 1997. Freeze-desiccation survival in wild yeasts in the bloom of icewine grapes. Food Res Int. 30(6):435-439. doi:10.1016/S0963-9969(97)00070-7.

Chambers PF, Pretorius IS. 2010. Fermenting knowledge: the history of winemaking, science and yeast research. EMBO Rep. 11(12):914-920. doi:10.1038/embor.2010.179.

Charoenchai C, Fleet GH, Henschke PA, Todd BENT. 1997. Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. Aust J Grape Wine Res. 3(1):2-8. doi:10.1111/j.1755-0238.1997.tb00109.x.

Charron G, Leducq JB, Bertin C, Dubé AK, Landry CR. 2014. Exploring the northern limit of the distribution of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* in North America. FEMS Yeast Res. 14(2):281-288. doi:10.1111/1567-1364.12100.

Chavan P, Mane S, Kulkarni G, Shaikh S, Ghormade V, Nerkar DP, Shouche Y, Deshpande MV. 2009. Natural yeast flora of different varieties of grapes used for wine making in India. Food Microbiol. 26(8):801-808. doi:10.1016/j.fm.2009.05.005.

Cheirsilp B, Umsakul K. 2008. Processing of banana-based wine product using pectinase and α-amylase. J Food Process Eng. 31(1):78-90. doi:10.1111/j.1745-4530.2007.00152.x.

Chen Y, Zhang W, Yi H, Wang B, Xiao J, Zhou X, Jiankun X, Jiang L, Shi X. 2020. Microbial community composition and its role in volatile compound formation during the spontaneous fermentation of ice wine made from Vidal grapes. Process Biochem. 92:365-377. doi:10.1016/j.procbio.2020.01.027.

Cheng E, Martiniuk JT, Hamilton J, McCarthy G, Castellarin SD, Measday V. 2020. Characterization of sub-regional variation in *Saccharomyces* populations and grape phenolic composition in Pinot Noir vineyards of a Canadian wine region. Front Genet. 11:908. doi:10.3389/fgene.2020.00908.

Cheraiti N, Guezenec S, Salmon JM. 2005. Redox interactions between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in mixed culture under enological conditions. Appl Environ Microbiol. 71(1):255-260. doi:10.1128/AEM.71.1.255-260.2005

Choudhary DK, Johri BN. 2009. Basidiomycetous yeasts: current status. In: Satyanarayana T, Kunze G, editors. Yeast Biotechnology: Diversity and Applications. Dordrecht (Netherlands): Springer; p. 19-46.

Chung HY, Fung PK, Kim JS. 2005. Aroma impact components in commercial plain sufu. J Agric Food Chem. 53(5):1684-1691. doi:10.1021/jf048617d.

Ciani M, Comitini F, Mannazzu I, Domizio P. 2010. Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Res. 10(2):123-133. doi:10.1111/j.1567-1364.2009.00579.x.

Ciani M, Mannazzu I, Marinangeli P, Clementi F, Martini A. 2004. Contribution of winery-resident *Saccharomyces cerevisiae* strains to spontaneous grape must fermentation. Antonie van Leeuwenhoek. 85(2):159-164. doi:10.1023/B:ANTO.0000020284.05802.d7.

Cioch-Skoneczny M, Satora P, Skotniczny M, Skoneczny S. 2018. Quantitative and qualitative composition of yeast microbiota in spontaneously fermented grape musts obtained from cool climate grape varieties ‘Rondo’ and ‘Regent’. FEMS Yeast Res. 18(8):foy089. doi:10.1093/femsyr/foy089.

Clemente-Jimenez JM, Mingorance-Cazorla L, Martínez-Rodríguez S, Heras-Vásquez FJ, Rodríguez-Vico F. 2004. Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. Food Microbiol. 21(2):149-155. doi:10.1016/S0740-0020(03)00063-7.

Clowers KJ, Heilberger J, Piotrowski JS, Will JL, Gasch AP. 2015. Ecological and genetic barriers differentiate natural populations of *Saccharomyces cerevisiae*. Mol Biol Evol. 32(9):2317-2327. doi:10.1093/molbev/msv112.

Combina M, Elía A, Mercado L, Catania C, Ganga A, Martinez C. 2005a. Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. 99(3):237-243. doi:10.1016/j.ijfoodmicro.2004.08.017.

Combina M, Mercado L, Borgo P, Elia A, Jofré V, Ganga A, Martinez C, Catania C. 2005b. Yeasts associated to Malbec grape berries from Mendoza, Argentina. J Appl Microbiol. 98(5):1055-1061. doi:10.1111/j.1365-2672.2005.02540.x.

Comeau AM, Douglas GM, Langille M. 2017. Microbiome helper: a custom and streamlined workflow for microbiome research. mSystems. 2(1):e00127-16. doi:10.1128/mSystems.00127-16.

Comitini F, Agarbati A, Canonico L, Ciani M. 2021. Yeast interactions and molecular mechanisms in wine fermentation: a comprehensive review. Int J Mol Sci. 22(14):7754. doi:10.3390/ijms22147754.

Comitini F, Capece A, Ciani M, Romano P. 2017. New insights on the use of wine yeasts. Curr Opin Food Sci. 13:44-49. doi:10.1016/j.cofs.2017.02.005.

Comitini F, Ciani M. 2008. Influence of fungicide treatments on the occurrence of yeast flora associated with wine grapes. Ann Microbiol. 58(3):489-493. doi:10.1007/BF03175547.

Comitini F, Gobbi M, Domizio P, Romani C, Lencioni L, Mannazzu I, Ciani M. 2011. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiol. 28(5):873-882. doi:10.1016/j.fm.2010.12.001.

Cordero-Bueso G, Arroyo T, Serrano A, Tello J, Aporta I, Vélez MD, Valero E. 2011. Influence of the farming system and vine variety on yeast communities associated with grape berries. Int J Food Microbiol. 145(1):132-139. doi:10.1016/j.ijfoodmicro.2010.11.040.

Costanigro M, Appleby C, Menke SD. 2014. The wine headache: consumer perceptions of sulfites and willingness to pay for non-sulfited wines. Food Qual Prefer. 31:81-89. doi:10.1016/j.foodqual.2013.08.002.

Costantini A, Vaudano E, Pulcini L, Boatti L, Gamalero E, Garcia-Moruno E. 2022. Yeast biodiversity in vineyard during grape ripening: comparison between culture dependent and NGS analysis. Processes. 10(5):901. doi:10.3390/pr10050901.

Coulon J, Husnik JI, Inglis DL, van der Merwe GK, Lonvaud A, Erasmus DJ, van Vuuren HJJ. 2006. Metabolic engineering of *Saccharomyces cerevisiae* to minimize the production of ethyl carbamate in wine. Am J Enol Vitic. 57(2):113-124. doi:10.5344/ajev.2006.57.2.113.

Craig B. 2022. Guide to Pest Management in Grapes. MacDonald J, Diez R, editors. Perennia Food and Agriculture Corporation.

Cravero MC. 2019. Organic and biodynamic wines quality and characteristics: a review. Food Chem. 295:334-340. doi:10.1016/j.foodchem.2019.05.149.

Csoma H, Zakany N, Capece A, Romano P, Sipiczki M. 2010. Biological diversity of *Saccharomyces* yeasts of spontaneously fermenting wines in four wine regions: comparative genotypic and phenotypic analysis. Int J Food Microbiol. 140(2-3):239-248. doi:10.1016/j.ijfoodmicro.2010.03.024.

Cureau N, Threlfall R, Carbonero F, Howard L, Lavefve L. 2021a. Fungal diversity and dynamics during grape wine fermentations with different sulfite levels and yeast inoculations. Am J Enol Vitic. 72(3):240-256. doi:10.5344/ajev.2021.20054.

Cureau N, Threlfall R, Marasini D, Lavefve L, Carbonero F. 2021b. Year, location, and variety impact on grape-associated mycobiota of Arkansas-grown wine grapes for wine production. Microb Ecol. 82(4):845-858. doi:10.1007/s00248-021-01705-y.

Čuš F, Zabukovec P, Schroers HJ. 2017. Indigenous yeasts perform alcoholic fermentation and produce aroma compounds in wine. Czech J Food Sci. 35(4):329-345. doi:10.17221/398/2016-CJFS.

D'Amico M, Di Vita G, Monaco L. 2016. Exploring environmental consciousness and consumer preferences for organic wines without sulfites. J Clean Prod. 120:64-71. doi:10.1016/j.jclepro.2016.02.014.

Daniel HM, Lachance MA, Kurtzman CP. 2014. On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. Antonie Van Leeuwenhoek. 106(1):67-84. doi:10.1007/s10482-014-0170-z

Davenport RR. 1974. Microecology of yeasts and yeast-like organisms associated with an English vineyard. Vitis. 13(2):123-130.

Davenport RR. 1976. Distribution of yeasts and yeast-like organisms from aerial surfaces of developing apples and grapes. In: Dickinson CH, Preece TF, editors. Microbiology of Aerial Plant Surfaces. London (UK): Academic Press; p. 325-359.

David V, Terrat S, Herzine K, Claisse O, Rousseaux S, Tourdot-Maréchal R, Masneuf-Pomarède I, Ranjard L, Alexandre H. 2014. High-throughput sequencing of amplicons for monitoring yeast biodiversity in must and during alcoholic fermentation. J Ind Microbiol Biotechnol. 41(5):811-821. doi:10.1007/s10295-014-1427-2.

De Cáceres L, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology. 90(12):3566-3574. doi:10.1890/08-1823.1.

De Filippis F, La Storia A, Blaiotta G. 2017. Monitoring the mycobiota during Greco di Tufo and Aglianico wine fermentation by 18S rRNA gene sequencing. Food Microbiol. 63:117-122. doi:10.1016/j.fm.2016.11.010.

DeKrey DH, Klodd AE, Clark MD, Blanchette RA. 2022. Grapevine trunk diseases of cold-hardy varieties grown in Northern Midwest vineyards coincide with canker fungi and winter injury. PLOS ONE. 17(6):e0269555. doi:10.1371/journal.pone.0269555.

Delaquis P, Cliff M, King M, Girard B, Hall J, Reynolds A. 2000. Effect of two commercial malolactic cultures on the chemical and sensory properties of Chancellor wines vinified with different yeasts and fermentation temperatures. Am J Enol Vitic. 51(1):42-48. doi:10.5344/ajev.2000.51.1.42.

De La Torre MJ, Millan MC, Perez-Juan P, Morales J, Ortega JM. 1999. Indigenous yeasts associated with two *Vitis vinifera* grape varieties cultured in Northern Spain. Microbios. 100(395):27-40.

Del Fresno JM, Cardona M, Ossorio P, Loira I, Escott C, Morata A. 2022. White must extraction methods. In: Morata A, editor. White Wine Technology. London (UK): Academic Press; p. 39-47.

De Mot R, Verachtert H. 1985. Purification and characterization of extracellular amylolytic enzymes from the yeast *Filobasidium capsuligenum*. Appl Environ Microbiol. 50(6):1474-1482. doi:10.1128/aem.50.6.1474-1482.1985.

Demuyter C, Lollier M, Legras JL, Le Jeune C. 2004. Predominance of *Saccharomyces uvarum* during spontaneous alcoholic fermentation, for three consecutive years, in an Alsatian winery. J Appl Microbiol. 97(6):1140-1148. doi:10.1111/j.1365-2672.2004.02394.x.

Deneulin P, Dupraz X. 2018. Vins sans sulfites ajoutés et labels environnementaux : quel prix pour les consommateurs suisses? Rev Suisse Vitic Arboric Hortic. 50(3):176-182.

de Ponzzes-Gomes CMPBS, de Mélo DLFM, Santana CA, Pereira GE, Mendonça MOC, Gomes FCO, Oliveira ES, Barbosa AM Jr, Trindade RC, Rosa CA. 2014. *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts in grape varieties of the São Francisco Valley. Braz J Microbiol. 45:411-416. doi:10.1590/S1517-83822014000200007.

Díaz C, Molina AM, Nähring J, Fischer R. 2013. Characterization and dynamic behavior of wild yeast during spontaneous wine fermentation in steel tanks and amphorae. BioMed Res Int. 2013:540465. doi:10.1155/2013/540465.

Diez-Zamudio F, Laytte R, Grallert C, Gutiérrez-Gamboa G. 2021a. Nutritional status differentially affect yield and must composition of hybrids and *V. vinifera* varieties established under cold climate conditions. Cienc e Tec Vitivinic. 36(1):89-103. doi:10.1051/ctv/20213601089.

Diez-Zamudio F, Laytte R, Grallert C, Ivit NN, Gutiérrez-Gamboa G. 2021b. Viticultural performance of hybrids and *Vitis vinifera* varieties established in Annapolis Valley (Nova Scotia). Horticulturae. 7(9):291. doi:10.3390/horticulturae7090291.

Di Maro E, Ercolini D, Coppola S. 2007. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. Int J Food Microbiol. 117(2):201-210. doi:10.1016/j.ijfoodmicro.2007.04.007.

Ding Y, Wei R, Wang L, Yang C, Li H, Wang H. 2021. Diversity and dynamics of microbial ecosystem on berry surface during the ripening of Ecolly (*Vitis vinifera* L.) grape in Wuhai, China. World J Microbiol Biotechnol. 37(12):214. doi:10.1007/s11274-021-03170-8.

Dissanayake AJ, Purahong W, Wubet T, Hyde KD, Zhang W, Xu H, Zhang G, Fu C, Liu M, Xing Q, et al. 2018. Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (*Vitis vinifera*). Fungal Divers. 90(1):85-107. doi:10.1007/s13225-018-0399-3.

Dizy M, Bisson LF. 2000. Proteolytic activity of yeast strains during grape juice fermentation. Am J Enol Vitic. 51(2):155-167. doi:10.5344/ajev.2000.51.2.155.

Domizio R, Romani C, Lencioni L, Comitini F, Gobbi M, Mannazzu I, Ciani M. 2011. Outlining a future for non-*Saccharomyces* yeasts: selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. Int J Food Microbiol. 147(3):170-180. doi:10.1016/j.ijfoodmicro.2011.03.020.

Drumonde-Neves J, Fernandes T, Lima T, Pais C, Franco-Duarte R. 2021. Learning from 80 years of studies: a comprehensive catalogue of non-*Saccharomyces* yeasts associated with viticulture and winemaking. FEMS Yeast Res. 21(3):foab017. doi:10.1093/femsyr/foab017.

Drumonde-Neves J, Franco-Duarte R, Lima T, Schuller D, Pais C. 2017. Association between grape yeast communities and the vineyard ecosystems. PLOS ONE. 12(1):e0169883. doi:10.1371/journal.pone.0169883.

Duley G, Ceci AT, Long E, Boselli E. 2023. Oenological potential of wines produced from disease-resistant grape cultivars. Compr. 22(4):2591-2610. doi:10.1111/1541-4337.13155.

Dutra-Silva L, Pereira GE, Batista LR, Matteoli FP. 2021. Fungal diversity and occurrence of mycotoxin producing fungi in tropical vineyards. W J Microbiol Biotechnol. 37(7):112. doi:10.1007/s11274-021-03081-8.

Egli CM, Edinger WD, Mitrakul CM, Henick-Kling T. 1998. Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. J Appl Microbiol. 85(5):779-789. doi:10.1046/j.1365-2672.1998.00521.x.

Elfeky N, Elmahmoudy M, Bao Y. 2020. Manipulation of culture conditions: tool for correlating/improving lipid and carotenoid production by *Rhodotorula glutinis*. Processes. 8(2):140. doi:10.3390/pr8020140.

Enache-Angoulvant A, Hennequin C. 2005. Invasive *Saccharomyces* infection: a comprehensive review. Clin Infect Dis. 41(11):1559-1568. doi:10.1086/497832.

Englezos V, Mota-Gutierrez J, Giacosa S, Río Segade S, Pollon M, Gambino G, Rolle L, Ferrocino I, Rantsiou K. 2022. Effect of alternative fungicides and inoculation strategy on yeast biodiversity and dynamics from the vineyard to the winery. Food Res Int. 162(A):111935. doi:10.1016/j.foodres.2022.111935.

Environment and Climate Change Canada Monthly Climate Summaries. 1840–. Government of Canada. [updated monthly on the 5th of the month]. https://climate.weather.gc.ca/prods_servs/cdn_climate_summary_e.html.

Erasmus DJ, Cliff M, van Vuuren HJJ. 2004. Impact of yeast strain on the production of acetic acid, glycerol, and the sensory attributes of icewine. Am J Enol Vitic. 55(4):371-378. doi:10.5344/ajev.2004.55.4.371.

Escribano-Viana R, López-Alfaro I, López R, Santamaría P, Gutiérrez AR, González-Arenzana L. 2018. Impact of chemical and biological fungicides applied to grapevine on grape biofilm, must, and wine microbial diversity. Front Microbiol. 9:59. doi:10.3389/fmicb.2018.00059.

Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J Syst Evol Microbiol. 49(1):329-337. doi:10.1099/00207713-49-1-329.

Fabbrizzi S, Alampi Sottini V, Cipollaro M, Menghini S. 2021. Sustainability and natural wines: an exploratory analysis on consumers. Sustainability. 13(14):7645. doi:10.3390/su13147645.

Fadiji AE, Babalola OO. 2020. Metagenomics methods for the study of plant-associated microbial communities: a review. J Microbiol Methods. 170:105860. doi:10.1016/j.mimet.2020.105860.

Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2

domain sequence analysis. Int J Syst Evol Microbiol. 50(3):1351-1371. doi:10.1099/00207713-50-3-1351.
Feng CT, Du X, Wee J. 2021. Microbial and chemical analysis of non-*Saccharomyces* yeasts from Chambourcin hybrid grapes for potential use in winemaking. Fermentation 7(1):15. doi:10.3390/fermentation7010015.
Fernández M, Úbeda JF, Briones AI. 2000. Typing of non-*Saccharomyces* yeasts with enzymatic activities of interest in wine-making. Int J Food Microbiol. 59(1-2):29-36. doi:10.1016/S0168-1605(00)00283-X.
Fia G, Canuti V, Rosi I. 2014. Evaluation of potential side activities of commercial enzyme preparations used in winemaking. Int J Food Sci Technol. 49(8):1902-1911. doi:10.1111/ijfs.12508.
Fleet GH. 2003. Yeast interactions and wine flavour. Int J Food Microbiol. 86(1/2):11-22. doi:10.1016/S0168-1605(03)00245-9.
Fleet GH. 2008. Wine yeasts for the future. FEMS Yeast Res. 8(7):979-995. doi:10.1111/j.1567-1364.2008.00427.x.
Fleet GH, Lafon-Lafourcade S, Ribéreau-Gayon P. 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl Environ Microbiol. 48(5):1034-1038. doi:10.1128/aem.48.5.1034-1038.1984.
Fleet GH, Prakitchaiwattana C, Beh A, Heard G. 2002. The yeast ecology of wine grapes. In: Ciani M, editor. Biodiversity and Biotechnology of Wine Yeasts. Kerala: Research Signpost; p. 1-17.
Forge T, Munro P, Wright H, Moreau D. 2022. Plant-parasitic nematodes in Nova Scotia vineyards. Phytoprotection. 102(1):15-20. doi:10.7202/1088485ar.
Fuchs M, Martinson TE, Loeb GM, Hoch HC. 2009. Survey for the three major leafroll disease-associated viruses in Finger Lakes Vineyards in New York. Plant Dis. 93(4):395-401. doi:10.1094/PDIS-93-4-0395.
Furneaux B, Bahram M, Rosling A, Yorou NS, Ryberg M. 2021. Long- and short-read metabarcoding technologies reveal similar spatiotemporal structures in fungal communities. Mol Ecol Resour. 21(6):1833-1849. doi:10.1111/1755-0998.13387.
Gagnon S. 1999. “We’re swimming upstream” … into the mainstream? An overview of social, economic, and institutional aspects of organic farming in Nova Scotia [master’s thesis]. Ottawa (ON): Carleton University.
Galati A, Schifani G, Crescimanno M, Migliore G. 2019. “Natural wine” consumers and interest in label information: An analysis of willingness to pay in a new Italian wine market segment. J Clean Prod. 227:405-413. doi:10.1016/j.jclepro.2019.04.219.
Gao F, Chen J, Xiao J, Cheng W, Zheng X, Wang B, Shi X. 2019. Microbial community composition on grape surface controlled by geographical factors of different wine regions in Xinjiang, China. Food Res Int. 122:348-360. doi:10.1016/j.foodres.2019.04.029.
Gao J, Geng H, Chai R, Wu T, Huang W, You Y, Zhan J. 2024. Fungal community composition and its relationship with volatile compounds during spontaneous fermentation of Cabernet Sauvignon from two Chinese wine-growing regions. Foods. 13(1):106. doi:10.3390/foods13010106.

García M, Esteve-Zarzoso B, Cabellos JM, Arroyo T. 2020. Sequential non-*Saccharomyces* and *Saccharomyces cerevisiae* fermentations to reduce the alcohol content in wine. Fermentation. 6(2):60. doi:10.3390/fermentation6020060.

Garde-Cerdán T, Ancín-Azpilicueta C. 2006. Contribution of wild yeasts to the formation of volatile compounds in inoculated wine fermentations. Eur Food Res Technol. 222(1):15-25. doi:10.1007/s00217-005-0029-7.

Garijo P, López R, Santamariá P, Ocón E, Olarte C, Sanz S, Gutiérrez AR. 2011. Presence of enological microorganisms in the grapes and the air of a vineyard during the ripening period. Eur Food Res Technol. 233(2):359-365. doi:10.1007/s00217-011-1528-3.

Garofalo C, Russo P, Beneduce L, Massa S, Spano G, Capozzi V. 2016. Non-*Saccharomyces* biodiversity in wine and the 'microbial terroir': a survey on Nero di Troia wine from the Apulian region, Italy. Ann Microbiol. 66(1):143-150. doi:10.1007/s13213-015-1090-5.

Gayevskiy V, Goddard MR. 2012. Geographic delineations of yeast communities and populations associated with vines and wines in New Zealand. ISME J. 6(7):1281-1290. doi:10.1038/ismej.2011.195.

Ge X, Wang J, Wang X, Liu Y, Dang C, Suo R, Sun J. 2023. Evaluation of indigenous yeasts screened from Chinese vineyards as potential starters for improving wine aroma. Foods. 12(16):3073. doi:10.3390/foods12163073.

Giacosa S, Río Segade S, Cagnasso E, Caudana A, Rolle L, Gerbi V. 2019. SO_2 in wines: rational use and possible alternatives. In: Morata A, editor. Red Wine Technology. London (UK): Academic Press; p. 309-321.

Giffard B, Winter S, Guidoni S, Nicolai A, Castaldini M, Cluzeau D, Coll P, Cortet J, Le Cadre E, d'Errico G, et al. 2022. Vineyard management and its impacts on soil biodiversity, functions, and ecosystem services. Front Ecol Evol. 10:850272. doi:10.3389/fevo.2022.850272.

Gil-Sánchez I, Bartolomé Suáldea B, Moreno-Arribas MV. 2019. Malolactic fermentation. In: Morata A, editor. Red Wine Technology. London (UK): Academic Press; p. 85-98.

Gjelaj M. 2019. Characterization of Nova Scotia Grape and Wine [master's thesis]. Wolfville (NS): Acadia University.

Gobbi M, Comitini F, Domizio P, Romani C, Lencioni L, Mannazzu I, Ciani M. 2013. *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: a strategy to enhance acidity and improve the overall quality of wine. Food Microbiol. 33(2):271-281. doi:10.1016/j.fm.2012.10.004.

Goddard MR, Anfang N, Tang R, Gardner RC, Jun C. 2010. A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-aided global dispersal in oak barrels. Environ Microbiol. 12(1):63-73. doi:10.1111/j.1462-2920.2009.02035.x.

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, et al. 1996. Life with 6000 genes. Science. 274(5287):546-567. doi:10.1126/science.274.5287.546.

Gómez-Albarrán C, Melguizo C, Patiño B, Vázquez C, Gil-Serna J. 2021. Diversity of mycobiota in Spanish grape berries and selection of *Hanseniaspora uvarum* U1 to prevent mycotoxin contamination. Toxins, 13(9):649. doi:10.3390/toxins13090649.

González AP, Parga-Dans E. 2020. Natural wine: do consumers know what it is, and how natural it really is? J Clean Prod. 251:119635. doi:10.1016/j.jclepro.2019.119635.
Gonzalez R, Quirós M, Morales P. 2013. Yeast respiration of sugars by non-*Saccharomyces* yeast species: a promising and barely explored approach to lowering alcohol content of wines. Trends Food Sci Technol. 29(1):55-61. doi:10.1016/j.tifs.2012.06.015.
González-Royo E, Pascual O, Kontoudakis N, Esteruelas M, Esteve-Zarzoso B, Mas A, Canals JM, Zamora F. 2015. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. Eur Food Res Technol. 240(5):999-1012. doi:10.1007/s00217-014-2404-8.
Goodwin S, McPherson JD, McCombie WR. 2016. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet. 17(6):333-351. doi:10.1038/nrg.2016.49.
Goold HD, Kroukamp H, Williams TC, Paulsen IT, Varela C, Pretorius IS. 2017. Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. Microb Biotechnol. 10(2):264-278. doi:10.1111/1751-7915.12488.
Goto S. 1980. Changes in the wild yeast flora of sulfited grape musts. J Inst Enol Vitic Yamanashi Univ. 15:29-32.
Gottlieb PD, Moscovici D. 2015. Sustainability practices at New Jersey wineries. Exploring the roles played by agricultural tourism and firm size. Riv Sci Turismo. 6(1-2):23-38. doi:107358/rst-2015-03-gomo.
Granchi L, Bosco M, Messini A, Vincenzini M. 1999. Rapid detection and quantification of yeast species during spontaneous wine fermentation by PCR–RFLP analysis of the rDNA ITS region. J Appl Microbiol. 87(6):949-956. doi:10.1046/j.1365-2672.1999.00600.x.
Grangeteau C, David V, Hervé A, Guilloux-Benatier M, Rousseaux S. 2017. The sensitivity of yeasts and yeast-like fungi to copper and sulfur could explain lower yeast biodiversity in organic vineyards. FEMS Yeast Res. 17(8):fox092. doi:10.1093/femsyr/fox092.
Griggs RG, Steenwerth KL, Mills DA, Cantu D, Bokulich NA. 2021. Sources and assembly of microbial communities in vineyards as a functional component of winegrowing. Front Microbiol. 12:673810. doi:10.3389/fmicb.2021.673810.
Guerrini L, Corti F, Angeloni G, Masella P, Spadi A, Calamai L, Parenti A. 2022. The effects of destemming/crushing and pressing conditions in rosé wine production. Aust J Grape Wine Res. 2022:9853264. doi:10.1155/2022/9853264.
Guibourdenche L, Stevenson R, Pedneault K, Poirier A, Widory D. 2020. Characterizing nutrient pathways in Quebec (Canada) vineyards: insight from stable and radiogenic strontium isotopes. Chem Geol. 532:119375. doi:10.1016/j.chemgeo.2019.119375.
Hall B, Durall DM, Stanley G. 2011. Population dynamics of *Saccharomyces cerevisiae* during spontaneous fermentation at a British Columbia winery. Am J Enol Vitic. 62(1):66-72. doi:10.5344/ajev.2010.10054.
Hall JF. 1971. Detection of wild yeasts in the brewery. J Inst Brew. 77(6):513-516. doi:10.1002/j.2050-0416.1971.tb03413.x.
Halwachs B, Madhusudhan N, Krause R, Nilsson RH, Moissl-Eichinger C, Högenauer C, Thallinger GG, Gorkiewicz G. 2017. Critical issues in mycobiota analysis. Front Microbiol. 8:180. doi:10.3389/fmicb.2017.00180.

Hawkins DL, Ryder J, Parish-Virtue K, Fedrizzi B, Goddard MR, Knight SJ. 2023. Mixed yeast communities contribute to regionally distinct wine attributes. FEMS Yeast Res. 23:foad005. doi:10.1093/femsyr/foad005.

Hayward L, Jantzi H, Smith A, McSweeney MB. 2020. How do consumers describe cool climate wines using projective mapping and ultra-flash profile? Food Qual Prefer. 86:104026. doi:10.1016/j.foodqual.2020.104026.

He Y, Wang X, Li P, Lv Y, Nan H, Wen L, Wang Z. 2023. Research progress of wine aroma components: a critical review. Food Chem. 402:134491. doi:10.1016/j.foodchem.2022.134491,

Heard GM, Fleet GH. 1985. Growth of natural yeast flora during the fermentation of inoculated wines. Appl Environ Microbiol. 50(3):727-728. doi:10.1128/aem.50.3.727-728.1985.

Herraiz T, Reglero G, Herraiz M, Martin-Alvarez PJ, Cabezudo MD. 1990. The influence of the yeast and type of culture on the volatile composition of wines fermented without sulfur dioxide. Am J Enol Vitic. 41(4):313-318. doi:10.5344/ajev.1990.41.4.313.

Hillier NK, Lefebvre J. 2012. Detection of insect pests of grapes, *Vitis vinifera*, in vineyards of Nova Scotia through pheromone trapping. J Acad Entomol Soc. 8:30-35.

Hinnen A, Hicks JB, Fink GR. 1978. Transformation of yeast. Proc Natl Acad Sci USA. 75(4):1929-1933. doi:10.1073/pnas.75.4.1929.

Homolka L. 2014. Preservation of live cultures of basidiomycetes – recent methods. Fungal Biol. 118(2):107-125. doi:10.1016/j.funbio.2013.12.002.

Huang L, Ma Y, Tian X, Li JM, Li LX, Tang K, Xu Y. 2018. Chemosensory characteristics of regional Vidal icewines from China and Canada. Food Chem. 261:66-74. doi:10.1016/j.foodchem.2018.04.021.

Husnik JI, Volschenk H, Bauer J, Colavizza D, Luo Z, van Vuuren HJJ. 2006. Metabolic engineering of malolactic wine yeast. Metab Eng. 8(4):315-323. doi:10.1016/j.ymben.2006.02.003.

Inglis DL, Kelly JM, van Dyk SA, Dowling LK, Pickering GJ, Kemp B. 2020. *Saccharomyces uvarum* yeast isolate consumes acetic acid during fermentation of high sugar juice and juice with high starting volatile acidity. OENO One. 54(2). doi:10.20870/oeno-one.2020.54.2.2594.

Ioannou P, Vamvoukaki R, Samonis G. 2019. *Rhodotorula* species infections in humans: a systematic review. Mycoses. 62(2):90-100. doi:10.1111/myc.12856.

Ivit NN, Kemp B. 2018. The impact of non-*Saccharomyces* yeast on traditional method sparkling wine. Fermentation. 4(3):73. doi:10.3390/fermentation4030073.

Ivit NN, Longo R, Kemp B. 2020. The effect of non-*Saccharomyces* and *Saccharomyces* non-*cerevisiae* yeasts on ethanol and glycerol levels in wine. Fermentation. 6(3):77. doi:10.3390/fermentation6030077.

Jantzi HA, McSweeney MB. 2019. An overview of wine consumers in Nova Scotia, Canada: a conjoint analysis study. J Wine Res. 30(1):48-61. doi:10.1080/09571264.2019.1573356.

Jara C, Laurie VF, Mas A, Romero J. 2016. Microbial terroir in Chilean valleys: diversity of non-conventional yeast. Front Microbiol. 7:663. doi:10.3389/fmicb.2016.00663.

Jiang J, Liu Y, Li H, Yang Q, Wu Q, Chen S, Tang J, Xu Y. 2019. Modeling and regulation of higher alcohol production through the combined effects of the C/N ratio and microbial interaction. J Agric Food Chem. 67(38):10694-10701. doi:10.1021/acs.jafc.9b04545.

Jiraska L, Jones B, Knight SJ, Lennox J, Goddard MR 2023. Soil and bark biodiversity forms discrete islands between vineyards that are not affected by distance or management regime. Environ Microbiol. 25(12):3655-3670. doi:10.1111/1462-2920.16513.

Johnson EA. 2013. Biotechnology of non-*Saccharomyces* yeasts – the basidiomycetes. Appl Microbiol Biotechnol. 97(17):7563-7577. doi:10.1007/s00253-013-5046-z.

Jolly NP, Augustyn OPH, Pretorius IS. 2006. The role and use of non-*Saccharomyces* yeasts in wine production. S Afr J Enol Vitic. 27(1):15-39. doi:10.21548/27-1-1475.

Jolly N, Mehlomakulu NN, Nortje S, Beukes L, Hoff J, Booyse M, Erten H. 2022. Non-*Saccharomyces* yeast for lowering wine alcohol levels: partial aeration versus standard conditions. FEMS Yeast Res. 22(1):foac002. doi:10.1093/femsyr/foac002.

Jolly NP, Varela C, Pretorius IS. 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res. 14(2):215-237. doi:10.1111/1567-1364.12111.

Jones G, Grandjean E. 2018. Creating the market for organic wine: sulfites, certification and green values. In: Jones G, editor. Varieties of Green Business: Industries, Nations and Time. Cheltenham (UK): Edward Elgar Publishing; p. 154-193.

Jones GV, Schultz RH. 2016. Climate change and emerging cool climate wine regions. Wine Vitic J. V31N6.

Jusino MA, Palmer JM, Banik MT, Edgar RC, Lindner DL. 2016. Everything is not everywhere: designing a synthetic fungal ITS mock community for NGS of environmental samples. Inoculum. 67(4):130.

Kačániová M, Kunová S, Sabo J, Ivanišová E, Žiarovská J, Felsöciová S, Terentjeva M. 2020. Identification of yeasts with mass spectrometry during wine production. Fermentation. 6(1):5. doi:10.3390/fermentation6010005.

Kachalkin AV, Abdullabekova DA, Magomedova ES, Magomedov GG, Chernov IYu. 2015. Yeasts of the vineyards in Dagestan and other regions. Microbiol. 84(3):425-432. doi:10.1134/S002626171503008X.

Kamilari E, Mina M, Karallis C, Tsaltas D. 2021. Metataxonomic analysis of grape microbiota during wine fermentation reveals the distinction of Cyprus regional terroirs. Front Microbiol. 12:726483. doi:10.3389/fmicb.2021.726483.

Kántor A, Mareček J, Ivanišová E, Terentjeva M, Kačániová M. 2017. Microorganisms of grape berries. Proceedings LAS. 71(6):502-208. doi:10.1515/prolas-2017-0087.

Kapsopoulou K, Mourtzini A, Anthoulas M, Nerantzis E. 2007. Biological acidification during grape must fermentation using mixed cultures of *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae*. World J Microbiol Biotechnol. 23(5):735-739. doi:10.1007/s11274-006-9283-5.

Karimi B, Cahurel JY, Gontier L, Charlier L, Chovelon M, Mahé H, Ranjard L. 2020. A meta-analysis of the ecotoxicological impact of viticultural practices on soil biodiversity. Environ Chem Lett. 18(6):1947-1966. doi:10.1007/s10311-020-01050-5.

Kecskeméti E, Berkelmann-Löhnertz B, Reineke A. 2016. Are epiphytic microbial communities in the carposphere of ripening grape clusters (*Vitis vinifera* L.) different between conventional, organic, and biodynamic grapes? PLOS ONE. 11(8):e0160852. doi:10.1371/journal.pone.0160852.

Kelly J, Inglis DL, Pickering GJ. 2020. Sensorial and volatile analysis of wines made from partially dehydrated grapes: an Ontario case study. J Food Qual. 2020:e8861185. doi:10.1155/2020/8861185.

Kelly J, Yang F, Dowling L, Nurgel C, Beh A, Di Profio G, Pickering G, Inglis DL. 2018. Characterization of *Saccharomyces bayanus* CN1 for fermenting partially dehydrated grapes grown in cool climate winemaking regions. Fermentation. 4(3):77. doi:10.3390/fermentation4030077.

Kemp B, Plante J, Inglis DL. 2020. Nutrient addition to low pH base wines (L. cv. Riesling) during yeast acclimatization for sparkling wine: its influence on yeast cell growth, sugar consumption and nitrogen usage. Beverages. 6(1):10. doi:10.3390/beverages6010010.

Kernaghan G, Mayerhofer M, Griffin A. 2017. Fungal endophytes of wild and hybrid *Vitis* leaves and their potential for vineyard biocontrol. Can J Microbiol. 63(7):583-595. doi:10.1139/cjm-2016-0740.

Keszthelyi A, Ohkusu M, Takeo K, Pfeiffer I, Litter J, Kucsera J. 2006. Characterisation of the anticryptococcal effect of the FC-1 toxin produced by *Filobasidium capsuligenum*. Mycoses. 49(3):176-183. doi:10.1111/j.1439-0507.2006.01227.x.

Kidd SE, Abdolrasouli A, Hagen F. 2023. Fungal nomenclature: managing change is the name of the game. Open Forum Infect Dis. 10(1):ofac559. doi:10.1093/ofid/ofac559.

Kioroglou D, Kraeva-Deloire E, Schmidtke LM, Mas A, Portillo MC. 2019. Geographical origin has a greater impact on grape berry fungal community than grape variety and maturation state. Microorganisms. 7(12):669. doi:10.3390/microorganisms7120669.

Klein M, Swinnen S, Thevelein JM, Nevoight E. 2017. Glycerol metabolism and transport in yeast and fungi: established knowledge and ambiguities. Environ Microbiol. 19(3):878-893. doi:10.1111/1462-2920.13617.

Knight S, Goddard MR. 2015. Quantifying separation and similarity in a *Saccharomyces cerevisiae* metapopulation. ISME J. 9(2):361-370. doi:10.1038/ismej.2014.132.

Knight S, Klaere S, Fedrizzi B, Goddard MR. 2015. Regional microbial signatures positively correlate with differential wine phenotypes: evidence for a microbial aspect to terroir. Sci Rep. 5:14233. doi:10.1038/srep14233.

Knight SJ, Klaere S, Morrison-Whittle P, Goddard MR. 2018. Fungal diversity during fermentation correlates with thiol concentration in wine. Aust J Grape Wine Res. 24(1):105-112. doi:10.1111/ajgw.12304.

Kontkanen D, Inglis DL, Pickering GJ, Reynolds A. 2004. Effect of yeast inoculation rate, acclimatization, and nutrient addition on icewine fermentation. Am J Enol Vitic. 55(4):363-370. doi:10.5344/ajev.2004.55.4.363.

Kot AM, Błażejak S, Kurcz A, Gientka I, Kieliszek M. 2016. *Rhodotorula glutinis* – potential source of lipids, carotenoids, and enzymes for use in industries. Appl Microbiol Biotechnol. 100(14):6103-6117. doi:10.1007/s00253-016-7611-8.

Kot AM, Kieliszek M, Piwowarek K, Błażejak S, Mussagy CU. 2021. *Sporobolomyces* and *Sporidiobolus* – non-conventional yeasts for use in industries. Fungal Biol Rev. 37:41-58. doi:10.1016/j.fbr.2021.06.001.

Koulougliotis D, Eriotou E. 2016. Isolation and identification of endogenous yeast strains in grapes and must solids of Mavrodafni Kefalonias and antioxidant activity of the produced red wine. J Ferment Technol. 5(1):1000125. doi:10.4172/2167-7972.1000125.

Kumar KR, Cowley MJ, Davis RL. 2019. Next-generation sequencing and emerging technologies. Semin Thromb Hemost. 45(7):661-673. doi:10.1055/s-0039-1688446.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 35(6):1547-1549. doi:10.1093/molbev/msy096.

Kurtzman CP. 2011. Discussion of teleomorphic and anamorphic ascomycetous yeasts and yeast-like taxa. In: Kurtzman CP, Fell JW, Boekhout T, editors. Yeasts: A Taxonomic Study. London (UK): Elsevier; p. 293-310.

Kurtzman CP, Fell JW. 2006. Yeast systematics and phylogeny – implications of molecular identification methods for studies in ecology. In: Rosa C, Péter G, editors. Biodiversity and Ecophysiology of Yeasts. Heidelberg: Springer-Verlag; p. 11-30.

Kurtzman CP, Mateo RQ, Kolecka A, Theelen B, Robert V, Boekhout T. 2015. Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways. FEMS Yeast Res. 15(6):fov050. doi:10.1093/femsyr/fov050.

Kurtzman CP, Robnett CJ. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek. 73(4):331-371. doi:10.1023/A:1001761008817.

Kutyna DR, Varela C, Henschke PA, Chambers PJ, Stanley GA. 2010. Microbiological approaches to lowering ethanol concentration in wine. Trends Food Sci Technol. 21(6):293-302. doi:10.1016/j.tifs.2010.03.004.

Lagassey JB. 2014. Eastern US wine and yeast study; kinetics and composition [major qualifying project]. Worcester (MA): Worcester Polytechnic Institute.

Lallemand Oenology. 2024. Lallemand Inc. [accessed 2024 Jan 21]. https://www.lallemandwine.com/en/north-america/.

Lambrechts MG, Pretorius IS. 2000. Yeast and its importance to wine aroma: a review. S Afr J Enol Vitic. 21:97-129.

Lange JN. 2012. Yeast population dynamics during inoculated and spontaneous fermentations at three local British Columbia wineries [master's thesis]. Kelowna (BC): University of British Columbia.

Lange JN, Faasse E, Tantikachornkiat M, Gustafsson FS, Halvorsen LC, Kluftinger A, Ledderhof D, Durall DM. 2014. Implantation and persistence of yeast inoculum in Pinot noir fermentations at three Canadian wineries. Int J Food Microbiol. 180:56-61. doi:10.1016/j.ijfoodmicro.2014.04.003.

Lappa IK, Kachrimanidou V, Pateraki C, Koulougliotis D, Eriotou E, Kopsahelis N. 2020. Indigenous yeasts: emerging trends and challenges in winemaking. Curr Opin Food Sci. 32:133-143. doi:10.1016/j.cofs.2020.04.004.

Leach A, Leach H. 2020. Characterizing the spatial distributions of spotted lanternfly (Hemiptera: Fulgoridae) in Pennsylvania vineyards. Sci Rep. 10(1):20588. doi:10.1038/s41598-020-77461-9.

Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. 2006. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J Clin Microbiol. 44(3):693-699. doi:10.1128/jcm.44.3.693-699.2006.

Lederer MA, Nielsen DS, Toldam-Andersen TB, Herrmann JV, Arneborg N. 2013. Yeast species associated with different wine grape varieties in Denmark. Acta Agric Scand B Soil Plant Sci. 63(1):89-96. doi:10.1080/09064710.2012.723738.

Lee H, To RJB, Latta RK, Biely P, Schneider H. 1987. Some properties of extracellular acetylxylan esterase produced by the yeast *Rhodotorula mucilaginosa*. Appl Environ Microbiol. 53(12):2831-2834. doi:10.1128/aem.53.12.2831-2834.1987.

Lee SB, Banda C, Park HD. 2019. Effect of inoculation strategy of non-*Saccharomyces* yeasts on fermentation characteristics and volatile higher alcohols and esters in Campbell Early wines. Aust J Grape Wine Res. 25(4):384-395. doi:10.1111/ajgw.12405.

Legeron I. 2020. Natural wine: an introduction to organic and biodynamic wines made naturally. 3rd ed. London (UK): CICO Books.

Lehman ED, Nawrocki A. 2011. A History of Connecticut Wine: Vineyard in Your Backyard. Charleston (SC): The History Press.

Lennon JT, Muscarella ME, Pacella SA, Lehmkuhl BK. 2018. How, when, and where relic DNA affects microbial diversity. mBio. 9(3):e00637-18. doi:10.1128/mbio.00637-18.

Li AH, Yuan FX, Groenewald M, Bensch K, Yurkov AM, Han PJ, Guo LD, Aime MC, Sampaio JP, et al. 2020. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: proposal of two new orders, three new families, eight new genera and one hundred and seven new species. Stud Mycol. 96(1):17-140. doi:10.1016/j.simyco.2020.01.002.

Li E, Liu A, Xue B, Liu Y. 2011. Yeast species associated with spontaneous wine fermentation of Cabernet Sauvignon from Ningxia, China. World J Microbiol Biotechnol. 27(10):2475-2482. doi:10.1007/s11274-011-0711-9.

Li H, Tao YS, Wang H, Zhang L. 2008. Impact odorants of Chardonnay dry white wine from Changli County (China). Eur Food Res Technol. 227(1):287-292. doi:10.1007/s00217-007-0722-9.

Li J, Hu W, Huang X, Xu Y. 2018. Investigation of yeast population diversity and dynamics in spontaneous fermentation of Vidal blanc icewine by traditional culture-dependent and high-throughput sequencing methods. Food Res Int. 112:66-77. doi:10.1016/j.foodres.2018.06.011.

Li J, Hu WZ, Xu YP. 2019. Diversity and dynamics of yeasts during Vidal Blanc icewine fermentation: a strategy of the combination of culture-dependent and high-throughput sequencing approaches. Front Microbiol. 10:1588. doi:10.3389/fmicb.2019.01588.

Li R, Lin M, Guo S, Yang S, Han X, Ren M, Song Y, You Y, Zhan J et al. 2021. A fundamental landscape of fungal biogeographical patterns across the main Chinese wine-producing regions and the dominating shaping factors. Food Res Int. 150(A):110736. doi:10.1016/j.foodres.2021.110736.

Li R, Yang S, Lin M, Guo S, Han X, Ren M, Du L, Song Y, You Y, Zhan J et al. 2022. The biogeography of fungal communities across different Chinese wine-producing regions associated with environmental factors and spontaneous fermentation performance. Front Microbiol. 12:636639. doi:10.3389/fmicb.2021.636639.

Li SS, Cheng C, Li Z, Chen JY, Yan B, Han BZ, Reeves M. 2010. Yeast species associated with wine grapes in China. Int J Food Microbiol. 138(1):85-90. doi:10.1016/j.ijfoodmicro.2010.01.009.

Liang L, Ma Y, Jiang Z, Sam FE, Peng S, Li M, Wang J. 2023. Dynamic analysis of microbial communities and flavor properties in Merlot wines produced from inoculation and spontaneous fermentation. Food Res Int. 164:112379. doi:10.1016/j.foodres.2022.112379.

Lindegren CC, Lindegren G. 1943. Segregation, mutation, and copulation in *Saccharomcyes cerevisiae*. Ann Mo Bot Gard. 30(4):453-468. doi:10.2307/2394308.

Liu D, Chen Q, Zhang P, Chen D, Howell K. 2020. The fungal microbiome is an important component of vineyard ecosystems and correlates with regional distinctiveness of wine. mSphere. 5(4):e00534-20. doi:10.1128/msphere.00534-20.

Liu D, Howell K. 2021. Community succession of the grapevine fungal microbiome in the annual growth cycle. Environ Microbiol. 23(4):1842-1857. doi:10.1111/1462-2920.15172.

Liu D, Legras JL, Zhang P, Chen D, Howell K. 2021. Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine. Int J Food Microbiol. 338:108983. doi:10.1016/j.ijfoodmicro.2020.108983.

Liu D, Zhang P, Chen D, Howell K. 2019. From the vineyard to the winery: how microbial ecology drives regional distinctiveness of wine. Front Microbiol. 10:2679. doi:10.3389/fmicb.2019.02679.

Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M. 2012. Comparison of next-generation sequencing systems. BioMed Res Int. 2012:e251364. doi:10.1155/2012/251364.

Liu PT, Lu L, Duan CQ, Yan GL. 2016. The contribution of indigenous non-*Saccharomyces* wine yeast to improved aromatic quality of Cabernet Sauvignon wines by spontaneous fermentation. LWT – Food Sci Technol. 71:356-363. doi:10.1016/j.lwt.2016.04.031.

Liu XZ, Wang QM, Theelan B, Groenewald M, Bai FY, Boekhout T. 2015. Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses. Stud Mycol. 81(1):1-26. doi:10.1016/j.simyco.2015.08.001.

Longo E, Cansado J, Agrelo D, Villa TG. 1991. Effect of climatic conditions on yeast diversity in grape musts from Northwest Spain. Am J Enol Vitic. 42(2):141-144. doi:10.5344/ajev.1991.42.2.141.

Louw C, La Grange D, Pretorius IS, van Rensburg P. 2006. The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and wine flavour. J Biotechnol. 125(4):447-461. doi:10.1016/j.jbiotec.2006.03.029.

Lu Y, Sun F, Wang W, Liu Y, Wang J, Sun J, Mu J, Gao Z. 2020. Effects of spontaneous fermentation on the microorganisms diversity and volatile compounds during 'Marselan' from grape to wine. LWT. 134:110193. doi:10.1016/j.lwt.2020.110193.

Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, et al. 2020. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? IMA Fungus. 11(1):14. doi:10.1186/s43008-020-00033-z.

Lyons SMA. 2021. The diversity and function of indigenous yeasts in the Okanagan region of British Columbia [master's thesis]. Kelowna (BC): University of British Columbia.

Lyons SM, Morgan SC, McCann S, Sanderson S, Newman BL, Watson TL, Jiranek V, Durall DM, Zandberg WF. 2021. Unique volatile chemical profiles produced by indigenous and commercial strains of *Saccharomyces uvarum* and *Saccharomyces cerevisiae* during laboratory-scale Chardonnay fermentations. OENO One. 55(3):101-122. doi:10.20870/oeno-one.2021.55.3.4551.

Ma N, Wang X, Kong C, Tao Y. 2021a. Effect of mixed culture fermentation with *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* on the aroma and color of red wine. Food Sci. 42(2):97-104. doi:10.7506/spkx1002-6630-20191112-158.

Ma Y, Li T, Xu X, Ji Y, Jiang X, Shi X, Wang B. 2021b. Investigation of volatile compounds, microbial succession, and their relation during spontaneous fermentation of Petit Manseng. Front Microbiol. 12:717387. doi:10.3389/fmicb.2021.717387.

Ma Y, Peng S, Mi L, Li M, Jiang Z, Wang J. 2023. Correlation between fungi and volatile compounds during different fermentation modes at the industrial scale of Merlot wines. Food Res Int. 174:113638. doi:10.1016/j.foodres.2023.113638.

Madalinska K, McDougall R, Nielsen AL. 2022. Characterizing location of spotted lanternfly egg masses in wooded habitat during early invasion stages. Front Insect Sci. 2:964736. doi:10.3389/finsc.2022.964736.

Maicas S, Mateo J. 2015. Enzyme contribution of non-*Saccharomyces* yeasts to wine production. Univers J Microbiol Res. 3:17-25. doi:10.13189/ujmr.2015.030202.

Mandakovic D, Pulgar R, Maldonado J, Mardones W, González M, Cubillos FA, Cambiazo V. 2020. Fungal diversity analysis of grape musts from Central Valley-Chile and characterization of potential new starter cultures. Microorganisms. 8(6):956. doi:10.3390/microorganisms8060956.

Mann S, Ferjani A, Reissig L. 2012. What matters to consumers of organic wine? Brit Food J. 114(2):272-284. doi:10.1108/00070701211202430.

Mannazzu I, Domizio P, Carboni G, Zara S, Zara G, Comitini F, Budroni M, Ciani M. 2019. Yeast killer toxins: from ecological significance to application. Crit Rev Biotechnol. 39(5):603-617. doi:10.1080/07388551.2019.1601679.

Mansfield AK. [2015]. Yeast trials for improved wine quality in cold climate cultivars. Ithaca (NY): Cornell University. Northern Grapes Project: Viticulture, enology and marketing for cold-hardy grapes.

Marr RA, Moore J, Formby S, Martiniuk JT, Hamilton J, Ralli S, Konwar K, Rajasundaram N, Hahn A, Measday V. 2023. Whole genome sequencing of Canadian *Saccharomyces cerevisiae* strains isolated from spontaneous wine fermentations reveals a new Pacific

West Coast Wine clade. G3-GENES GENOM GENET. 13(8):jkad130. doi: doi:10.1093/g3journal/jkad130.
Marsit S, Dequin S. 2015. Diversity and adaptive evolution of *Saccharomyces* wine yeast: a review. FEMS Yeast Res. 15(7):fov067. doi:10.1093/femsyr/fov067.
Martínez C, Gertosio C, Labbe A, Pérez R, Ganga MA. 2006. Production of *Rhodotorula glutinis*: a yeast that secretes alpha-L-arabinofuranosidase. Electron J Biotechnol. 9(4).
Martiniuk JT. 2020. A survey of wine- and vineyard-associated yeast and fungal populations at a British Columbian winery [master's thesis]. Vancouver (BC): University of British Columbia.
Martiniuk JT, Hamilton J, Dodsworth T, Measday V. 2023. Grape-associated fungal community patterns persist from berry to wine on a fine geographical scale. FEMS Yeast Res. 23:foac067. doi:10.1093/femsyr/foac067.
Martiniuk JT, Pacheco B, Russell G, Tong S, Backstrom I, Measday V. 2016. Impact of commercial strain use on *Saccharomyces cerevisiae* population structure and dynamics in Pinot Noir vineyards and spontaneous fermentations of a Canadian winery. PLOS ONE. 11(8):e0160259. doi:10.1371/journal.pone.0160259.
Martins G, Vallance J, Mercier A, Albertin W, Stamatopoulos P, Rey P, Lonvaud A, Masneuf-Pomarède I. 2014. Influence of the farming system on the epiphytic yeasts and yeast-like fungi colonizing grape berries during the ripening process. Int J Food Microbiol. 177:21-28. doi:10.1016/j.ijfoodmicro.2014.02.002.
Martins V, Costa L, Soares R, Ayogu P, Teixeira A, Gerós H. 2022. A catalogue of cultivable yeasts from the microbiota of grape berries cv. Vinhão and Loureiro. OENO One. 56(3):257-270. doi:10.20870/oeno-one.2022.56.3.5462.
Martins V, Szakiel A, Pączkowski C, Teixeira A, Gerós H. 2021. The restructuring of grape berry waxes by calcium changes the surface microbiota. Food Res Int. 150:110812. doi:10.1016/j.foodres.2021.110812.
Mateo JJ, Garcerà P, Maicas S. 2020. Unusual non-*Saccharomyces* yeasts isolated from unripened grapes without antifungal treatments. Fermentation. 6(2):41. doi:10.3390/fermentation6020041.
Mateo JJ, Maicas S. 2016. Application of non-*Saccharomyces* yeasts to wine-making process. Fermentation. 2(3):14. doi:10.3390/fermentation2030014.
Maturano YP, Rodríguez Assaf LA, Toro ME, Nally MC, Vallejo M, Castellanos de Figueroa LI, Combina M, Vazquez F. 2012. Multi-enzyme production by pure and mixed cultures of *Saccharomyces* and non-*Saccharomyces* yeasts during wine fermentation. Int J Food Microbiol. 155(5):43-50. doi:10.1016/j.ijfoodmicro.2012.01.015.
Maygar I, Tóth T. 2011. Comparative evaluation of some oenological properties in wine strains of *Candida stellata*, *Candida zemplinina*, *Saccharomyces uvarum*, and *Saccharomyces cerevisiae*. Food Microbiol. 28(1):94-100. doi:10.1016/j.fm.2010.08.011.
McBee RH. 1940. A study of some yeasts occurring on Oregon grapes [master's thesis]. Corvallis (OR): Oregon State College.
McCarthy GC, Morgan SC, Martiniuk JT, Newman BL, McCann SE, Measday V, Durall DM. 2021. An indigenous *Saccharomyces uvarum* population with high genetic diversity

dominates uninoculated Chardonnay fermentations at a Canadian winery. PLOS ONE. 16(2):e0225615. doi:10.1371/journal.pone.0225615.
McCole D. 2022. Hybrid wine grapes and emerging wine tourism regions. In: Dixit SK, editor. Routledge Handbook of Wine Tourism. 1st ed. London (UK): Routledge; p. 603-613. doi:10.4324/9781993143628-54.
McGovern PE, Fleming SJ, Katz SH, editors. 2003. The origins and ancient history of wine. Vol. 11, Food and nutrition in history and anthropology. London (UK): Routledge.
McKnight DT, Huerlimann R, Bower DS, Schwarzkopf L, Alford RA, Zenger KR. 2019. Methods for normalizing microbiome data: an ecological perspective. Methods Ecol Evol. 10(3):389-400. doi:10.1111/2041-210X.13115.
McMurdie PJ, Holmes S. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLOS Comput Biol. 10(4):e1003531. doi:10.1371/journal.pcbi.1003531.
Medina K, Boido E, Fariña L, Gioia O, Gomaz ME, Barquet M, Gaggero C, Dellacassa E, Carrau F. 2013. Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. Food Chem. 141(3):2513-2521. doi:10.1016/j.foodchem.2013.04.056.
Mendes SDC, Ramírez-Castrillón M, Feldberg NP, Bertoldi FC, Valente P. 2017. Environmental yeast communities in vineyards in the mountains of Santa Catarina State, Brazil. World J Microbiol Biotechnol. 33(6):33-128. doi:10.1007/s11274-017-2298-2.
Mendes Ferreira A, Climaco MC, Mendes Faia A. 2001. The role of non-*Saccharomyces* species in releasing glycosidic bound fraction of grape aroma components – a preliminary study. J Appl Microbiol. 91(1):67-71. doi:10.1046/j.1365-2672.2001.01348.x.
Merín MG, Martín MC, Rantsiou K, Cocolin L, de Ambrosini VIM. 2015. Characterization of pectinase activity for enology from yeasts occurring in Argentine Bonarda grape. Braz J Microbiol. 46:815-823. doi:10.1590/S1517-838246320140160.
Merín MG, Mendoza LM, Morata de Ambrosini VI. 2014. Pectinolytic yeasts from viticultural and enological environments: novel finding of *Filobasidium capsuligenum* producing pectinases. J Basic Microbiol. 54(8):835-842. doi:10.1002/jobm.201200534.
Messiga AJ, Gallant KS, Sharifi M, Hammermeister A, Fuller K, Tango M, Fillmore S. 2016. Grape yield and quality response to cover crops and amendments in a vineyard in Nova Scotia, Canada. Am J Enol Vitic. 67(1):77-85. doi:10.5344/ajev.2015.15013.
Messiga Aj, Sharifi M, Hammermeister A, Fuller K, Tango M. 2015. Soil quality response to cover crops and amendments in a vineyard in Nova Scotia, Canada. Sci Hortic. 188:6-14. doi:10.1016/j.scienta.2015.02.041.
Mestre Furlani MV, Maturano YP, Combina M, Mercado LA, Toro ME, Vazquez F. 2017. Selection of non-*Saccharomyces* yeasts to be used in grape musts with high alcoholic potential: a strategy to obtain wines with reduced ethanol content. FEMS Yeast Res. 17(2):fox101. doi:10.1093/femsyr/fox010.
Mezzasalma V, Sandionigi A, Guzzetti L, Galimberti A, Grando MS, Tardaguila J, Labra M. 2018. Geographical and cultivar features differentiate grape microbiota in Northern Italy and Spain vineyards. Front Microbiol. 9:946. doi:10.3389/fmicb.2018.00946.

Migliore G, Thrassou A, Crescimanno M, Schifani G, Galati A. 2020. Factors affecting consumer preferences for “natural wine”: An exploratory study in the Italian market. Brit Food J. 122(8):2463-2479. doi:10.1108/BFJ-07-2019-0474.

Milanović V, Cardinali F, Ferrocino I, Boban A, Franciosa I, Gajdoš Kljusurić J, Mucalo A, Osimani A, Aquilanti L, Garofalo C, et al. 2022. Croatian white grape variety Maraština: first taste of its indigenous mycobiota. Food Res Int. 162:111917. doi:10.1016/j.foodres.2022.111917.

Milanović V, Comitini F, Ciani M. 2013. Grape berry yeast communities: influence of fungicide treatments. Int J Food Microbiol. 161(3):240-246. doi:10.1016/j.ijfoodmicro.2012.12.019.

Mills DA, Johannsen EA, Cocolin L. 2002. Yeast diversity and persistence in *Botrytis*-affected wine fermentations. Appl Environ Microbiol. 68(10):4884-4893. doi:10.1128/AEM.68.10.4884-4893.2002.

Miura T, Sánchez R, Casteñeda LE, Godoy K, Barbosa O. 2017. Is microbial terroir related to geographic distance between vineyards? Appl Microbiol Int. 9(6):742-749. doi:10.1111/1758-2229.12589.

Montoya MC, Magwene PM, Perfect JR. 2021. Associations between *Cryptococcus* genotypes, phenotypes, and clinical parameters of human disease: a review. J Fungi. 7(4):260. doi:10.3390/jof7040260.

Morata A, González C, Tesfaye W, Loira I, Suárez-Lepe JA. 2019. Maceration and fermentation: new technologies to increase extraction. In: Morata A, editor. Red Wine Technology. London (UK): Academic Press; p. 35-49.

Moreira N, Mendes F, Guedes de Pinho P, Hogg T, Vasconcelos I. 2008. Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. Int J Food Microbiol. 124(3):231-238. doi:10.1016/j.ijfoodmicro.2008.03.025.

Moreira N, Mendes F, Hogg T, Vasconcelos I. 2005. Alcohols, esters and heavy sulphur compounds production by pure and mixed cultures of apiculate wine yeasts. Int J Food Microbiol. 103(3):285-294. doi:10.1016/j.ijfoodmicro.2004.12.029.

Morgan SC, Haggert JJ, Jiranek V, Durall DM. 2020. Competition between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in controlled Chardonnay wine fermentations. Am J Enol Vitic. 71(3):198-207. doi:10.5344/ajev.2020.19072.

Morgan SC, McCarthy GC, Watters BS, Tantikachornkiat M, Zigg I, Cliff MA, Durall DM. 2019. Effect of sulfite addition and pied de cuve inoculation on the microbial communities and sensory profiles of Chardonnay wine: dominance of indigenous *Saccharomyces uvarum* at a commercial winery. FEMS Yeast Res. 19(5):foz049. doi:10.1093/femsyr/foz049.

Morris EO, Eddy AA. 1957. Method for the measurement of wild yeast infection in pitching yeast. J Inst Brew. 63(1):34-35. doi:10.1002/j.2050-0416.1957.tb02902.x.

Morrison-Whittle P, Goddard MR. 2018. From vineyard to winery: a source map of microbial diversity driving wine fermentation. Environ Microbiol. 20(1):75-84. doi:10.1111/1462-2920.13960.

Morrison-Whittle P, Lee SA, Goddard MR. 2017. Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. Agric Ecosyst Environ. 246:306-313. doi:10.1016/j.agee.2017.05.022.
Mortimer R, Polsinelli M. 1999. On the origins of wine yeast. Res Microbiol. 150(3):199-204. doi:10.1016/S0923-2508(99)80036-9.
Moscovici D, Gottlieb PD. 2017. Finding a state of sustainable wine: implications for sustainable viticulture and oenology in New Jersey, USA. Int J Sustain. 3(3):196-214. doi:10.1504/IJSAMI.2017.090295.
Moss R, Barker S, McSweeney MB. 2021. Using check-all-that-apply to evaluate wine and food pairings: an investigation with white wines. J Sens Stud. 37(1):e12720. doi:10.1111/joss.12720.
Muñoz-González C, Martín-Álvarez, Moreno-Arribas MV, Pozo-Bayón MÁ. 2014. Impact of the nonvolatile wine matrix composition on the *in vivo* aroma release from wines. J Agric Food Chem. 62(1):66-73. doi:10.1021/jf405550y.
Museum of Natural History Staff. 1996. Natural History of Nova Scotia. Vol. 1, Topics and Habitats. Halifax (NS): Nova Scotia Museum. Topic 5, The dynamics of Nova Scotia's climate; p. 94-103.
Muysson J, Miller L, Allie R, Inglis DL. 2019. The use of CRISPR-Cas9 genome editing to determine the importance of glycerol uptake in wine yeast during icewine fermentation. Fermentation. 5(4):93. doi:10.3390/fermentation5040093.
Nemcová K, Breierová E, Vadkertiová R, Molnárová J. 2015. The diversity of yeasts associated with grapes and musts of the Strekov winegrowing region, Slovakia. Folia Microbiol. 60(2):103-109. doi:10.1007/s12223-014-0347-x.
Neugebauer KA, Perkins JA, Sysak R, Isaacs R, Miles TD. 2024. Reducing cluster rots in Michigan wine grapes using combined pathogen and vinegar fly control. Crop Prot. 177:106528. doi:10.1016/j.cropro.2023.106528.
Neuner M. 2016. Yeast population and community dynamics: their effect on chemical and sensory profiles of inoculated and spontaneous Pinot Noir and Chardonnay fermentations at a Canadian winery [master's thesis]. Kelowna (BC): University of British Columbia.
Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson KH. 2008. Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol Bioinform. 4:193-201. doi:10.4137/EBO.S653.
Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson KH, Kõljalg U. 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLOS ONE. 1(1):e59. doi:10.1371/journal.pone.0000059.
Nisbet MA, Martinson TE, Mansfield AK. 2014. Accumulation and prediction of yeast assimilable nitrogen in New York winegrape cultivars. Am J Enol Vitic. 65(3):325-332. doi:10.5344/ajev.2014.13130.
Nisiotou AA, Nychas GJE. 2007. Yeast populations residing on healthy or *Botrytis*-infected grapes from a vineyard in Attica, Greece. Appl Environ Microbiol. 73(8):2765-2768. doi:10.1128/AEM.01864-06.

Nisiotou AA, Spiropoulos AE, Nychas GJE. 2007. Yeast community structures and dynamics in healthy and *Botrytis*-affected grape must fermentations. Appl Environ Microbio. 73(21):6705-6713. doi:10.1128/AEM.01279-07.

Nova Scotia. 2023. [accessed 2024 Feb 20]. https://www.novascotia.com/.

Nova Scotia L'Acadie Vineyards. 2024. Gaspereau (NS): L'Acadie Vineyards; [accessed 2024 Feb 16]. https://www.lacadievineyards.ca/.

Nova Scotia Liquor Corporation. 2021. Annual Report.

Nova Scotia Liquor Corporation. 2023. Annual Report.

Nurgel C, Erten H, Canbas A, Cabaroglu T, Selli S. 2005. Yeast flora during the fermentation of wines made from *Vitis vinifera* L. cv. Emir and Kalecik Karasi grown in Anatolia. World J Microbiol Biotechnol. 21:1187-1194. doi:10.1007/s11274-005-1106-6.

Nurgel C, Inglis DL, Pickering GJ, Reynolds AG, Brindle I. 2004. Dynamics of indigenous and inoculated yeast populations in Vidal and Riesling icewine fermentations. Am J Enol Vitic. 55(4):435A.

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Henry MHH, Szoecs E, et al. 2022. _vegan: Community Ecology Package_ [program]. Version 2.6-2. R package. https://CRAN.R-project.org/package=vegan.

O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 44(D1):D733-D745. doi:10.1093/nar/gkv1189.

Oluwa SW. 2020. Structure and foaming properties of viscous exopolysaccharides from a wild grape-associated basidiomycetous yeast *Papiliotrema flavescens* formerly known as *Cryptococcus flavescens*. J Microbiol Biotechnol. 30(11):1739-1749. doi:10.4014/jmb.2002.02065.

Op De Beeck M, Lievens B, Busschaert P, Declerck S, Vangronsveld J, Colpaert JV. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. PLOS ONE. 9(6):e97629. doi:10.1371/journal.pone.0097629.

Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G, Arvanitidis C, Iliopoulos I. 2015. Metagenomics: tools and insights for anlayzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insights. 9. doi:10.4137/BBI.S12462.

Oztekin S, Dikmetas DN, Devecioglu D, Acar EG, Karbancioglu-Guler F. 2023. Recent insights into the use of antagonistic yeasts for sustainable biomanagement of postharvest pathogenic and mycotoxigenic fungi in fruits with their prevention strategies against mycotoxins. J Agric Food Chem. 71(26):9923-9950. doi:10.1021/acs.jafc.3c00315.

PacBio. 2024. Sequencing Systems. [accessed 2024 Mar 7]. https://www.pacb.com/sequencing-systems/.

Padilla B, García-Fernández D, González B, Isidoro I, Esteve-Zarzoso B, Beltran G, Mas A. 2016. Yeast biodiversity from DOQ Priotat uninoculated fermentations. Front Microbiol. 7:930. doi:10.3389/fmicb.2016.00930.

Padilla B, Zulian L, Ferreres A, Pastor R, Esteve-Zarzoso B, Beltran G, Mas A. 2017. Sequential inoculation of native non-*Saccharomyces* and *Saccharomyces cerevisiae* strains for wine making. Front Microbiol. 8:1293. doi:10.3389/fmicb.2017.01293.

Pagliarini E, Laureati M, Gaeta D. 2013. Sensory descriptors, hedonic perception and consumer's attitudes to Sangiovese red wine deriving from organically and conventionally grown grapes. Front Psychol. 4:896. doi:10.3389/fpsyg.2013.00896.

Palmieri N, Perito MA, Pesce A. 2023. Young consumers' preferences for natural wine: an Italian exploratory study. J Int Food Agribusiness Mark. 74(1):477-495. doi:10.1080/08974438.2023.2284909.

Panagopoulos I. 2002. Identification and comparison of indigenous yeast on grapes located within the Lake Eerie appellation by sequencing PCR amplified rDNA [master's thesis]. Youngstown (OH): Youngstown State University.

Parish ME, Carroll DE. 1985. Indigenous yeasts associated with Muscadine (*Vitis rotundifolia*) grapes and musts. Am J Enol Vitic. 36(2):165-169. doi:10.5344/ajev.1985.36.2.165.

Pataro C, Guerra JB, Petrillo-Peixoto ML, Mendonça-Hagler LC, Linardi VR, Rosa CA. 2000. Yeast communities and genetic polymorphism of *Saccharomyces cerevisiae* strains associated with artisanal fermentation in Brazil. J Appl Microbiol. 89(1):24-31. doi:10.1046/j.1365-2672.2000.01092.x.

Perpetuini G, Rossetti AP, Battistelli N, Zulli C, Cichelli A, Arfelli G, Tofalo R. 2022. Impact of vineyard management on grape fungal community and Montepulciani d'Abruzzo wine quality. Food Res Int. 158:111577. doi:10.1016/j.foodres.2022.111577.

Petitgonnet C, Klein GL, Roullier-Gall C, Schmitt-Kopplin P, Quintanilla-Casas B, Vichi S, Julien-David D, Alexandre H. 2019. Influence of cell-cell contact between *L. thermotolerans* and *S. cerevisiae* on yeast interactions and the exo-metabolome. Food Microbiol. 83:122-133. doi:10.1016/j.fm.2019.05.005.

Piago K, O'Donnell R, Osborne J, Curtin C. 2021. Enrichment of *Brettanomyces* and other non-*Saccharomyces* fermentative yeasts from vineyard samples in Oregon. Am J Enol Vitic. 72(3): 257-266. doi:10.5344/ajev.2021.20071.

Pickering GJ, Spink M, Kotseridis Y, Inglis D, Brindle ID, Sears M, Beh AL. 2008. Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine. Aust J Grape Wine Res. 14(3):230-237. doi:10.1111/j.1755-0238.2008.00026.x.

Pinto C, Pinho D, Cardoso R, Custódio V, Fernandes J, Sousa S, Pinheiro M, Egas C, Gomes AC. 2015. Wine fermentation microbiome: a landscape from different Portuguese wine appellations. Front Microbiol. 6:905. doi:10.3389/fmicb.2015.00905.

Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC. 2014. Unravelling the diversity of grapevine microbiome. PLOS ONE. 9(1):e85622. doi:10.1371/journal.pone.0085622.

Poojari S, Moreau DL, Kahl D, Ritchie M, Ali S, Úrbez-Torres JR. 2020. Disease incidence and genetic variability of economically important grapevine viruses in Nova Scotia. Can J Plant Pathol. 42(4):584-594. doi:10.1080/07060661.2020.1730443.

Portillo MDC, Mas A. 2016. Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing. LWT – Food Sci Technol. 72:317-321. doi:10.1016/j.lwt.2016.05.009.

Povhe Jemec K, Cadez N, Zagorc T, Bubic V, Zupec A, Raspor P. 2001. Yeast population dynamics in five spontaneous fermentations of Malvasia must. Food Microbiol. 18(3):247-259. doi:10.1006/fmic.2001.0396.

Prakitchaiwattana CJ, Fleet GH, Heard GM. 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. FEMS Yeast Res. 4(8):865-877. doi:10.1016/j.femsyr.2004.05.004.

Provost C, Pedneault K. 2016. The organic vineyard as a balanced ecosystem: improved organic grape management and impacts on wine quality. Sci Hortic. 208:43-56. doi:10.1016/j.scienta.2016.04.024.

Qiagen. 2017. DNeasy UltraClean Microbial Kit [equipment]. https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-ultraclean-novipure-microbial-kits.

Quirós M, Rojas V, Gonzalez R, Morales P. 2014. Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. Int J Food Microbiol. 181:85-91. doi:10.1016/j.ijfoodmicro.2014.04.024.

R Core Team. 2022. R: A language and environment for statistical computing [software]. Vienna (Austria):R Foundation for Statistical Computing. https://R-project.org.

Rantsiou K, Giacosa S, Pugliese M, Englezos V, Ferrocino I, Río Segade S, Monchiero M, Gribaudo I, Gambino G, Gullino ML, et al. 2020. Impact of chemical and alternative fungicides applied to grapevine cv. Nebbiolo on microbial ecology and chemical-physical grape characteristics at harvest. Front Plant Sci. 11:700. doi:10.3389/fpls.2020.00700.

Raspor P, Cus F, Povhe Jemec K, Zagorc T, Cadez N, Nemanic J. 2002. Yeast population dynamics in spontaneous and inoculated alcoholic fermentations of Zametovka must. Food Technol Biotechnol. 40(2):95-102. https://hrcak.srce.hr/178425.

Raspor P, Milek DM, Polanc J, Smole Možina S, Čadež N. 2006. Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. Int J Food Microbiol. 109(1):97-102. doi:10.1016/j.ijfoodmicro.2006.01.017.

Regecová I, Marcinčák S, Nagy J, Popelka P, Semjon B, Jevinová P, Pipová M, Král M, Kovalčík M. 2019. Detection of microbiota in the vineyards of the Tokaj wine region. Slovak J Food Sci. 13(1):984-992. doi:10.5219/1246.

Rementeria A, Rodriguez JA, Cadaval A, Amenabar R, Muguruza JR, Hernando FL, Sevilla MJ. 2003. Yeast associated with spontaneous fermentations of white wines from the “Txakoli de Bizkaia” region (Basque Country, North Spain). Int J Food Microbiol. 86(1):201-207. doi:10.1016/S0168-1605(03)00289-7.

Renault P, Coulon J, de Revel G, Barbe JC, Bely M. 2015. Increase of fruity aroma during mixed *T. delbrueckii*/*S. cerevisiae* wine fermentation is linked to specific esters enhancement. Int J Food Microbiol. 207:40-48. doi:10.1016/j.ijfoodmicro.2015.04.037.

Renault P, Miot-Sertier C, Marullo P, Hernández-Orte P, Lagarrigue L, Lonvaud-Funel A, Bely M. 2009. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: potential applications in the wine industry. Int J Food Microbiol. 134(3):201-210. doi:10.1016/j.ijfoodmicro.2009.06.008.

Renner B, Sproesser G, Strochback S, Schupp HT. 2012. Why we eat what we eat. The Eating Motivation Survey (TEMS). Appetite. 59(1):117-128. doi:10.1016/j.appet.2012.04.004.

Renouf V, Claisse O, Lonvaud-Funel A. 2005. Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. Aust J Grape Wine Res. 11(3):316-327. doi:10.1111/j.1755-0238.2005.tb00031.x.

Renouf V, Claisse O, Lonvaud-Funel A. 2007a. Inventory and monitoring of wine microbial consortia. Appl Microbiol Biotechnol. 75(1):149-164. doi:10.1007/s00253-006-0798-3.

Renouf V, Perello MC, Strehaiano P, Lonvaud-Funel A. 2006. Global survey of the microbial ecosystem during alcoholic fermentation in winemaking. OENO One. 40(2):101-116. https://doi.org10.20870/oeno-one.2006.40.2.878.

Renouf V, Strehaiano P, Lonvaud-Funel A. 2007b. Yeast and bacteria analysis of grape, wine and cellar equipments by PCR-DGGE. OENO One. 41(1):51-61. doi:10.20870/oeno-one.2007.41.1.858.

Rigou P, Mekoue J, Sieczkowski N, Doco T, Vernhet A. 2021. Impact of industrial yeast derivative products on the modification of wine aroma compounds and sensorial profile. A review. Food Chem. 358:129760. doi:10.1016/j.foodchem.2021.129760.

Río Segade S, Giacosa S, Gerbi V, Rolle L. 2019. Grape maturity and selection: automatic grape selection. In: Morata A, editor. Red Wine Technology. London (UK): Academic Press; p. 1-16.

Rodríguez ME, Lopes CA, Barbagelata RJ, Barda NB, Caballero AC. 2010. Influence of *Candida pulcherrima* Patagonian strain on alcoholic fermentation behaviour and wine aroma. Int J Food Microbiol. 138(1):19-25. doi:10.1016/j.ijfoodmicro.2009.12.025.

Rojas V, Gil JV, Piñaga F, Manzanares P. 2001. Studies on acetate ester production by non-*Saccharomyces* wine yeasts. Int J Food Microbiol. 70(3):283-289. doi:10.1016/S0168-1605(01)00552-9.

Rolle L, Río Segade S, Paissoni MA Giacosa S, Gerbi V. 2022. Assessment and control of grape maturity and quality. In: Morata A, editor. White Wine Technology. London (UK): Academic Press; p. 1-16.

Rollero S, Bloem A, Ortiz-Julien A, Camarasa C, Divol B. 2018. Altered fermentation performances, growth, and metabolic footprints reveal competition for nutrients between yeast species inoculated in synthetic grape juice-like medium. Front Microbiol. 9:196. doi:10.3389/fmicb.2018.00196.

Romano P, Caruso M, Capece A, Lipani G, Paraggio M, Fiore C. 2003. Metabolic diversity of *Saccharomyces cerevisiae* strains from spontaneously fermented grape musts. World J Microbiol Biotechnol. 19:311-315. doi:10.1023/A:1023666510737.

Romano P, Suzzi G, Comi G, Zironi R. 1992. Higher alcohol and acetic acid production by apiculate wine yeasts. J Appl Bacteriol. 73(2):126-130. doi:10.1111/j.1365-2672.1992.tb01698.x.

Rosini G, Federici F, Martini A. 1982. Yeast flora of grape berries during ripening. Microb Ecol. 8(1):83-89. doi:10.1007/BF02011464.

Rossetti AP, Perpetuini G, Battistelli N, Zulli C, Arfelli G, Suzzi G, Cichelli A, Tofalo R. 2023. Capturing the fungal community associated with conventional and organic Trebbiano Abruzzese grapes and its influence on wine characteristics. Food Biosci. 52:102382. doi:10.1016/j.fbio.2023.102382.

Rossi R. 2020. “Farm to Fork” strategy: striving for healthy and sustainable food (PE 651.971). European Parliamentary Research Service. https://www.europarl.europa.eu/RegData/etudes/ATAG/2020/651971/EPRS_ATA(2020)651971_EN.pdf.

Rossouw D, Bauer FF. 2016. Exploring the phenotypic space of non-*Saccharomyces* wine yeast biodiversity. Food Microbiol. 55:32-46. doi:10.1016/j.fm.2015.11.017.

Rozin P, Spranca M, Krieger Z, Neuhaus R, Surillo D, Swerdlin A, Wood K. 2004. Preference for natural: instrumental and ideational/moral motivations, and the contrast between foods and medicines. Appetite. 43(2):147-154. doi:10.1016/j.appet.2004.03.005.

Ruiz J, Kiene F, Belda I, Fracassetti D, Marquina D, Navascués E, Calderón F, Benito A, Rauhut D, Santos A, et al. 2019. Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine. Appl Microbiol Biotechnol. 103(18):7415-7450. doi:10.1007/s00253-019-10008-9.

Sabate J, Cano J, Esteve-Zarzoso B, Guillamón JM. 2002. Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. Microbiol Res. 157(4):267-274. doi:10.1078/0944-5013-00163.

Sadoudi M, Tourdot-Maréc R, Rousseaux S, Steyer D, Gallardo-Chacón JJ, Ballester J, Vichi S, Guérin-Schneider R, Caixach J, Alexandre H. 2012. Yeast–yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. Food Microbiol. 32(2):243-253. doi:10.1016/j.fm.2012.06.006.

Saha DC, Xess I, Biswas A, Bhowmik DM, Padma MV. 2009. Detection of *Cryptococcus* by conventional, serological and molecular methods. J Med Microbiol. 58(8):1098-1105. doi:10.1099/jmm.0.007328-0.

Salvadó Z, Arroyo-López FN, Guillamón JM, Salazar G, Querol A, Barrio E. 2011. Temperature adaptation markedly determines evolution within the genus *Saccharomyces*. Appl Environ Microbiol. 77(7):2292-2302. doi:10.1128/AEM.01861-10.

Scacco A, Oliva D, Di Maio S, Polizzotto G, Genna G, Tripodi G, Lanza CM, Verzera A. 2012. Indigenous *Saccharomyces cerevisiae* strains and their influence on the quality of Cataratto, Inzolia and Grillo white wines. Food Res Int. 46(1):1-9. doi:10.1016/j.foodres.2011.10.038.

Schäfele I, Hamm U. 2018. Organic wine purchase behaviour in Germany: exploring the attitude-behaviour-gap with data from a household panel. Food Qual Prefer. 63:1-11. doi:10.1016/j.foodqual.2017.07.010.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Nat Acad Sci. 109(16):6241-6246. doi:10.1073/pnas.1117018109.

Scholl CM, Morgan SC, Stone ML, Tantikachornkiat M, Neuner M, Durall DM. 2016. Composition of *Saccharomyces cerevisiae* strains in spontaneous fermentations of Pinot Noir and Chardonnay. Aust J Grape Wine Res. 22(3):384-390. doi:10.1111/ajgw.12221.

Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A. 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Res. 2(4):495-517. doi:10.1111/j.1567-1364.2002.tb00117.x.

Seguinot P, Ortiz-Julien A, Camarasa C. 2020. Impact of nutrient availability on the fermentation and production of aroma compounds under sequential inoculation with *M. pulcherrima* and *S. cerevisiae*. Front Microbiol. 11:305. doi:10.3389/fmicb.2020.00305.

Serpaggi V, Remize F, Recorbet G, Gaudot-Dumas E, Sequeira-Le Grand A, Hervé A. 2012. Characterization of the "viable but nonculturable" (VBNC) state in the wine spoilage yeast *Brettanomyces*. Food Microbiol. 30(2):438-447. doi:10.1016/j.fm.2011.12.020.

Setati ME, Jacobson D, Andong UC, Bauer F. 2012. The vineyard yeast microbiome, a mixed model microbial map. PLOS ONE 7(12):e52609. doi:10.1371/journal.pone.0052609.

Setati ME, Jacobson D, Bauer FF. 2015. Sequence-based analysis of the Vitis vinifera L. cv Cabernet Sauvignon grape must mycobiome in three South African vineyards employing distinct agronomic systems. Front Microbiol. 6:1358. doi:10.3389/fmicb.2015.01358.

Shaw AB. 1999. The emerging cool climate wine regions of eastern Canada. J Wine Res. 10(2):79-94. doi:10.1080/09571269908718164.

Shendure J, Ji H. 2008. Next-generation DNA sequencing. Nat Biotechnol. 26:1135-1145. doi:10.1038/nbt1486.

Shinohara T, Kubodera S, Yanagida F. 2000. Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. J Biosci Bioeng. 90(1):90-97. doi:10.1016/S1389-1723(00)80040-7.

Silva Ferreira AC, Rodrigues P, Hogg T, Guedes de Pinho P. 2003. Influence of some technological parameters on the formation of dimethyl sulfide, 2-mercaptoethanol, methionol, and dimethyl sulfone in port wines. J Agric Food Chem. 51(3):727-732. doi:10.1021/jf025934g.

Singer GaC, Fahner NA, Barnes JG, McCarthy A, Hajibabaei M. 2019. Comprehensive biodiversity analysis via ultra-deep patterned flow cell technology: a case study of eDNA metabarcoding seawater. Sci Rep. 9(1):5991. doi:10.1038/s41598-019-42455-9.

Slegers A, Angers P, Ouellet É, Truchon T, Pedneault K. 2015. Volatile compounds from grape skin, juice and wine from five interspecific hybrid grape cultivars grown in Québec (Canada) for wine production. Molecules. 20(6):10980-11016. doi:10.3390/molecules200610980.

Spencer MW, Shek KL, Meyer K, Weisz J, Jones G, McGuire KL. 2022. Teasing apart terroir: the influence of management style on native yeast communities within Oregon wineries and vineyards. IVES Conference Series; Terclim; Jun 3-8; Bordeaux (France).

Slatko BE, Gardner AF, Ausubel FM. 2018. Overview of next generation sequencing technologies. Curr Protoc Mol Biol. 122(1):e59. doi:10.1002/cpmb.59.

Standards Council of Canada. 2021. Organic production systems: general principles and management standards. Ottawa (ON): Canadian General Standards Board. National Standard of Canada; CAN/CGSB-32.310-2020, Corrigendum No.1, March 2021.

Steenwerth KL, Morelan I, Stahel R, Figueroa-Balderas R, Cantu D, Lee J, Runnebaum RC, Poret-Peterson AT. 2021. Fungal and bacterial communities of 'Pinot noir' must: effects

of vintage, growing region, climate, and basic must chemistry. PeerJ. 9:e10836. doi:10.7717/peerj.10836.
Stefanini I, Albanese D, Cavazza A, Franciosi E, De Filippo C, Donati C, Cavalieri D. 2016. Dynamic changes in microbiota and mycobiota during spontaneous 'Vino Santo Trentino' fermentation. Microb Biotechnol. 9(2):195-208. doi:10.1111/1751-7915.12337.
Stefanini I, Cavalieri D. 2018. Metagenomic approaches to investigate the contribution of the vineyard environment to the quality of wine fermentation: potentials and difficulties. Front Microbiol. 9:991. doi:10.3389/fmicb.2018.00991.
Sterflinger K. 2006. Black yeasts and meristematic fungi: ecology, diversity and identification. In: Rosa C, Péter G, editors. Biodiversity and Ecophysiology of Yeasts. Heidelberg: Springer-Verlag; p. 501-514.
Sternes PR, Lee D, Kutyna DR, Borneman AR. 2017. A combined meta-barcoding and shotgun metagenomic analysis of spontaneous wine fermentation. GigaScience. 6(7):gix040. doi:10.1093/gigascience/gix040.
Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D, et al. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Pers: Mol Phylogeny Evol Fungi. 35(1):242-263. doi:10.3767/003158515X689135.
Strauss MLA, Jolly NP, Lambrechts MG, van Rensburg P. 2001. Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. J Appl Microbiol. 91(1):182-190. doi:10.1046/j.1365-2672.2001.01379.x.
Sturm J, Grossmann M, Schnell S. 2006. Influence of grape treatment on the wine yeast populations isolated from spontaneous fermentations. J Appl Microbiol. 101(6):1241-1248. doi:10.1111/j.1365-2672.2006.03034.x.
Subden RE, Husnik JI, van Twest R, van der Merwe G, van Vuuren HJJ. 2003. Autochthonous microbial population in a Niagara Peninsula icewine must. Food Res Int. 36(7):747-751. doi:10.1016/S0963-9969(03)00034-6.
Sumby KM, Caliani NS, Jiranek V. 2021. Yeast diversity in the vineyard: how it is defined, measured and influenced by fungicides. Aust J Grape Wine Res. 27(2):169-193. doi:10.1111/ajgw.12479.
Sun D, Qu J, Huang Y, Lu J, Yin L. 2021. Analysis of microbial community diversity of muscadine grape skins. Food Res Int. 145:110417. doi:10.1016/j.foodres.2021.110417.
Sun H, Loeb G, Walter-Peterson H, Martinson T, Scott JG. 2019. Insecticide resistance in *Drosophila melanogaster* (Diptera: Drosophilidae) is associated with field control failure of sour rot disease in a New York vineyard. J Econ Entomol. 112(3):1498-1501. doi:10.1093/jee/toz039.
Šuranská H, Vránová D, Omelková J, Vadkertiová R. 2012. Monitoring of yeast population isolated during spontaneous fermentation of Moravian wine. Chem Zvesti. 66(9):861-868. doi:10.2478/s11696-012-0198-3.
Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust J Grape Wine Res. 11(2):139-173. doi:10.1111/j.1755-0238.2005.tb00285.x.

Swofford DL. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods) [software]. Ver. 4. Sunderland (MA): Sinauer Associates.
Tait P, Saunders C, Dalziel P, Rutherford P, Criver T, Guenther M. 2019. Estimating wine consumer preferences for sustainability attributes: a discrete choice experiment of Californian Sauvignon blanc purchasers. J Clean Prod. 233:412-420. doi:10.1016/j.jclepro.2019.06.076.
Takashima M, Sugita T. 2022. Taxonomy of pathogenic yeasts *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon*. Med Mycol. 63(4):119-132. doi:10.3314/mmj.22.004.
Takush DG, Osborne JP. 2012. Impact of yeast on the aroma and flavour of Oregon Pinot Noir wine. Aust J Grape Wine Res. 18(2):131-137. doi:10.1111/j.1755-0238.2012.00181.x.
Tantikachornkiat M, Morgan SC, Lepitre M, Cliff MA, Durall DM. 2020. Yeast and bacterial inoculation practices influence the microbial communities of barrel-fermented Chardonnay wines. Aust J Grape Wine Res. 26(3):279-289. doi:10.1111/ajgw.12438.
Taylor MW, Tsai P, Anfang N, Ross HA, Goddard MR. 2014. Pyrosequencing reveals regional differences in fruit-associated fungal communities. Environ Microbiol. 16(9):2848-2858. doi:10.1111/1462-2920.12456.
Tello J, Cordero-Bueso G, Aporta I, Cabellos JM, Arroyo T. 2012. Genetic diversity in commercial wineries: effects of the farming system and vinification management on wine yeasts: yeast diversity in commercial wineries. J Appl Microbiol. 112(2):302-315. doi:10.1111/j.1365-2672.2011.05202.x.
Tempère S, Marchal A, Barbe JC, Bely M, Masneuf-Pomarède I, Marullo P, Albertin W. 2018. The complexity of wine: clarifying the role of microorganisms. Appl Microbiol Biotechnol. 102(9):3995-4007. doi:10.1007/s00253-018-8914-8.
Theelen B, Cafarchia C, Gaitanis G, Bassukas ID, Boekhout T, Dawson TL Jr, 2018. *Malassezia* ecology, pathophysiology, and treatment. Med Mycol. 56(suppl_1):S10-S25. doi:10.1093/mmy/myx134.
Thomas D, Vandegrift R, Bailes G, Roy B. 2017. Understanding and mitigating some limitations of Illumina© MiSeq for environmental sequencing of Fungi. bioRxiv. 184960. doi:10.1101/184960.
Thomas L. 2002. The identification and characterization of indigenous yeast in a Chardonnay fermentation of an Ohio winery [master's thesis]. Youngstown (OH): Youngstown State University.
Toju H, Tanabe AS, Yamamoto S, Sato H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLOS ONE. 7(7):e40863. doi:10.1371/journal.pone.0040863.
Töpfer R, Trapp O. 2022. A cool climate perspective on grapevine breeding: climate change and sustainability are driving forces for changing varieties in a traditional market. Theor Appl Genet. 135:3947-3960. doi:10.1007/ s00122-022-04077-0.
Torija MJ, Rozès N, Poblet M, Guillamón JM, Mas Albert. 2001. Yeast population dynamics in spontaneous fermentations: comparison between two different wine-producing areas over a period of three years. Antonie van Leeuwenhoek. 79(3):345-352. doi:10.1023/A:1012027718701.

Tosi E, Azzolini M, Guzzo F, Zapparoli G. 2009. Evidence of different fermentation behaviours of two indigenous strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* isolated from Amarone wine. J Appl Microbiol. 107(1):210-218. doi:10.1111/j.1365-2672.2009.04196.x

Tronchoni J, Setati ME, Fracassetti D, Valdetara F, Maghradze D, Foschino R, Curiel JA, Morales P, Gonzalez R, Vigentini I, et al. 2022. Identifying the main drivers in microbial diversity for Cabernet Sauvignon cultivars from Europe to South Africa: evidence for a cultivar-specific microbial fingerprint. J Fungi. 8(10):1034. doi:10.3390/jof8101034.

Turenne CY, Sanche SE, Hoban DJ, Karlowsky JA, Kabani AM. 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J Clin Microbiol. 37(6):1846-1851. doi:10.1128/jcm.37.6.1846-1851.1999.

Ultee A, Wacker A, Kunz D, Löwenstein R, König H. 2013. Microbial succession in spontaneously fermented grape must before, during and after stuck fermentation. S Afr J Enol Vitic. 34(1):68-78. doi:10.21548/34-1-1082.

Urso R, Rantsiou K, Dolci P, Rolle L, Comi G, Cocolin L. 2008. Yeast biodiversity and dynamics during sweet wine production as determined by molecular methods. FEMS Yeast Res. 8(7):1053-1062. doi:10.1111/j.1567-1364.2008.00364.x.

Usyk M, Zolnik CP, Patel H, Levi MH, Burk RD. 2017. Novel ITS1 fungal primers for characterization of the mycobiome. mSphere. 2(6):e00488-17. doi:10.1128/msphere.00488-17.

Valentini B, Barbero F, Casacci LP, Luganini A, Stefanani I. 2022. Forest influence yeast populations vectored by insects into vineyards. Front Microbiol. 13:1039939. doi:10.3389/fmicb.2022.1039939.

Valenzuela L, Ortega R, Moscovici D, Gow J, Alonso Ugaglia A, Mihailescu R. 2022. Consumer willingness to pay for sustainable wine – The Chilean case. Sustainability. 14(17):10910. doi:10.3390/su141710910.

Valero E, Schuller D, Cambon B, Casal M, Dequin S. 2005. Dissemination and survival of commercial wine yeast in the vineyard: a large-scale, three-years study. FEMS Yeast Res. 5(10):959-969. doi:10.1016/j.femsyr.2005.04.007.

Van Leeuwen C, Seguin G. 2006. The concept of terroir in viticulture. J Wine Res. 17(1):1-10. doi:10.1080/09571260600633135.

Varela C, Borneman AR. 2017. Yeasts found in vineyards and wineries. Yeast. 34(3):111-128. doi:10.1002/yea.3219.

Varela C, Sengler F, Solomon M, Curtin C. 2016. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chem. 209:57-64. doi:10.1016/j.foodchem.2016.04.024.

Varela C, Siebert T, Cozzolino D, Rose L, Mclean H, Henschke PA. 2009. Discovering a chemical basis for differentiating wines made by fermentation with ‘wild’ indigenous and inoculated yeasts: role of yeast volatile compounds. Aust J Grape Wine Res. 15(3):238-248. doi:10.1111/j.1755-0238.2009.00054.x.

Vasseur L, Catto N. 2008. Atlantic Canada. In: Lemmen DS, Warren FJ, Lacroix J, Bush E, editors. From Impacts to Adaptation: Canada in a Changing Climate 2007. Ottawa (ON): Government of Canada; p. 119-170.

Vaudano E, Quinterno G, Costantini A, Pulcini L, Pessione E, Garcia-Moruno E. 2019. Yeast distribution in Grignolino grapes growing in a new vineyard in Piedmont and the technological characterization of indigenous *Saccharomyces* spp. strains. Int J Food Microbiol. 289:154-161. doi:10.1016/j.ijfoodmicro.2018.09.016.

Vaudour E. 2002. The quality of grapes and wine in relation to geography: notions of terroir at various scales. J Wine Res. 13(2):117-141. doi:10.1080/0957126022000017981.

Vecchio R, Annunziata A, Parga Dans E, Alonso González P. 2023. Drivers of consumer willingness to pay for sustainable wines: natural, biodynamic, and organic. Org Agric. 13(2):247-260. doi:10.1007/s13165-023-00425-6.

Verginer M, Leitner E, Berg G. 2010. Production of volatile metabolites by grape-associated microorganisms. J Agric Food Chem. 58(14):8344-8350. doi:10.1021/jf100393w.

Vernhet A. 2019. Red wine clarification and stabilization. In: Morata A, editor. Red Wine Technology. London (UK): Academic Press; p. 237-251.

Větrovský T, Baldrian P, Morais D. 2018. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinform. 34(13):2292-2294. doi:10.1093/femsyr/foaa013.

Viana F, Gil J, Genoves S, Valles S, Manzanares P. 2008. Rational selection of non-*Saccharomyces* wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiol. 25(6):778-785. doi:10.1016/j.fm.2008.04.015.

Viana F, Gil J, Vallés S, Manzanares P. 2009. 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 Microbio. 135(1):68-74. doi:10.1016/j.ijfoodmicro.2009.07.025.

Vigenti I, De Lorenzis G, Fabrizio V, Valdetara F, Faccincani M, Panot CA, Picozzi C, Imazio S, Failla O, Foschino R. 2015. The vintage effect overcomes the terroir effect: a three year survey on the wine yeast biodiversity in Franciacorta and Oltrepò Pavese, two northern Italian vine-growing areas. Microbiology. 161(2):362-373. doi:10.1099/mic.0.000004.

Vilela A. 2020. Modulating wine pleasantness throughout wine-yeast co-inoculation or sequential inoculation. Fermentation. 6(1):22. doi:10.3390/fermentation6010022.

Villanueva EC, Moscovici D. 2017. Sustainable wine tourism development in burgeoning regions: lessons from New Jersey and Connecticut. Int J Econ Bus Res. 12(4):313-333. doi:10.1504/IJEBR.2016.081226.

Vu D, Groenewald M, Szöke S, Cardinali G, Eberhardt U, Stielow B, de Vries M, Verkleij GJM, Crous PW, Boekhout, et al. 2016. DNA barcoding analysis of more than 9 000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. Stud Mycol. 85:91-105. doi:10.1016/j.simyco.2016.11.007.

Wang C, García-Fernández D, Mas A, Esteve-Zarzoso B. 2015a. Fungal diversity in grape must and wine fermentation assessed by massive sequencing, quantitative PCR and DGGE. Front Microbiol. 6:1156. doi:10.3389/fmicb.2015.01156.

Wang HL, Hopfer H, Cockburn DW, Wee J. 2021a. Characterization of microbial dynamics and volatile metabolome changes during fermentation of Chambourcin hybrid grapes from two Pennsylvania regions. Front Microbiol. 11:614278. doi:10.3389/fmicb.2020.614278.

Wang K, Chen J, Martiniuk J, Ma X, Li Q, Measday V. Lu X. 2023a. Species identification and strain discrimination of fermentation yeasts *Saccharomyces cerevisiae* and *Saccharomyces uvarum* using Raman spectroscopy and convolutional neural networks. Appl Environ Microbiol. 89(12):e01673-23. doi:10.1128/aem.01673-23.

Wang QM, Yurkov AM, Göker M, Lumbsch HT, Leavitt SD, Groenewald M, Theelen B, Liu XZ, Boekhout T, Bai FY. 2015b. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. Stud Mycol. 81(1):149-189. doi:10.1016/j.simyco.2015.12.002.

Wang X, Fan G, Peng Y, Xu N, Xie Y, Zhou H, Liang H, Zhan J, Huang W, You Y. 2023b. Mechanisms and effects of non-*Saccharomyces* yeast fermentation on the aromatic profile of wine. J Food Compos Anal. 124:105660. doi:10.1016/j.jfca.2023.105660.

Wang X, Glawe DA, Kramer E, Weller D, Okubara PA. 2018. Biological control of *Botrytis cinerea*: interactions with native vineyard yeasts from Washington State. Phytopathology. 108(6):691-701. doi:10.1094/PHYTO-09-17-0306-R.

Wang X, Glawe DA, Weller D, Okubara PA. 2020. Real-time PCR assays for the quantification of native yeast DNA in grape berry and fermentation extracts. J Microbiol Methods. 168:105794. doi:10.1016/j.mimet.2019.105794.

Wang X, Schlatter DC, Glawe DA, Edwards CG, Weller DM, Paulitz TC, Abatzoglou JT, Okubara PA. 2021b. Native yeast and non-yeast fungal communities of Cabernet Sauvignon berries from two Washington State vineyards, and persistence in spontaneous fermentation. Int J Food Microbiol. 350:109225. doi:10.1016/j.ijfoodmicro.2021.109225.

Wang XC, Li AH, Dizy M, Ullah N, Sun WX, Tao YS. 2017. Evaluation of aroma enhancement for “Ecolly” dry white wines by mixed inoculation of selected *Rhodotorula mucilaginosa* and Saccharomyces cerevisiae. Food Chem. 228:550-559. doi:10.1016/j.foodchem.2017.01.113.

Wang XC, Liu C, Huang L, Bengtsson-Palme J, Chen H, Zhang JH, Cai D, Li JQ. 2015c. ITS1: a DNA barcode better than ITS2 in eukaryotes? Mol Ecol Resour. 15(3):573-586. doi:10.1111/1755-0998.12325.

Wei RT, Chen N, Ding YT, Wang L, Liu YH, Gao FF, Zhang L, Li H, Wang H. 2022. Correlations between microbiota with physicochemical properties and volatile compounds during the spontaneous fermentation of Cabernet Sauvignon (*Vitis vinifera* L.) wine. LWT. 163:113529. doi:10.1016/j.lwt.2022.113529.

Wei YJ, Wu Y, Yan YZ, Zou W, Xue J, Ma WR, Wang W, Tian G, Wang LY. 2018. High-throughput sequencing of microbial community diversity in soil, grapes, leaves, grape juice and wine of grapevine from China. PLOS ONE. 13(3):e0193097. doi:10.1371/journal.pone.0193097.

Weiss S, Xu ZZ, Peddada S, Amir A, Bitttinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, et al. 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5(1):27. doi:10.1186/s40168-017-0237-y.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TF, editors. PCR Protocols: A Guide to Methods and Applications. New York (NY): Academic Press Inc.; p. 315-322.

Whitener MEB, Stanstrup J, Carlin S, Divol B, Du Toit M, Vrhovsek U. 2017. Effect of non-*Saccharomyces* yeasts on the volatile chemical profile of Shiraz wine. Aust J Grape Wine Res. 23(2):179-192. doi:10.1111/ajgw.12269.

Whitmore BA. McCann SE, Noestheden M, Dennia EG, Lyons SM, Durall DM, Zandberg WF. 2021. Glycosidically-bound volatile phenols linked to smoke taint: stability during fermentation with different yeasts and in finished wine. Molecules. 26(15):4519. doi:10.3390/molecules26154519.

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. New York (NY): Springer-Verlag.

Windholtz S, Dutilh L, Lucas M, Maupeu J, Vallet-Courbin A, Farris L, Coulon J, Masneuf-Pomarède I. 2021a. Population dynamics and yeast diversity in early winemaking stages without sulfites revealed by three complementary approaches. Appl Sci. 11:2494.

Windholtz S, Vinsonneau E, Farris L, Thibon C, Masneuf-Pomarède I. 2021b. Yeast and filamentous fungi microbial communities in organic red grape juice: effect of vintage, maturity stage, SO_2, and bioprotection. Front Microbiol. 12:748416. doi:10.3389/fmicb.2021.748416.

Wines of Nova Scotia. 2024. [accessed 2024 Feb 20]. https://winesofnovascotia.ca/.

Wright AH, Ali S, Migicovsky Z, Douglas GM, Yurgel S, Bunbury-Blanchette A, Franklin J, Adams S, Walker AK. 2022. A characterization of a cool-climate organic vineyard's microbiome. Phytobiomes J. 6(1):69-82. doi:10.1094/PBIOMES-03-21-0019-R.

Wu G, Zhao H, Li C, Rajapakse MP, Wong WC, Xu J, Saunders CW, Reeder NL, Reilman RA, Scheynius A, et al. 2015. Genus-wide comparative genomics of *Malassezia* delineates its phylogeny, physiology, and niche adaptation on human skin. PLOS Gen. 11(11):e1005614. doi:10.1371/journal.pgen.1005614

Wu X, Cai W, Zhu P, Peng Z, Zheng T, Li D, Li J, Zhou G, Zhou J, Du G. 2023. Function-driven design of *Bacillus kochii* and *Filobasidium magnum* co-culture to improve quality of flue-cured tobacco. Front Microbiol. 13:1024005. doi:10.3389/fmicb.2022.1024005.

Xu J. 2016. Fungal DNA barcoding. Genome. 59:913-932. doi:10.1139/gen-2016-0046.

Xu W, Liu B, Wang C, Kong X. 2020. Organic cultivation of grape affects yeast succession and wine sensory quality during spontaneous fermentation. LWT. 120:108894. doi:10.1016/j.lwt.2019.108894.

Yan H, Ge C, Zhou J, Li J. 2022. Diversity of soil fungi in the vineyards of Changli region in China. Can J Microbiol. 68(5):341-352. doi:10.1139/cjm-2021-0337.

Yanagida F, Ichinose F, Shinohara T, Goto S. 1992. Distribution of wild yeasts in the white grape varieties at central Japan. J Gen Appl Microbiol. 38(5):501-504. doi:10.2323/jgam.38.501.

Yang F, Heit C, Inglis DL. 2017. Cytosolic redox status of wine yeast (*Saccharomyces cerevisiae)* under hyperosmotic stress during icewine fermentation. Fermentation. 3(4):61. doi:10.3390/fermentation3040061.

Yang RH, Su JH, Shang JJ, Wu YY, Li Y, Bao DP, Yao YJ, Cullen D. 2018. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PLOS ONE. 13(10):e0206428. doi:10.1371/journal.pone.0206428.

Yang S, Choi SJ, Kwak J, Kim K, Seo M, Moon T, Lee YW. 2013. *Aspergillus oryzae* strains from traditional Korean Nuruk: fermentation properties and influence on rice wine quality. Food Sci Biotechnol. 22:425-432. doi:10.1007/s10068-013-0097-6.

Yurkov AM, Golubev WI. 2013. Phylogenetic study of *Cryptococcus laurentii* mycocinogenic strains. Mycol Prog. 4(12):777-782. doi:10.1007/s11557-012-0873-3.

Zhang S, Chen X, Zhong Q, Zhuang X, Bai Z. 2019. Microbial community analyses associated with nine varieties of wine grape carposphere based on high-throughput sequencing. Microorganisms. 7(12):668. doi:10.3390/microorganisms7120668.

Zhu L, Li T, Xu X, Shi X, Wang B. 2021. Succession of fungal communities at different developmental stages of Cabernet Sauvignon grapes from an organic vineyard in Xinjiang. Front Microbiol. 12:718261. doi:10.3389/fmicb.2021.718261.

Zilelidou EA, Nisiotou A. 2021. Understanding wine through yeast interactions. Microorganisms. 9(8):1620. doi:10.3390/microorganisms9081620.

Zohre DE, Erten H. 2002. The influence of *Kloeckera apiculata* and *Candida pulcherrima* yeasts on wine fermentation. Process Biochem. 38(3):319-324. doi:10.1016/S0032-9592(02)00086-9.

Zott K, Miot-Sertier C, Claisse O, Lonvaud-Funel A, Masneuf-Pomarede I. 2008. Dynamics and diversity of non-*Saccharomyces* yeasts during the early stages in winemaking. Int J Food Microbiol. 125(2):197-203. doi:10.1016/j.ijfoodmicro.2008.04.001.

Appendix

Table A1. Current yeast taxonomy according to www.indexfungorum.org and www.mycobank.org of species names updated in this thesis. Additional literature was checked on a case-by-case basis as needed. Alternate taxonomy is provided when the current name was indefinite at the time of writing.

Current taxonomy	Invalid taxonomy	Alternate taxonomy
Anthracocystis penniseti	*Sporisorium penniseti*	
Brettanomyces bruxellensis		*Dekkera bruxellensis*
Buckleyzyma aurantiaca	*Rhodotorula aurantiaca*	
Bullera alba	*Bulleromyces alb(a)/(idus)*	
Curvibasidium cygneicollum		*Apiotrichum futronense*
		Rhodotorula fujisanensis
		Rhodotorula futronensis
Curvibasidium nothofagi	*Rhodotorula nothofagi*	*Apiotrichum nothofagi*
Cystobasidium laryngis	*Rhodotorula laryngis*	
Cystobasidium minutum	*Rhodotorula minuta*	
Cystobasidium oligophagum	*Rhodotorula oligophaga*	
Cystobasidium pallidum	*Rhodotorula pallida*	
Cystobasidium sloofiae	*Rhodotorula slooffiae*	
Cystofilobasidium macerans	*Cryptococcus macerans*	
Dioszegia hungarica	*Cryptococcus hungaricus*	
Filobasidium magnum	*Cryptococcus ater*	
	Cryptococcus magnus	
Filobasidium oeirense	*Cryptococcus oeriensis*	
Filobasidium stepposum	*Cryptococcus stepposum*	
Filobasidium uniguttulatum		*Cryptococcus uniguttulatus*
Filobasidium wieringae	*Cryptococcys wieringae*	
Globoramichloridium indicum	*Ramichloridium indicum*	
Hannaella luteola	*Cryptococcus luteola*	
Hanaella zeae	*Cryptococcus zeae*	
Hanseniaspora uvarum	*Kloeckera apiculata*	*Cryptococcus vini*
Holtermanniella festucosa		*Cryptococcus festucosus*
Krasilnikovozyma huempii	*Cryptococcus huempii*	

Kurtzmaniella quercitrusa	*Candida quercitrusa*	
Kwoniella dendrophila	*Bullera dendrophila*	
Lachancea thermotolerans	*Kluyveromyces thermotolerans*	
Metschnikowia pulcherrima	*Candida pulcherrima*	
Microstroma bacarum	*Rhodotorula bacarum*	
Mrakia aquatica	*Cryptococcus aquaticus*	*Mrakiella aquatica*
Naganishia adeliensis	*Cryptococcus adeliensis*	
Naganishia albida	*Cryptococcus albidus*	
Naganishia albidosimilis	*Cryptococcus albidosimilis*	
Naganishia bhutanensis	*Cryptococcus bhutanensis*	
Naganishia diffluens	*Cryptococcus diffluens*	
Naganishia globosa	*Cryptococcus saitoi*	
Naganishia randhawae	*Cryptococcus randhaw(ae)/(ai)/(ii)*	
Naganishia uzbekistanensis	*Cryptococcus uzbekistanensis*	
Papiliotrema aurea	*Cryptococcus aureus*	
Papiliotrema flavescens	*Cryptococcus flavescens*	
Papiliotrema fusca	*Auriculibuller fuscus*	
	Papiliotrema fuscus	
Papiliotrema laurentii	*Cryptococcus laurentii*	
Papiliotrema nemorosa	*Cryptococcus nemorosus*	
	Papiliotrema nemorosus	
Papiliotrema pseudoalba	*Bullera pseudoalba*	
Papiliotrema terrestris	*Cryptococcus terrestris*	
Pichia kudriavzevii	*Candida krusei*	
	Issatachenkia orientalis	
Pichia terricola	*Issatchenkia terricola*	
Piskurozyma capsuligena	*Filobasidium capsuligenum*	
Pseudomicrostroma phylloplanum	*Rhodotorula phylloplana*	
Rhodotorula babjevae	*Rhodosporidium babjevae*	
Rhodotorula diobovata	*Rhodosporidium diobovatum*	
Rhodotorula kratochvilovae	*Rhodosporidium kratochvilovae*	
Rhodotorula mucilaginosa	*Rhodotorula rubra*	
Rhodotorula toruloides	*Rhodosporidium toruloides*	

	Rhodotorula gracilis	Taxonomic confusion with *R. glutinis*
Saitozyma flava	*Cryptococcus flavus*	
Solicoccozyma terrea	*Cryptococcus terre(a)/(us)*	
	Solicoccozyma terreus	
Sporobolomyces japonicus	*Sporobolomyces pararoseus*	
Sporobolomyces roseus	*Sporidiobolus metaroseus*	
Sporobolomyces salmonicolor	*Sporidiobolus salmonicolor*	
Sporobolomyces shibatanus	*Sporidiobolus pararoseus*	
Starmerella apicola	*Candida apicola*	
Starmerella bacillaris	*Candida zemplinina*	
Starmerella stellata	*Candida stellata*	
Symmetrospora coprosmae	*Bullera coprosmae*	*Hannaella coprosmae()/(nsis)*
Symmetrospora oryzicola	*Sporobolomyces oryzicola*	
Symmetrospora symmetrica	*Sporobolomyces symmetric(a)/(us)*	
Torulaspora delbrueckii		*Debaryomyces delbrueckii*
Ustilago bullata	*Ustilago bromivora*	
Ustilago maydis	*Pseudozyma prolifica*	
Ustilentyloma graminis	*Rhodotorula hordea*	
Vanrija humicola	*Cryptococcus humicol(a)/(us)*	
Vishniacozyma carnescens	*Cryptococcus carnescens*	
Vishniacozyma dimennae	*Cryptococcus dimennae*	
Vishniacozyma foliicola	*Cryptococcus foliicola*	
Vishniacozyma globispora	*Vishniacozyma globospora*	
Vishniacozyma heimaeyensis	*Cryptococcus heimaeyensis*	
Vishniacozyma taibaiensis	*Cryptococcus taibaiensis*	
Vishniacozyma tephrensis	*Cryptococcus tephrensis*	
Vishniacozyma victoriae	*Cryptococcus victoriae*	
Wickerhamomyces anomalus	*Hansenula anomala*	
	Pichia anomala	
Zygosaccharomyces florentina	*Zygosaccharomyces florentinus*	

Table A2. List of yeast genera (legitimate and invalid taxonomy) used to filter yeasts from filamentous fungi. Upon evaluation of abundant genera in the data analysed for Chapter 2 and cited in other works, relevant genera of Ustilagomycotina with yeast or yeast-like forms were also considered yeasts (*Anthracocystis*, *Entyloma*, *Exobasidium*, *Golubevia*, and *Sporisorium*) in Chapters 2 & 4.

Saccharomycetales		**Other ascomycete**	**Basidiomycete**	
Aciculoconidium	*Macrorhabdus*	*Aureobasidium*	*Acaromyces*	*Malassezia*
Alloascoidea	*Magnusiomyces*	*Burenia*	*Agaricostilbum*	*Mastigobasidium*
Allodekkera	*Martiniozyma*	*Capronia*	*Apiotrichum*	*Meira*
Ambrosiozyma	*Menezesia*	*Cephaloascus*	*Aurantiosporium*	*Meniscomyces*
Aphidomyces	*Metahyphopichia*	*Cladophialophora*	*Auriculibuller*	*Meredithblackwellia*
Arthroascus	*Metschnikowia*	*Coniosporium*	*Ballistosporomyces*	*Microbotryozyma*
Arxiozyma	*Metschnikowiella*	*Exophiala*	*Bandonia*	*Microbotryum*
Arxula	*Meyerozyma*	*Fonsecea*	*Bannoa*	*Microsporomyces*
Ascobotryozyma	*Microanthomyces*	*Globoramichloridium*	*Bannozyma*	*Microstroma*
Ascocephalophora	*Middelhovenomyces*	*Hortaea*	*Bauerago*	*Mixia*
Ascocybe	*Millerozyma*	*Lalaria*	*Begerowomomyces*	*Moesziomyces*
Ascoidea	*Monospora*	*Neolecta*	*Bensingtonia*	*Moniliella*
Ashbya	*Monosporella*	*Phaeoanellomyces*	*Boekhoutia*	*Mrakia*
Asporomyces	*Myceloblastanon*	*Phaeococcomyces*	*Buckleyzyma*	*Mrakiella*
Aureomyces	*Mycelorrhizodes*	*Phialophora*	*Bullera*	*Mycogloea*
Azymocandida	*Mycocandida*	*Pneumocystis*	*Bulleribasidium*	*Naematelia*
Azymohansenula	*Mycokluyveria*	*Protomyces*	*Bulleromyces*	*Naganishia*
Azymomyces	*Mycotorula*	*Protomycopsis*	*Camptobasidium*	*Naohidea*
Azymoprocandida	*Mycotoruloides*	*Ramichloridium*	*Carcinomyces*	*Nielozyma*
Babjevia	*Myriogonium*	*Rhinocladiella*	*Carlosrosaea*	*Oberwinklerozyma*
Babjeviella	*Myxozyma*	*Saitoella*	*Chionosphaera*	*Occultifur*
Bacillopsis	*Nadsonia*	*Sarcinomyces*	*Chrysozyma*	*Papiliotrema*
Barnettozyma	*Nakaseomyces*	*Schizosaccharomyces*	*Colacogloea*	*Pascua*
Basidioascus	*Nakazawaea*	*Taphridium*	*Cryptococcus*	*Phaeotremella*
Berkhoutia	*Naumovia*	*Taphrina*	*Cryptotrichosporon*	*Phaffia*
Blastobotrys	*Naumovozyma*	*Volkartia*	*Cuniculitrema*	*Phenoliferia*
Blastodendrion	*Nectaromyces*		*Curvibasidium*	*Phyllozyma*
Blastoschizomyces	*Nematodospora*		*Cutaneotrichosporon*	*Piskurozyma*
Botryoascus	*Nematospora*		*Cyrenella*	*Prillingera*

Botryozyma
Brettanomyces
Byrrha
Candida
Carpozyma
Castellania
Cephaloascus
Chlamydozyma
Cicadomyces
Citeromyces
Clavispora
Coccidiascus
Crebrothecium
Cyberlindnera
Cyniclomyces
Debaryolipomyces
Debaryomyces
Debaryozyma
Dekkera
Dekkeromyces
Diddensiella
Dipodascopsis
Dipodascus
Diutina
Dolichoascus
Eeniella
Enantiothamnus
Endoblastoderma
Endoblastomyces
Endomyces
Endomycodes
Endomycopsella
Endomycopsis
Octomyces
Ogataea
Oleina
Oleinis
Oosporoidea
Pachysolen
Pachytichospora
Parasaccharomyces
Paratorulopsis
Parendomyces
Petasospora
Peterozyma
Phaffomyces
Phialoascus
Pichia
Polymorphomyces
Priceomyces
Procandida
Prosaccharomyces
Pseudohansenula
Pseudomonilia
Pseudomycoderma
Psyllidomyces
Saccharomyces
Saccharomycodes
Saccharomycopsis
Sachsia
Saeenkia
Saprochaete
Saturnispora
Savitreea
Scheffersomyces
Schizoblastosporion
Cystobasidiopsis
Cystobasidium
Cystofilobasidium
Derxomyces
Dimennazyma
Dioszegia
Effuseotrichosporon
Erythrobasidium
Farysizyma
Fellomyces
Fellozyma
Fibulobasidium
Filobasidiella
Filobasidium
Fonsecazyma
Fulvisporium
Gelidatrema
Genolevuria
Glaciozyma
Goffeauzyma
Guehomyces
Haglerozyma
Halobasidium
Hamamotoa
Hannaella
Heterocephalacria
Holtermannia
Holtermanniella
Itersonilia
Jianyunia
Kalmanozyma
Kockovaella
Kondoa
Pseudobensingtonia
Pseudohyphozyma
Pseudomicrostroma
Pseudosterigmatospora
Pseudotremella
Pseudozyma
Quambalaria
Reniforma
Rhodosporidiobolus
Rhodosporidium
Rhodotorula
Rhynchogastrema
Robertozyma
Rosettozyma
Ruinenia
Rustroemia
Saitozyma
Sakaguchia
Sampaiozyma
Septobasidium
Sirobasidium
Slooffia
Solicoccozyma
Sphacelotheca
Spiculogloea
Sporidiobolus
Sporobolomyces
Sterigmatomyces
Sterigmatospora
Sterigmatosporium
Stilbum
Sugitazyma
Symmetrospora

Endyllium
Entelexis
Ephebella
Eremothecium
Eutorula
Eutorulopsis
Fabospora
Fermentotrichon
Fragosia
Galactomyces
Geotrichum
Grigorovia
Groenewaldozyma
Guilliermondella
Hagleromyces
Hansenia
Hanseniaspora
Hansenula
Helicogonium
Hemisphaericaspora
Holleya
Hormoascus
Hyphopichia
Isomyces
Issatchenkia
Kawasakia
Kazachstania
Kloeckera
Kloeckeraspora
Kluyveromyces
Kockiozyma
Kodamaea
Komagataea
Schwanniomyces
Smithozyma
Spathaspora
Spencermartinsiella
Spermophthora
Sporopachydermia
Starmera
Starmerella
Stephanoascus
Sugiyamaella
Suhomyces
Sympodiomyces
Syringospora
Tetrapisispora
Teunomyces
Thailandia
Thelis
Tortispora
Torulaspora
Torulopsis
Trichomonascus
Trigonopsis
Vanderwaltia
Vanderwaltozyma
Waltiozyma
Waltomyces
Wickerhamia
Wickerhamiella
Wickerhamomyces
Willia
Williopsis
Wingea
Yamadazyma
Krasilnikovozyma
Kriegeria
Kurtzmanomyces
Kwoniella
Leucosporidiella
Leucosporidium
Libkindia
Lichenozyma
Liroa
Sympodiomycopsis
Syzygospora
Takashimella
Tausonia
Teunia
Tilletiaria
Tilletiopsis
Tremella
Trichosporon
Trichosporonoides
Trigonosporomyces
Trimorphomyces
Tsuchiyaea
Udeniomyces
Urendiniomyces
Ustilago
Ustilentyloma
Vanrija
Vishniacozyma
Vonarxula
Vustinia
Xanthophyllomyces
Xenogloea
Yamadamyces
Yunzhangia
Yurkovia
Zundeliomyces

Komagataella	*Yarrowia*		
Kregervanrija	*Yueomyces*		
Kuraishia	*Zendera*		
Kurtzmaniella	*Zonosporis*		
Lachancea	*Zygoascus*		
Limtongella	*Zygofabospora*		
Limtongia	*Zygohansenula*		
Limtongozyma	*Zygolipomyces*		
Lindnera	*Zygopichia*		
Lipomyces	*Zygorenospora*		
Lodderomyces	*Zygosaccharis*		
	Zygosaccharomyces		
	Zygosaccharomycodes		
	Zygotorulaspora		
	Zygowillia		
	Zygowilliopsis		
	Zygozyma		
	Zymodebaryomyces		
	Zymopichia		

Table A3. List of yeast classes, orders, and families (legitimate and invalid taxonomy) used to filter yeasts from filamentous fungi.

Ascomycete		**Basidiomycete**	
Classes	**Families**	**Classes**	**Families**
Neolectomycetes	Alloascoideaceae	Agaricostilbomycetes	Agaricostilbaceae
Pneumocystidomycetes	Ascoideaceae	Cystobasidiomycetes	Brachybasidiaceae
Saccharomycetes	Cephaloascaceae	Exobasidiomycetes	Buckleyzymaceae
Schizosaccharomycetes	Debaryomycetaceae	Microbotryomycetes	Bulleraceae
Taphrinomycetes	Dipodascaceae	Mixiomycetes	Bulleribasidiaceae
	Endomycetaceae	Spiculogloeomycetes	Camptobasidiaceae
Orders	Eremotheciaceae	Tremellomycetes	Chionosphaeraceae
Neolectales	Lipomycetaceae	Ustilaginomycetes	Chrysozymaceae
Pneumocystidales	Metshcnikowiaceae		Colacogloeaceae

Saccharomycetales	Neolectaceae	**Orders**	Cryptobasidiaceae
Schizosaccharomycetales	Phaffomycetaceae	Agaricostilbales	Cryptococcaceae
Taphrinales	Pichiaceae	Cystobasidiales	Cuniculitremaceae
	Pneumocystidaceae	Cystofilobasidiales	Cystobasidiaceae
	Protomycetaceae	Doassansiales	Cystofilobasidiaceae
	Saccharomycetaceae	Entylomatales	Entylomataceae
	Saccharomycodaceae	Erythrobasidiales	Erythrobasidiaceae
	Saccharomycopsidaceae	Exobasidiales	Filobasidiaceae
	Schizosaccharomycetaceae	Filobasidiales	Holtermanniaceae
	Taphrinaceae	Georgefisherales	Jianyuniaceae
	Trichomonacsaceae	Heitmaniales	Kondoaceae
	Trigonopsidaceae	Leucosporidiales	Kriegeriaceae
	Wickerhamomycetaceae	Malasseziales	Leucosporidiaceae
	Wickerhamomyceteae	Microbotryales	Malasseziaceae
		Microstromatales	Microbotryaceae
		Mixiales	Microsporomycetaceae
		Naohideales	Microstromataceae
		Rosettozymales	Mixiaceae
		Spiculogloeales	Moniliellaceae
		Sporidiobolales	Mrakiaceae
		Tremellales	Naemateliaceae
		Trichosporonales	Naohideaceae
		Ustilaginales	Phaeotremellaceae
			Piskurozymaceae
			Quambalariaceae
			Rhynchogastremaceae
			Rosettozymaceae
			Ruineniaceae
			Rutstroemiaceae
			Sakaguchiaceae
			Septobasidiaceae
			Sirobasidiaceae
			Spiculogloeaceae

	Sporidiobolaceae Symmetrosporaceae Tilletiariaceae Tremellaceae Trichosporonaceae Trimorphomycetaceae Ustilaginaceae Ustilentylomataceae