A PRACTICAL WINEMAKER'S GUIDE TO ISOLATING WILD YEAST SPECIES TO PRODUCE UNIQUE IN-HOUSE STRAINS FOR WINE FERMENTATIONS

A Project Paper

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by

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ABSTRACT

This informational guide provides instructional assistance for boutique and do-it-yourself winemakers who would like to explore wild yeast strain options used for the alcoholic fermentation of grape must/juice to wine. Yeasts are important for fermentation since they affect many sensory characteristics of wine, such as aroma, taste (flavor), color, clarity, mouthfeel, heat perception (alcohol) and fermentation kinetics. This paper details a plan that may be used by any winemaker to isolate wild yeast strains for building a starter culture, in order to ferment grape must/juice into wine. Producing unique in-house yeast strains creates an interesting story that may be used for marketing purposes in addition to wine augmentation. Different yeasts influence wine fermentation and microbial ecology in their own unique ways, providing interesting aroma and flavor characteristics throughout fermentation. There are thousands of yeast strains, many of which have not been discovered yet, containing characteristics unknown.

BIOGRAPHICAL SKETCH

Jacob Tannenbaum is a member of the Gibney lab and a Master's student in Food Science with a concentration in Viticulture and Enology at Cornell University. He received his Bachelor of Business from Farleigh Dickenson University in 2015. After discovering his passion for the art and science of winemaking, he returned to school to pursue his Post-Bachelors Studies in biology and chemistry in order to apply to Cornell's Enology program. Jacob then took a position as an assistant winemaker in Northern Israel, where he held that position for 3 consecutive harvest seasons, working under the guidance and direction of the owners before deciding to pursue his Master's education. His responsibilities included vineyard management and irrigation, vintner and cellar master. There, he received a thorough education of the wine making process from vine to bottle. He also had the opportunity to aid in the growing of other fruits such as: peaches, nectarines, plums, pears, pomegranates and apples, thus broadening his horizon of the agriculture industry - not only relating to red and white grape varietals. Formerly a technician in his family's HVAC company, Jacobs experience with mechanics has shown to be useful when operating, maintaining and repairing winery equipment. After his graduation in August 2019, Jacob will pursue a career in the wine industry with an ambition to build his own wine brand.

DEDICATION

This project is dedicated to all past, present and future Viticulturists, Enologists and Winemakers. Hard work, commitment and devotion is a righteous tool in this tribal industry of art, science and technology. I wish you all great success.

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CHAPTER 1

INTRODUCTION

Goal of This Project

The purpose of this project is to make the yeast isolation techniques presented in this paper accessible to anyone who is interested in exploring wild yeast strains and their impact on the flavor and aroma profile of a wine by fermentation. The goal is to create a practical step by step guide on how to properly isolate wild yeast strains from a vineyard location. Isolating wild yeasts is an exercise within the grasp of any winemaker.

History of Yeast in Fermentation

Since ancient Mesopotamian times, yeast have been used to ferment wine (Bisson 2012). Yeasts are single-celled eukaryotes and are members of the fungus kingdom. There are approximately 1,500 identified yeast species, compromising 1% of all fungi (Kurtzman *et al* 2006). We live in a microbial world; thus, yeasts are found all around us. Wine, beer, cider, sake, vodka, whiskey and other fermented beverages, require yeast to produce alcohol and other flavors.

In 1860, Louis Pasteur observed that yeast cells were required for fermentation of sugar into alcohol. Pasteur and other scientists at the time believed that living cells contained a vital force catalyzing fermentation (Pasteur 1860). In 1897, Eduard Buchner ground up yeast cells releasing their internal contents and discovered that this extract could perpetuate fermentation without any living organism present. This discovery opened the door to understand the chemical nature of fermentation. Buchner was awarded the Nobel Prize in 1907 for his findings (Buchner 1897).

Role of Yeast in Fermentation

One role that yeasts have during fermentation is to convert carbohydrates, such as grape sugars like glucose and fructose to carbon dioxide and ethanol. Fermentation kinetics are dependent upon many variables, including temperature, yeast assimilable nitrogen composition levels (YAN), vitamin levels, mineral levels, other nutrients and microbial interactions within the grape must/juice matrix (Celorrio *et al* 2016, Bisson 1999). When released from the must/juice into the air by yeast fermentation, carbon dioxide creates a protective layer of gas above the grape must/juice, thus inhibiting oxygen exposure and safeguarding the fermentation from oxidation and microbial spoilage.

It is crucial to understand that not only is yeast required to convert sugars into carbon dioxide and ethanol, additionally many other metabolites that contribute to a wine's final sensory flavor and aroma profile are released throughout fermentation. Yeast fermentation influences the sensory profile of wine by contributing to the flavor/aroma complexity and quality of the finished product (Bisson 1999, Bisson *et al* 2010).

Yeasts are important because they affect many sensory characteristics of wine, such as aroma, taste (flavor), color, clarity, heat perception (alcohol) and mouthfeel. Many compounds are converted and released by yeast metabolism throughout the fermentation of grape must/juice to wine. These compounds contribute to a wines final sensory flavor and aroma profile.

Growth of Yeast

Yeast generally grow asexually by budding. A small daughter cell will bud outward from the mother cell. When the mother cell has completely duplicated its DNA, its nucleus will divide. Then, the separated nucleus containing its corresponding DNA will be transferred into the daughter cell. Once the daughter cell reaches its appropriate size, it will split off from the mother cell

becoming an individual cell. This process of DNA replication and cell division is called the cell cycle. It is important to note that yeast cells can also reproduce sexually, when two cells of opposite mating types conjugate in a process called shmooing (Mell *et al* 2002). Budding yeast cells prefer to grow by fermentation and will continue to reproduce asexually so long as sufficient amounts of nutrients such as sugar, nitrogen and phosphate are available (Waterhouse *et al* 2016).

Yeast have three growth phases during fermentation: lag, exponential growth, and stationary. Lag phase is the period when the yeast population in a must/juice starts to build biomass. At this time there is minimal conversion of sugars into ethanol and effective carbon dioxide levels are not easily measurable. Exponential growth phase is the period when yeast populations are highly active, readily converting must/juice sugars to ethanol and carbon dioxide. This growth phase continues until the maximum yeast population is attained. Stationary phase is the final stage of alcoholic fermentation when yeast cell division decreases, and sugars have been relatively depleted. The majority of fermentation occurs after cell division stops. At this point, the wine is considered fermented to dryness as fermentation slows and eventually stops. It is important to note that these fermentation parameters are highly dependent on many variables during the fermentation process, including temperature and available nutrient resources, which are crucial for a healthy and non-sluggish fermentation. After the stationary phase, carbon dioxide production ceases and sugars have been converted to their final ethanol concentration. Ethanol is a product of fermentation containing flavor properties that influence the sensory profile of wine. Ethanol in wine can be perceived as having a viscous texture, sweetish aromas, sweet tasting, pungent (heat) and bitter (Bisson *et al* 2010).

Commercialized Yeast Strains vs. Wild Yeast Strains

The yeasts found in grape must/juice, are categorized as *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* yeasts. Currently, there are greater than 300 commercialized yeast strains available on the market that have been isolated for decades from wineries in different geographical regions all over the world (Richter *et al* 2013). *Saccharomyces cerevisiae* yeasts are typically used in commercial wine strains, due to their reliable strong fermentation kinetics, however there are some commercialized non-*Saccharomyces cerevisiae* yeast strains available on the market. Wild yeast strains are ubiquitous in nature and are found on wine grapes. They are transferred from the vineyard to the winery crush pad and are found in the must/juice. There is an increasing interest to use wild yeast strains in the wine industry. Some winemakers advocate that wild yeast strains have a significant impact on the aroma and flavor development of wine. These winemakers are experimenting with wild yeast fermentations, in hopes to produce wine that has unique aroma and flavor complexity when compared to other wines launched by a commercialized yeast strain.

Non-Inoculated vs. Inoculated

Vineyards foster a complex microbiome including fungi and bacteria, containing a unique set of physiological and metabolic characteristics. The natural microflora present in a vineyard location and on the grapes processed into must/juice, changes from vintage to vintage, due to the dependency of several variables, such as environmental weather conditions, geographic location, grape variety and chemical spray applications (Pinto *et al* 2015). Types and amounts of microflora found in the vineyard will change from season to season depending on weather conditions. Harsh climatic states will change the natural microflora in any environment. Microbial terroir can be defined as the microflora present in a certain vineyard location at a given time, which then evolves

during the fermentation and winemaking process. Different winegrowing regions will maintain different microbial communities; thus, these site-specific fungi and bacteria may influence and contribute to the flavor development of a wine.

Non-inoculated fermentations are initiated by the various yeast genera ubiquitous in nature and on grapes, such as *Candida, Debaryomyces, Dekkera, Hanseniaspora, Metschnikowia, Pichia, Torulaspora, Lachancea, Aureobasidium* and *Zygosaccharomyces* (Manzanares *et al* 2011). There are several common terms used in winemaking literature when discussing non-inoculated fermentations. Wild, native, indigenous, autochthonous, natural and spontaneous are common words used to describe non-inoculated fermentations.

Inoculated fermentations are typically characterized by the intentional addition of microbes to the juice/must; launched by a commercialized yeast strain starter culture most often containing *Saccharomyces cerevisiae*. It is interesting to note that many non-inoculated fermentations are completed by commercial *Saccharomyces cerevisiae* yeast already present in the winery as environmental contaminants from previous fermentations; even if the winemaker believes that wild/natural yeasts are performing the fermentation.

Consumer Appeal

Consumer enthusiasm has increased for winemakers to experiment with non-inoculated fermentations, thus utilizing the wild yeasts found on wine grapes (Manzanares *et al* 2011). Furthermore, there has been a growing interest from winemakers to experiment with non-*Saccharomyces* yeasts, due to their interesting contributions of flavor and aroma produced by their metabolism, that may differ from a typical inoculated fermentation (Medina *et al* 2018).

Winemakers who produce non-inoculated wine, allow the existing yeasts found on the fruit to begin fermenting independently, without additions of sulfites (SO_2) that might inhibit wild yeast

growth. Winemakers who produce wines made by this process suggest that non-inoculated fermentations allow the wild yeast strains to develop unique flavor complexity, thus enhancing the aromatics and overall flavor profile of the finished wine product. Commonly, non-inoculated fermentations will have several wild yeast strains present in the grape must/juice (Manzanares et *al* 2011). These wild strain combinations start to ferment, and develop a distinctive taste profile, in contrast to wines that are fermented by a dominant commercialized yeast strain - typically *Saccharomyces cerevisiae*. Wild yeasts may have positive or negative effects, or a combination of both. It is important to monitor non-inoculated fermentations closely to make sure that the flavors and aromas are developing without flaws (e.g. unpleasant smells or taste). In addition, one must continuously analyze the fermentation kinetics to ensure that the wine is protected by carbon dioxide or another inert gas during the fermentation process. Some non-*Saccharomyces cerevisiae* yeasts have slow fermentation kinetics, which may lead to a stuck fermentation; this allows for the possibility of microbial spoilage organisms to develop in the grape must/juice.

Saccharomyces cerevisiae are found at a very low frequency on grapes in the wild but are routinely present in wineries. Once a non-inoculated fermentation begins, wild *Saccharomyces cerevisiae* found in the natural grape must/juice will typically dominate the fermentation; due to its high ethanol tolerance and ability to rapidly consume glucose/fructose, thus growing its cell numbers. In most cases, non-*Saccharomyces cerevisiae* yeast strains will have a hard time completing the fermentation to dryness, due to their low ethanol tolerance.

The Complex Formation of a Wine's Aroma Profile can be Derived from Three Classification Categories

The formation of a wine's aroma profile is complex and depends on many volatile compounds present in the wine. Odor-active compounds in wine can be derived from three aroma

classification categories: Primary, Secondary or Tertiary. Primary aromas, such as methoxypyrazines (vegetal/bell pepper), rotundone (black pepper) and linalool or geraniol (floral), are compounds derived from specific grape varieties that can remain intact throughout the fermentation process and are found in the final sensory profile of a wine. Secondary aromas are compounds formed from alcoholic or malolactic fermentation by the means of yeast or bacteria metabolism, respectively. Tertiary aromas are derived from oak extractable compounds during barrel fermentation or aging, such as cis/trans oak lactones or whiskey lactones, that impart desirable sensory characteristics, such as caramel/sweet, coconut and vanilla notes. Microbial spoilage is also considered a tertiary aroma that might cause unpleasant odors and flavors in wine, for example, wine faults like mouse taint (ACTPY; 2-acetyl-3,4,5,6-tetrahydropyridine) or cork taint (TCA; 2,4,6-trichloroanisole), are considered negative tertiary aromas (Waterhouse *et al* 2016). Another negative tertiary aroma is acetic acid (vinegar). It is formed when acetic acid bacteria (*Acetobacter, Gluconobacter, etc.*) found in wine converts ethanol to acetic acid (vinegar) when in the presence of oxygen. Another negative tertiary aroma is high concentrations of ethyl acetate (perceived as smells of nail polish remover), which is the ester of ethanol and acetic acid (vinegar). Ethyl acetate and acetic acid (vinegar) contribute to the accumulation of VA (volatile acidity) in wine.

How Yeast Fermentation Contributes to the Flavors and Aromatics of Wine

There are many flavor and aroma compounds found in wine derived from different reactions within the chemistry of grape must/juice during yeast fermentation. Some of these compounds are esters, thiols, isoprenoids and other sulfur-containing compounds.

Esters:

Esters are important fruity aromatic compounds formed during alcoholic fermentation and impact the aroma profile of wine, beer, whiskey, rum, tequila and many other alcoholic beverages. In wine, ester aroma compounds are formed during Secondary or Tertiary stages by alcoholic fermentation or the wine aging process (Waterhouse *et al* 2016). The major esters in wine are ethyl esters (fruity, apple, peach, strawberry) and acetate esters (banana, cherry, green apple, honey, rose) (Waterhouse *et al* 2016). Esters formed during a healthy fermentation contribute significant fruity aromas to wine (Antalick *et al* 2014). This ester forming reaction is called esterification. Esterification occurs when carboxylic acids (R-COOH) react with alcohol (R-OH) (Richter *et al* 2013).

Thiols:

There are many thiol compounds found in grape must/juice and wine. Thiols have a major influence on wine by contributing pleasant tropical fruit and citrus aromas. Some thiol compounds are derived directly from the grape variety, such as 3-MH (grapefruit, passionfruit), 3-MHA (passionfruit, box tree) and 4-MMP (guava, box tree). Thiol compounds are commonly found in varietal wines, such as Sauvignon Blanc, Gewürztraminer, Riesling, Colombard, Muscat, Syrah, Cabernet Sauvignon and many other wines. These aromatic thiol compounds contain low odor detection thresholds and can intensify the sensory aroma profile of a wine in a positive way. During fermentation, varietal thiols can also be released from non-volatile grape precursors, such as amino acid sulfur-conjugates, thus impacting wine aroma (Waterhouse *et al* 2016).

Isoprenoids:

Other compounds that contribute enjoyable aromas to wine are isoprenoids, such as monoterpenoids (C_{10} compounds), sesquiterpenoids (C_{15} compounds) and norisoprenoids (C_{13}

compounds). Monoterpenoids commonly found in white wines notably in Muscat varieties, such as linalool and geraniol, contribute aromatics perceived as floral/citrus. (-)-cis-rose oxide is another well-known monoterpenoid that contributes the distinctive lychee aroma of Gewürztraminer. One famous sesquiterpenoid is rotundone, which contributes a black pepper aroma to wine. Rotundone is commonly found in varietals such as Shiraz, Cabernet Sauvignon, Mourvedre, Gruner Veltliner and many other wine varietals. Some important norisoprenoids are β-damascenone, β-ionone and TDN (1,1,6-Trimethyl-1,2 dihydronapthalene). β-damascenone contributes floral and cooked apple aromas, while β-ionone contributes aromas of violet, wood and raspberry. TDN is perceived as a petrol/kerosene aroma, most commonly known to be found in Riesling wine.

Many of these aromatic compounds are dependent on grape variety and are found as non-volatile glycosides in the grape, however some of these aromatic compounds are free volatiles. Enzymatic hydrolysis of non-volatile glycosides essentially breaks the glycosidic linkage causing the sugar to be separated from the complex, thus releasing the volatile compounds. This breakdown during yeast fermentation can potentially enhance the aromatic characteristics of a wine (Waterhouse *et al* 2016).

Other sulfur-containing compounds:

Acids, alcohols, carbonyl compounds, esters and sulfur-containing volatiles are the main compounds produced by yeast metabolism commonly found in wine above the sensory detection threshold, however some sulfur-containing volatiles are not desirable (Bisson 2010). Depending on the compound and concentration, sulfur-containing volatiles may contribute negative or positive aroma characteristics to a wines flavor profile. Some of these compounds have precursors

that are derived from the grape variety and others are formed during fermentation, wine aging or storage.

There are many volatile sulfur-containing compounds found in wine that are perceived at different detection thresholds. Yeast strain, fermentation kinetics and nutrient availability, plays a significant role regarding sulfur compound formation. Compounds such as hydrogen sulfide (H_2S; rotten eggs), methanethiol (MeSH; putrefaction) and dimethyl sulfide (DMS; cabbage/asparagus/ cooked vegetables), are compounds formed during poor fermentation conditions (e.g. insufficient nutrient availability). When these compounds are formed and perceived in wine, the wine is considered "reductive", due to the compounds containing reduced forms of sulfur. Other sulfur-containing aroma compounds in wine like aryl thiols may become detectable during barrel maturation and storage, such as Benzenemethanethiol (BMT; smoke/struck flint), 2-furfurylthiol (FFT; roasted coffee) and 2-methyl-3-furanthiol (MFT; cooked meat). Typically, wines aged for longer periods of time will contain these sensory aroma characteristics. For example, H_2S produced by fermentation is highly suspected to react with furfural in toasted oak barrels resulting in the formation of FFT (Waterhouse *et al* 2016).

Concluding Remarks on Yeast Metabolism, and its Impact on the Aroma and Flavor of Wine

Odor components bound by compounds in grape must/juice can be released through chemical reactions, thus imparting aromatic qualities to a wine. Yeast activity can impact many sensory attributes of wine aroma, taste, color, clarity, mouthfeel and oral tactile sensations, such as effervescence and perception of heat (Bisson 2010). Since many aromatic compounds are produced during fermentation by yeast metabolism, it appears that different yeast species indeed impact the final sensory aroma and flavor profile of wine, thus producing secondary aromas and flavors that were not initially present before fermentation. Fermentation kinetics, production of

alcohol, esterification, other yeast metabolites and many other reactions occurring during the transformation of must/juice to wine, are crucial components in the formation of the final sensory flavor and aroma profile of a wine.

CHAPTER 2

YEAST ISOLATION PROCEDURE EXAMPLE

The experiment presented in this chapter will illustrate an example procedure that may be conducted by any winemaker to discover the yeast populations present in a vineyard at any given time. By isolating and experimenting with wild yeasts using bench top trials, a winemaker will be able to identify and control wild yeast strains that contribute to the target flavor and aroma profile found in non-inoculated wines.

To elucidate the process of isolating wild yeasts, an experiment was conducted in which yeast strains were isolated from the Cornell Lansing vineyard and utilized to inoculate 10 samples of Cabernet Franc must. The grapes and wild yeast strains were sourced in-house from the same location (Cornell Lansing vineyard). The fermentation kinetics of these wild yeast strains were analyzed, and a sensory consensus test was performed to determine differences in wine aroma characteristics relating to the different yeast species and their respective model fermentations. The isolated wild yeast strains were then stored for future fermentations.

Purpose

The goal of this experiment was to discover and analyze wild yeasts, thereby producing unique in-house strains that can be used for wine fermentations, as well as creating an interesting story for marketing purposes. Isolating wild yeast strains and analyzing their impact on wine aroma, can become a tool utilized by winemakers to express the unique characteristics of individual microflora extracted from a certain vineyard location at a specific time.

Introduction

In the beginning of a non-inoculated fermentation, several various strains of non-*Saccharomyces cerevisiae* yeasts are present in the must/juice and influence the final sensory profile of a wine. In most cases, non-*Saccharomyces cerevisiae* yeast strains will have a hard time completing the fermentation to dryness, due to their low ethanol tolerance. Wild *Saccharomyces cerevisiae* yeasts are found at a very low frequency on grapes in nature but are routinely present in wineries. Commercialized *Saccharomyces cerevisiae* yeast strains are commonly used to inoculate grape must/juice due to their strong fermentation kinetics and stay viable on winery equipment long after the wine is bottled and stored. What the common wine enthusiast might not understand though, is that *Saccharomyces cerevisiae* will generally come to dominate a fermentation even in wineries that haven't used commercial strains - more because of their ethanol tolerance and rapid growth rate during fermentation; but wineries with commercial strain exposure, have a higher probability of those strains dominating the fermentation. Therefore, when non-inoculated wines are fermented, it is possible that most of the fermentation is completed by *Saccharomyces cerevisiae* strains already present in the winery environment, which may be commercial isolates.

Methods

8 environmental samples were obtained from Cornell University's vineyard, located in the town of Lansing, Tompkins County, Ithaca New York, Finger Lakes region. The specimens included: vineyard spider, flower, bark from a Cabernet Franc vine trunk, whole Cabernet Franc berries, crushed Cabernet Franc berries, earth, dead wasps from a trap and a Cabernet Franc leaf. These environmental samples were collected, inserted into plastic tubes, then brought to the

laboratory. The 8 specimens were then fermented in their respective tubes to increase cell populations (Table 1).

Tube #	Lansing Environmental Vineyard Sample
1	Lansing Flower
2	Cabernet Franc Leaf
3	Vineyard Spider
4	Whole berries Cabernet Franc
5	Wasps Lansing Vineyards
6	Scion Bark
7	Lansing Earth
8	Crushed Cabernet Franc Berries

Table 1. Environmental Vineyard Samples: Description of vineyard samples and their corresponding tube fermentation numbers.

Each tube was filled with 15ml of yeast enrichment liquid media containing 9% glucose, as well as a yeast nitrogen base (YNB) that contained ammonium sulfate as the nitrogen source, vitamins, trace metals and salts. Antibiotics streptomycin, kanamycin, and ampicillin were added as a preventative measure to inhibit unwanted bacterial growth; however, antibiotics are not required to successfully perform this procedure. 1 or 2 days later, the samples started to ferment. About a week into the fermentation, the tubes were vortexed, and 5 microliters of liquid was extracted from each sample and streaked out on growth media plates (YD and Lysine plates). The growth media plates were labeled corresponding to their environmental sample source. As a note, *S. cerevisiae* cannot utilize lysine as a nitrogen source, therefore these plates are often used to isolate non-*Saccharomyces* yeasts. Visual cell growth appeared on the growth media plates after 3 days of incubation at 30˚C. It was clear at this time that there were multiple different types of yeast colonies on the growth media plates. Some colonies were different colors, notably reddish/pinkish and others had unique shapes, sizes and various colony morphology descriptors (see Glossary).

A single representative of each yeast colony was isolated and selected from each of the initial growth media plates and re-streaked onto new growth media plates (YD plates). This re-streaking technique further separated the yeast colonies from unwanted microbes, such as mold and living bacteria that may have been present on the initial YD and Lysine growth media plates.

After visual cell growth appeared on the re-streaked growth media plates, the isolated yeast colonies were then selected and analyzed through microscopy to further determine cell morphology (see Glossary). One way to differentiate between yeast and bacteria cells is by size. Yeast cells are significantly bigger in size than bacteria when viewed under the microscope (40x / 100x). Another way to determine yeast apart from bacteria is by cell shape. Yeast cells will

typically have an oval or round shape. It is also possible to observe the buds, which can be seen on some yeast cells. The basic bacteria shapes are coccus (spherical; plural = cocci), bacillus (cylindrical-rod shaped; plural=bacilli), and spiral (corkscrew-shaped). This visual analysis may be used to identify the differences between yeast and bacteria cells, thus identifying the selected organism in question. Polymerase chain reaction (PCR) testing was performed on the isolated pure wild yeast colonies to further determine the genus of the strains and possible species (Figure 1, Table 2).

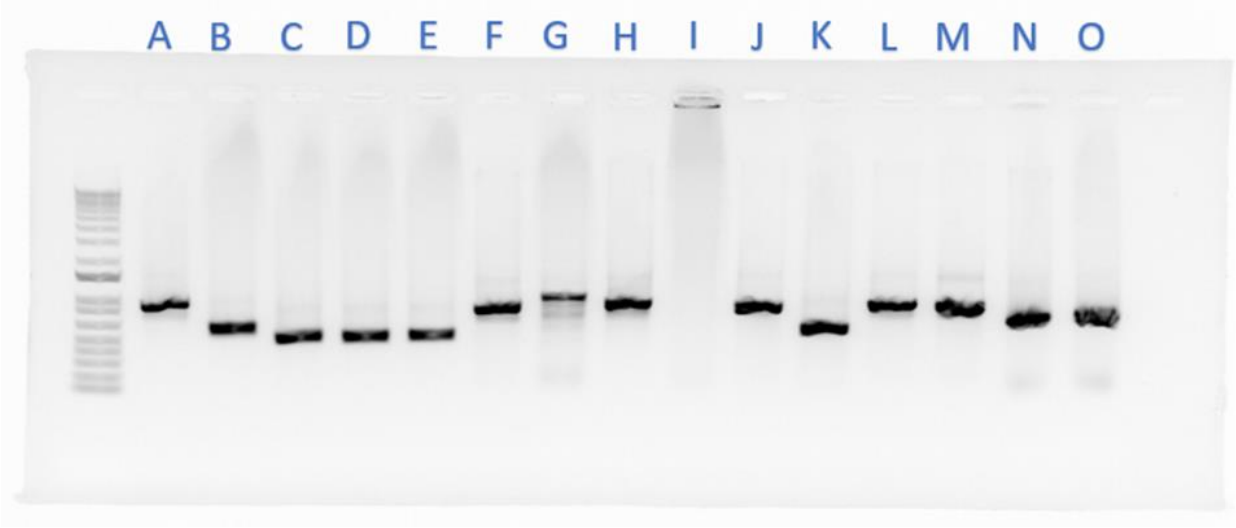

Figure 1. Gel Electrophoresis of ITS PCR from Isolated Yeasts. Genomic DNA was prepared using a protocol by Looke *et al* 2011. PCR was performed using ITS4 and ITS5 primers in PCR tube with 2X HS TAQ Master Mix (NEB). An aliquot of each PCR reaction was loaded on a 1% agarose gel with gel red DNA dye (VWR), then visualized in Bio-Rad ChemiDoc gel imaging station.

PCR Sample ID	Isolation Growth Media	Fermentation Number	Vineyard Samples	Genus	Possible Species (listed in order of top results)
A	YD	1	Flower	*Hanseniaspora*	*uvarum*
B	YD	2	Spider	*Pichia*	*kluyveri, fermentans*
C	YD	3	Spider	*Metschnikowia*	*sinensis, shanxiensis, pulcherrima, fructicola*
D	YD	4	Whole Berries	*Metschnikowia*	*bicuspidata, pulcherrima, sinensis*
E	YD	5	Wasps	*Metschnikowia*	*ziziphicola*
F	YD	6	Wasps	*Lachancea*	*thermotolerans*
G	YD	7	Scion Bark	*Hanseniaspora*	*uvarum*
H	YD	8	Scion Bark	*Candida*	*californica*
I	YD	9	Earth	*n.d.*	*n.d.*
J	YD	10	Crushed Berries, Cab Franc	*Hanseniaspora*	*uvarum*
K	YD	11	Crushed Berries, Cab Franc	*Pichia*	*fermentans, kluyveri*
L	Lysine		Whole Berries, Cab Franc	*Hanseniaspora*	*uvarum*
M	YD		Wasps	*Hanseniaspora*	*vineae, osmophila*
N	YD		Leaf, Cab Franc	*Aureobasidium*	*pullulans*
O	Lysine		Leaf, Cab Franc	*Aureobasidium*	*pullulans*

Table 2. Organism Genus and Respective Fermentations: This table shows the PCR sample ID, Isolation growth media type, model fermentation number, environmental vineyard sample, yeast strain Genus and possible species identified by ITS PCR. This experiment showed confidence in the genus with possible variations amongst the species. For some reason, PCR sample [I] did not yield results. n.d. = not determined.

The isolated yeast colonies were grown into starter cultures and used to inoculate a sterilized grape must, thus initiating alcoholic fermentation. 11 pure yeast colonies were harvested from the re-streaked growth media plates and grown separately with agitation in 10ml of Wegman's organic juice medium, with an addition of 0.5 mg/ml of diammonium phosphate (DAP), reaching a yeast assimilable nitrogen (YAN) level of 150 mg/L. Number 9 however, did not ferment for some unknown reason. When inoculating grape must/juice, Scott Labs recommends an initial yeast starter culture concentration of 3–4 million (3×10^6) viable cells per ml of must or juice. Under favorable conditions, the initial cell population may increase up to 100–150 million (10^8) viable cells per ml of must or juice, before growth stops and alcoholic fermentation begins. This biomass increase is critical for a healthy fermentation. Cell concentration was tested using spectrophotometry.

Beakers and equipment used in this experiment were sterilized ahead of time in an autoclave. The 10 fermentation experiments were performed under a ventilation hood at ambient temperature. Cabernet Franc clusters were de-stemmed/crushed and frozen on October 18th, 2018. Then, the must was thawed, sterilized at 212˚F and held at that temperature for 30 minutes on November 3rd, 2018. After the starter cultures were grown and measured to the required cell concentration, 300 ml of Cabernet Franc must was inserted into the beakers and inoculated with the 10 isolated wild yeast starter cultures on November 4th, 2018. Pre-fermentation Cabernet Franc grape chemistry was tested and recorded. Furthermore, chemical analyses revealed the final wine chemistry for all inoculated model fermentation experiments (Table 3). Fermaid K (complex yeast nutrient) additions were made according to Scott Labs recommendation of 25g/100L, which equated to 0.075g Fermaid K per 300ml of must [(25g/100L=0.25g/L) (0.25g/L x 0.3ml= 0.075g of Fermaid K per 300ml must)]. The model fermentations were conducted over one month, after

which a sensory consensus analysis was performed to determine aromatic differences between the fermentations (Table 4). One professor, two undergraduate and four graduate students from the Food Science department analyzed and described the aromas of each fermentation individually. Then, aroma descriptors found to be agreed upon by the panelists were compiled for each fermentation experiment.

Results

This experiment has shown that various non-*S. cerevisiae* yeast strains indeed possess different fermentation kinetics (Figure 2) and can influence grape fermentation and microbial ecology in their own unique ways, providing interesting and unique aroma characteristics throughout fermentation (Tables 3 & 4).

pH levels increased across all fermentations except for one (#6). One explanation for this pH increase might be due to the potassium (K) concentration leaked from the grape skins/pulp into the must/juice. Potassium (K) is released from grape skins into the must during early stages of fermentation and contributes to higher wine potassium and pH. Grape must/juice pH is positively correlated with grape juice K. Total acidity (TA) increased across all fermentations. In the United States, the legal limits of Volatile Acidity (VA) is 1.2g/L for red table wine and 1.1g/L for white table wine (Waterhouse *et al* 2016). Since this was an experiment conducted on red table wine, none of the fermentations reached the legal limit of VA concentration, however if the fermentations were left to finish until dry, it is possible that the VA concentration would have exceeded the legal limit. The malic acid levels across all fermentations increased but did not differ that much when compared to the initial Cab Franc grape chemistry, except for ferment number 5 which increased significantly. Tartaric acid, Brix and Glucose/Fructose levels decreased across all fermentations. Ethanol concentrations increased across all ferments (Table 4).

Kinetics varied greatly across all fermentations (Figure 2). All fermentations experienced loss of CO_2, however none of the experiments fermented to dryness. The experiment was stopped one month after initiation, due to time constrains. Figure 2 shows that each experiment fermented differently when compared to each other, thus showing how different yeast strains can affect wine fermentation by means of their respective metabolism.

The sensory consensus analysis results revealed a large mix of positive and negative aroma descriptors. The panelists analyzed each fermentation separately and recorded their sensory descriptors. These descriptors were compiled and recorded as the complete list of descriptors for each fermentation. Then, aroma descriptors found to be the same across the panelists for each fermentation, were compiled and listed as consensus descriptors (Table 4).

Fermentation # And Organism	TA g/L	pH	VA g/L	Malic acid g/L	Tartaric acid g/L	Brix	Glu+ Fru g/L	Ethanol at 20˚C % vol	YAN mg/L
Pre-Fermentation Cabernet Franc Juice Chemistry	5.7	3.42	n.d.	2.34	3.7	21.3	222	n.d.	33
#1 ***Hanseniaspora***	7.6	3.71	1.14	2.31	2.4	9.3	81	5.2	24
#2 ***Pichia***	6.6	3.72	0.75	2.56	2.2	8.09	78	7.4	18
#3 ***Metschnikowia***	6.3	3.68	0.16	2.56	2.3	11.2	96	6.2	25
#4 ***Metschnikowia***	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
#5 ***Metschnikowia***	9.0	3.57	<0.05	3.46	2.1	10.4	62	4.9	21
#6 ***Lachancea***	17.2	3.36	0.66	2.52	2.6	2.4	5.6	8.7	24
#7 ***Hanseniaspora***	7.3	3.69	0.79	2.47	2.3	11.4	113	6.6	31
#8 ***Candida***	7.2	3.71	0.78	2.44	2.1	10.4	98	6.6	35
#10 ***Hanseniaspora***	7.0	3.75	0.91	2.13	2.1	10.5	102	6.6	38
#11 ***Pichia***	8.5	3.61	0.63	3.69	2.1	19.1	180	4.2	34

Table 3. Chemical Analysis of Model Fermentations: Chemical analysis results of the bench-top trial fermentation experiments performed, including pre-fermentation Cabernet Franc grape chemistry. TA = total acidity. VA = volatile acidity. Glu+Fru = Glucose+Fructose. YAN = yeast assimilable nitrogen. n.d. = not determined.

Fermentation # and Organism	Consensus Descriptors	Complete List of Descriptors
#1 *Hanseniaspora*	Vanilla, potato chips, starch, bread, yeasty	Vanilla, flowers, caramel, fake candy, yeasty, oxidized, bread, sweet bread, sour cherry, sour milk, savory, potato chips, fruity, vinegar
#2 *Pichia*	Nail polish, cherry, cough medicine, fake fruit, artificial fruit	Nail polish, lychee, fresh paint, mango, blueberry, fruity, cherry, tutti fruity, CO_2, cotton candy, fake fruit, cough medicine, soda, artificial sweetener, hand sanitizer, moldy, wet, dark red fruit
#3 *Metschnikowia*	Chemical, cooked fruit, strawberry, raspberry, musty, rotten corpse, VA	Marshmallow, musty, woody, earthy, foul-smelling meat, chemical, cleaner, rubber band, sharpie marker, volatile acidity, cooked fruit, yeast, strawberry, soap, very light odor, raspberry, red fruits, cherry, nail polish, jammy, sharp
#4 *Metschnikowia*	Strawberry, chemical, sanitizer, green, fruit/grapey	Wet, nail polish, strawberry, green, yeasty, ethanol, grape, cherry cough syrup, hand sanitizer, floor cleaner, CO_2, cotton candy, cherry, tutti fruity, paint, apple
#5 *Metschnikowia*	Strawberry, red fruit, spicy, latex, medical, pomegranate, chemical, musty, less fruity aromas	Stinky, musty, ethanol, less fruit, strawberry, hint of mango, tropical fruit, spicy, chemical, varnish, mild fruity, red fruit, cherry, hard candy, pomegranate, Dr office, floral, rubber, smoke, latex, hazelnut
#6 *Lachancea*	Sweet candy, caramel, cherry, light berries,	Fruity, vanilla, cream, yeasty, maple syrup, caramel, sweet candied raspberries, caramel apple, sweet cherry, red fruit, jam, red plum, old yogurt, sour milk, rubber band, latex, pear, strawberry, berries, fresh baked pie
#7 *Hanseniaspora*	Dark cherry, oxidized fruit, herbaceous, ethanol, yeasty, red fruit	Strong ethanol, herbaceous, fruity, SO_2 smell, grassy, earthy, raisin, dark ripe cherry, tutti fruity, old yogurt sour milk, hospital, burnt plastic, light red fruit, artificial cherry, yeast, nail polish, grapey, sharp
#8 *Candida*	Strawberry, chemical, oxidation, ethanol, cooked sugar, dark fruit	Apricots, jammy, cherry, strawberry, cooked red fruits, oxidized, chemical, septic, medical, dark fruit, blackberry, insulin, sharpie marker, ripe cherry, burnt sugar, spicy
#10 *Hanseniaspora*	Chemical, sharpie, nail polish, neutral smelling, grassy, plant matter	Smells like plants, strawberry/more grassy, mild fruit, very neutral smelling, nail polish, chemical, chocolate, floral/lilacs, plum port/brandy, waffles, band aid, cooked apricots, yeast, canned cream
#11 *Pichia*	Sharp, vinegar, darker red ripe fruit, cherry pear, apple pie, cooked apple, sour, rotted fruit, nail polish	Nail polish, dark fruit, red ripe cherry, strawberry, sharp, vinegar, plum, herbal, weird, sour, rotten fruit, pear, fruity, floral (rose petal), metallic, hint of phenol, dead meat while decaying, apple that was left outside for too long, jammy, baked pie

Table 4. Sensory Consensus Analysis Results: Olfactory analysis was performed by 1 professor, 2 undergraduate and 4 graduate students from the food science department. Complete list of panelist descriptors was compiled, as well as consensus descriptors across panelists.

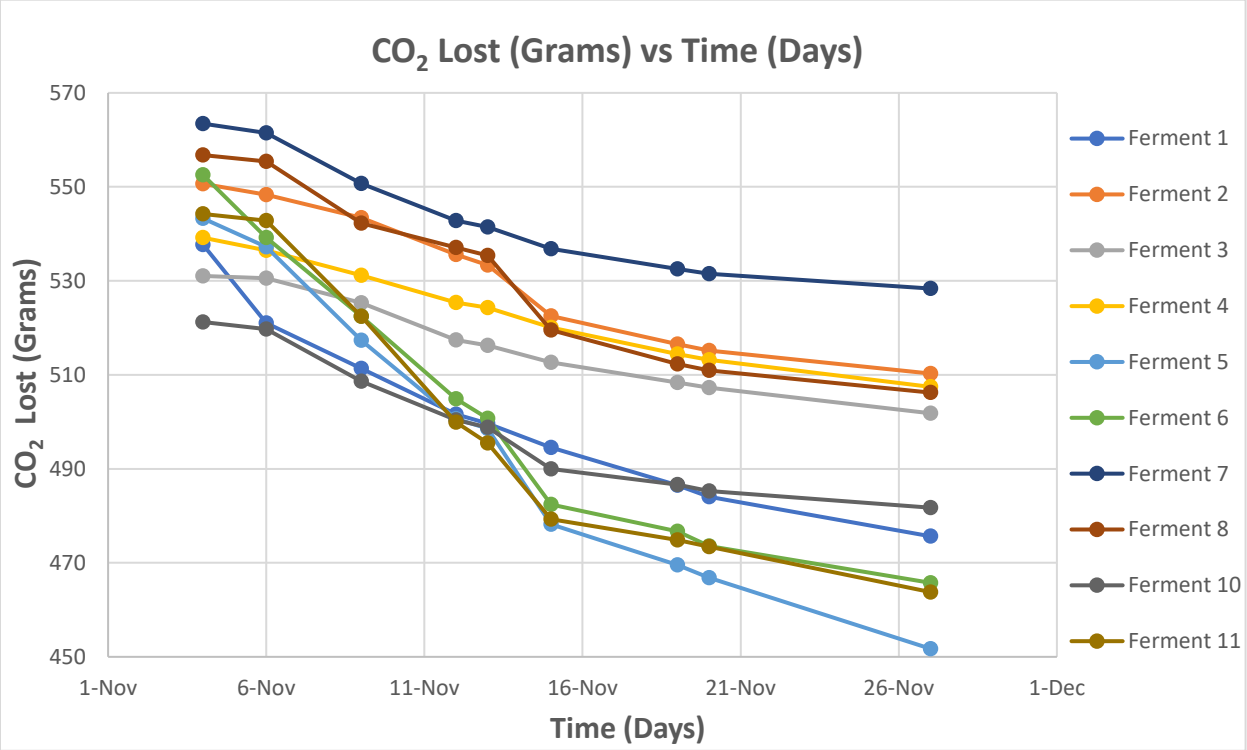

Figure 2. Fermentation Kinetics: Scatter plot illustrating loss of weight in grams over time measured by loss of CO_2, for all 10 experimental fermentations.

Conclusion

If wild yeast strains are discovered to provide desirable fermentation kinetics, wineries could experiment with wild yeast to enhance the flavor and aroma profile of a wine without having to worry about stuck fermentations. Although wine fermentations may come to a standstill for multiple reasons, wild yeast strains that are found to have desirable fermentation kinetics under optimal fermentation conditions, may also enhance the sensory characteristics of a wine, thereby creating an interesting flavor profile unique to the finished wine product. Co-inoculation could be used to prevent stuck fermentations from occurring by adding 10% of commercialized *Saccharomyces cerevisiae* to a 90% wild yeast fermentation. This will allow the wild yeasts to develop aroma/flavor complexity in the beginning of fermentation, while the commercialized *Saccharomyces cerevisiae* added will finish the fermentation to dryness.

Locating an alternative to the *Saccharomyces cerevisiae* winery workhorse is only one experiment away. There are thousands of known yeast strains and likely many which have not been discovered yet, containing characteristics unknown. Although there is some literature on wild *Saccharomyces cerevisiae* and non- *Saccharomyces cerevisiae* yeast strains regarding their fermentation kinetics, aroma and flavor influences on wine, more research needs to be explored to determine the true impacts that these two groups have on the characteristics of a wines sensory profile, including flavor, aroma, body and mouthfeel.

This experiment was conducted to provide useful information for boutique and do-it-yourself winemakers, who would like to explore different yeast strain options regarding the alcoholic fermentation of grapes. This scientific project found that different yeast strains influence grape fermentation, contributing unique aroma characteristics as well as varying fermentation kinetics.

CHAPTER 3

PRACTICAL WINEMAKER'S GUIDE TO ISOLATING WILD YEAST SPECIES

It is a matter of utmost importance that aseptic technique be utilized throughout the isolation procedure for this experiment to be successful. When extracting and working with microbes, contamination is a serious concern. To correctly isolate a yeast strain, one must be sure that the strain is free from unwanted microbes or contamination. Therefore, when selecting and streaking a microbe onto growth media, the tool used to extract the cells must be sterilized by flame, ethanol or both. This sterilization step will kill any microbes on the surface of the streaking tool (toothpick or metal loop), thus ensuring that the microbe is free from contaminants. This is a crucial and necessary measure for the experiment to work properly. The following section will go through the step-by-step yeast isolation procedure in more detail (Figure 3).

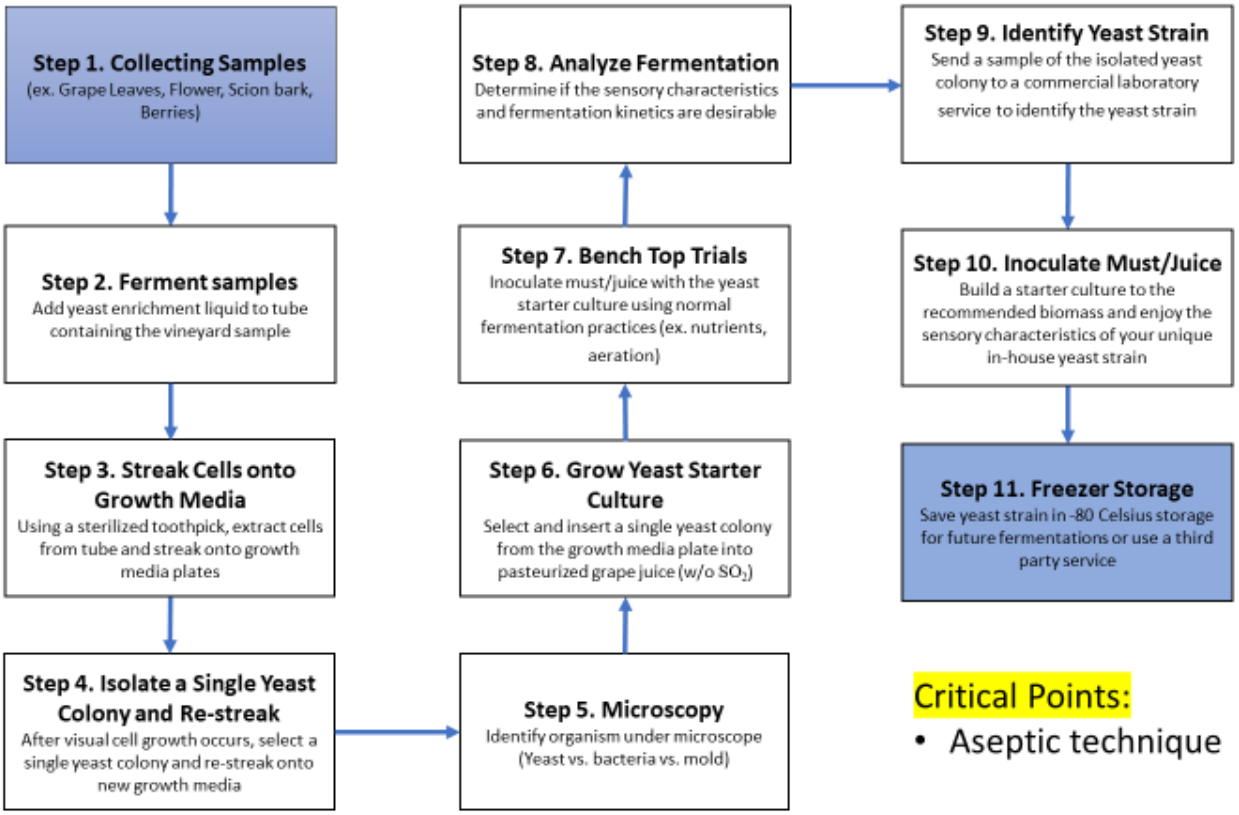

Figure 3. Flow Chart of Yeast Isolation Procedure: Flow chart illustrating the step-by-step procedure to isolating wild yeast from a vineyard.

1. Collecting Samples

Several environmental samples, such as grape berries, scion bark, a flower, vine leaves or earth should be extracted from the vineyard location and inserted separately into individual plastic tubes and closed. The samples can be stored at 4 degrees Celsius until ready for yeast enrichment. See Table 5 for a list of yeast isolation equipment.

2. Ferment Samples

Then, yeast enrichment liquid should be inserted into the tubes, ensuring that the respective vineyard samples are fully submerged under the yeast enrichment liquid (see Table 6 for yeast enrichment liquid recipe). Allow the samples to ferment in their respective tubes. They will start to ferment after a few days (Figure 4). If there is a closure on the tube, loosen it so that CO_2 can escape, thus preventing the fermentation pressure from bursting the tube open.

Yeast Isolation Equipment:	
Tubes (for environmental vineyard samples)	Required
Gloves	Optional
Wood Toothpicks or Metal Loop	Required
Empty Plastic Sterile Growth Media Streak Plates with Covers	Required
YD Growth Media Plate Recipe: (Page 27 - Table 6. Growth Media Types). - Alternatively, pre-prepared growth media plates can be purchased as YM, YD or YPD from Teknova.com instead of building the growth media from scratch.	Required
Enrichment Liquid: (Page 27 - Table 6. Growth Media Types)	Required
Pasteurized Juice: (Page 27 - Table 6. Growth Media Types)	Required
Compound Microscope	Optional
Glass Slides for Microbe Analysis	Optional
Immersion Oil for Compound Microscope	Optional
De-ionized Water	Optional

Table 5. Yeast Isolation Equipment. A list of optional and required yeast isolation materials.

Growth Media Type:	Compounds:	Use:
Enrichment Liquid	0.67% (w/v) YNB with Ammonium Sulfate 9% (w/v) Glucose	Used to enrich yeasts by fermenting vineyard samples in tubes with enrichment liquid.
YD Growth Media Plate Recipe	1% (w/v) Yeast Extract 1% (w/v) Glucose 2% (w/v) Agar	Grow and store yeasts on plates. Can also typically grow AAB (acetic acid bacteria), but not LAB (lactic acid bacteria).
Pasteurized Juice	Organic Grape Juice without SO_2 (Sulfites)	Grow pure starter cultures of selected yeasts in Pasteurized juice.

Table 6. Growth Media Types: A list of compounds necessary to create yeast enrichment liquid, and YD growth media plates. Pasteurized organic sulfite-free grape juice is used as a medium to grow the pure starter culture of selected yeast. There are many different types of growth media, YD is an example of one that is permissive. Most microbes can grow on YD, including yeast, bacteria and mold. To create the growth media type, the compounds are mixed with water and sterilized.

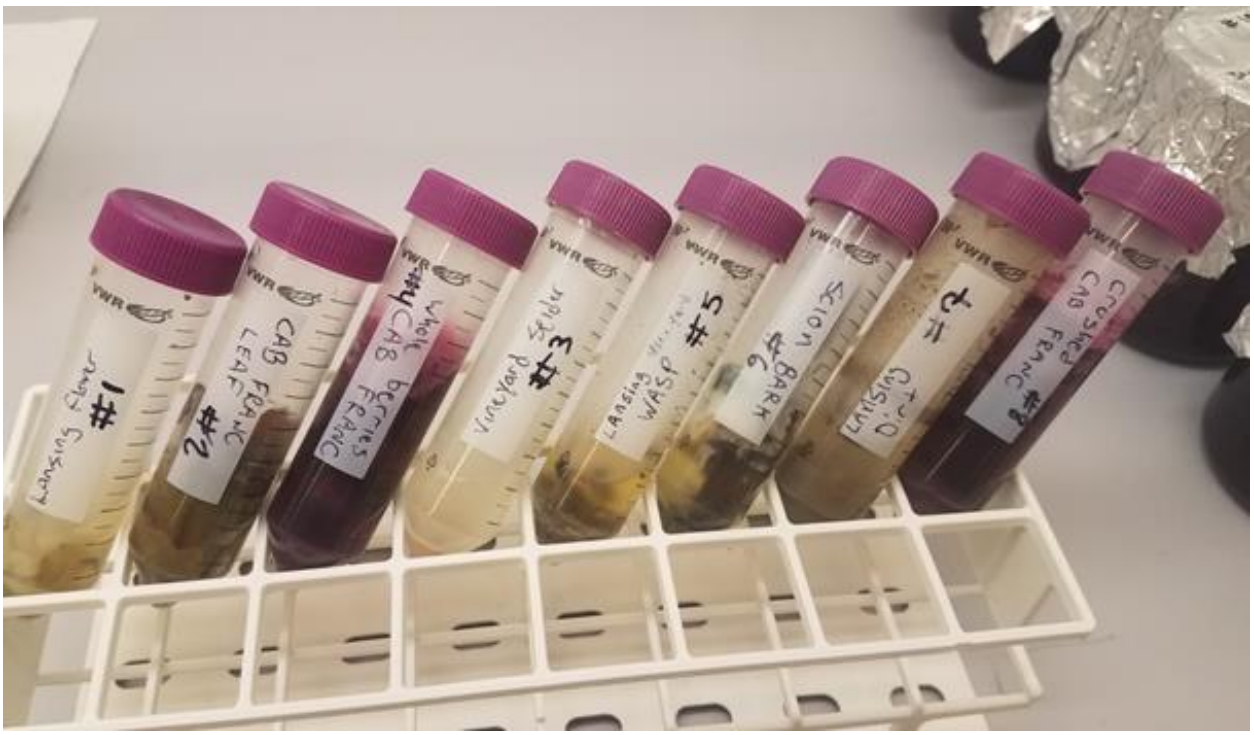

Figure 4. Yeast Enrichment of Vineyard Samples: Picture of vineyard samples in their respective tubes, fermenting in yeast enrichment liquid.

3. Streaking Cells onto Growth Media Plates

Primary Streak:

After visual fermentation (bubbling) slows down, the tubes should be shaken. Sterilize a wooden toothpick or a metal loop by running it through a flame. Flaming the toothpick will kill any microbes that may be present on the surface. Dip the toothpick into the yeast enrichment liquid, extract one drop of liquid from the tube and place it on the growth media plate (see Table 6 for growth media plate information). Streak or spread out the drop of liquid extracted from the tube with the sterilized wooden toothpick. Lightly drag the toothpick in a long sweeping motion back and forth across the surface of the growth media plate, until half of the growth media surface area is streaked out. This will create compact zigzag lines across half of the growth media plate (Figure 5). Once the toothpick has touched the growth media plate, do not lift it up until the primary streaking motion is complete. If the toothpick has been lifted from the growth media plate, discard the toothpick and use a new sterilized wooden toothpick to finish streaking.

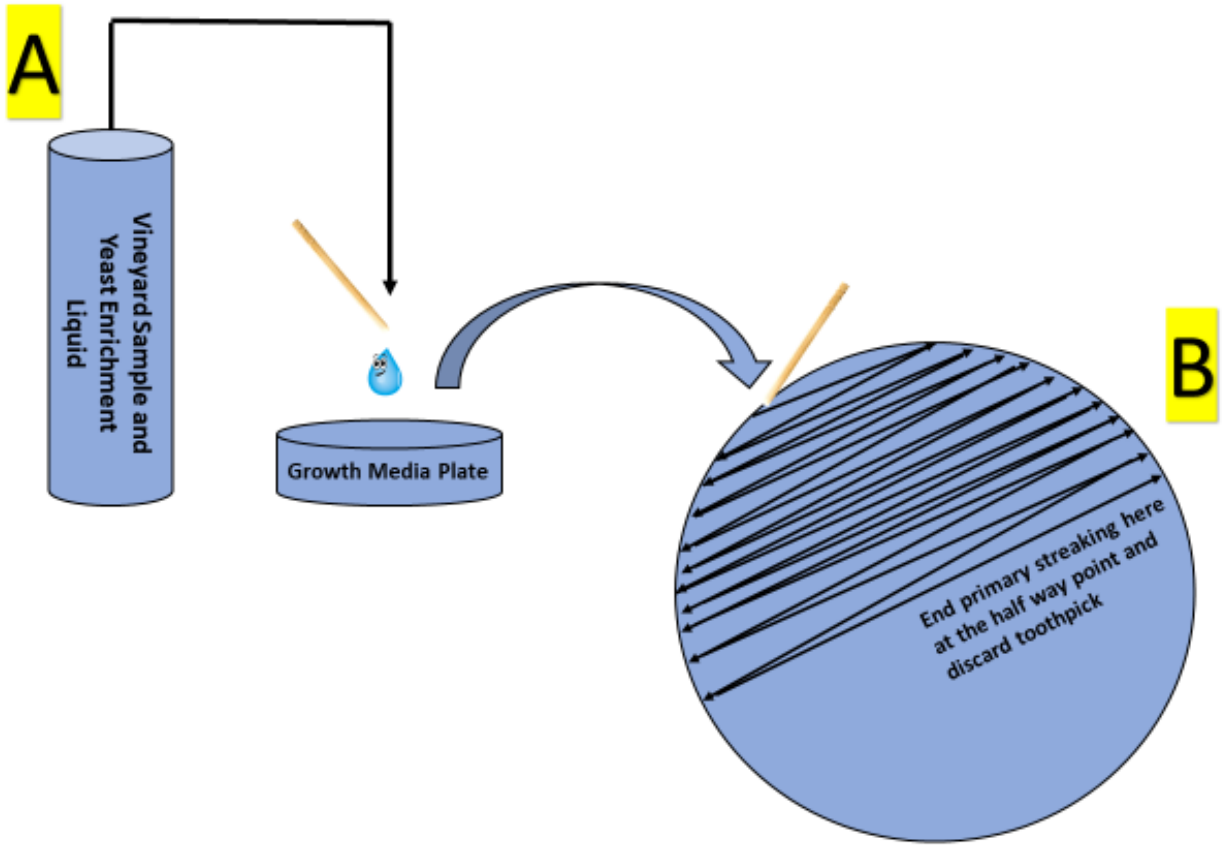

Figure 5. Primary Streak: One drop of liquid from the vineyard sample fermentation tube is extracted and placed onto the growth media plate using a wooden sterilized toothpick (A). The toothpick is then guided in a continuous streaking motion, thus dispersing the cells and creating compact zigzag lines across the surface of the growth media. The primary streak method is complete when half the growth media has been streaked out (B).

Secondary Streak:

The purpose of the secondary streak is to disperse and dilute the cells across the surface of the growth media. This technique will make it easier to separate and grow independent colonies of cells on the growth media, with the objective being to isolate and harvest a pure yeast culture.

Sterilize a new wooden toothpick by flaming it. Place the sterilized toothpick on the growth media towards the top-middle portion of the vertically positioned primary streak lines. Drag the toothpick in a horizontal streaking motion across to the right, thus dragging a portion of cells over onto the vacant half of the growth media surface. Without lifting the toothpick, streak out the remaining vacant space of the growth media by creating compact zig zag lines. Be careful not to overlap with the primary streak lines after the secondary streak has been initiated (Figure 6).

The streaking step has been completed when the liquid drop has been properly spread out across the surface of the growth media plate. Close the growth media plate with its cover to prevent possible microbial contamination. Store the recently streaked out growth media plate coverside facing down, to decrease growth media evaporation. Aseptic streaking techniques should be strictly followed to prevent contaminating the growth media plate by other unwanted microbes.

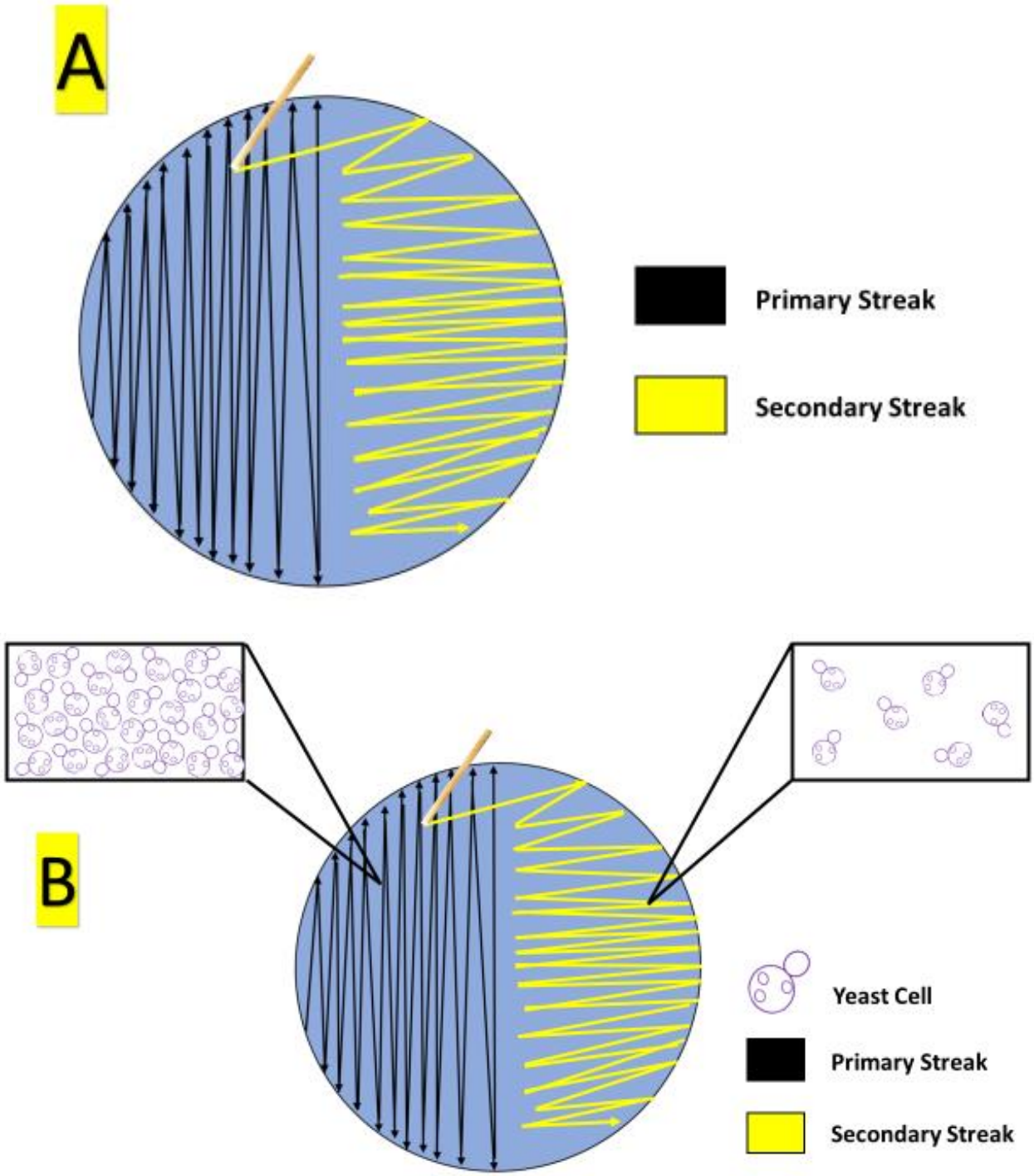

Figure 6. Secondary Streak: A sterilized wooden toothpick is utilized to drag a portion of cells toward the vacant half of the growth media. In a continuous motion, the cells are streaked and dispersed across the available growth media surface. It is important not to overlap with the primary streak lines once the secondary streak has been initiated, since the objective is to spread and dilute the cells over the growth media surface (A). This picture illustrates primary streak yeast cell concentration, compared to the dilution of yeast cells when utilizing the secondary streak (B).

4. Isolate a Single Yeast Colony and Re-Streak

After a few days, visible cell growth should appear on the growth media plate. It is possible at this time that multiple different yeast, bacteria or mold colonies will be present on the growth media plate. The main idea at this point in the procedure is to harvest and separate a pure yeast colony from the growth media plate, thus effectively isolating a pure yeast culture away from unwanted microbes such as bacteria or mold. Using a sterilized wooden toothpick, harvest what appears to be a single isolated yeast colony and re-streak it onto a new sterilized growth media plate, to obtain a pure yeast culture (Figure 7).

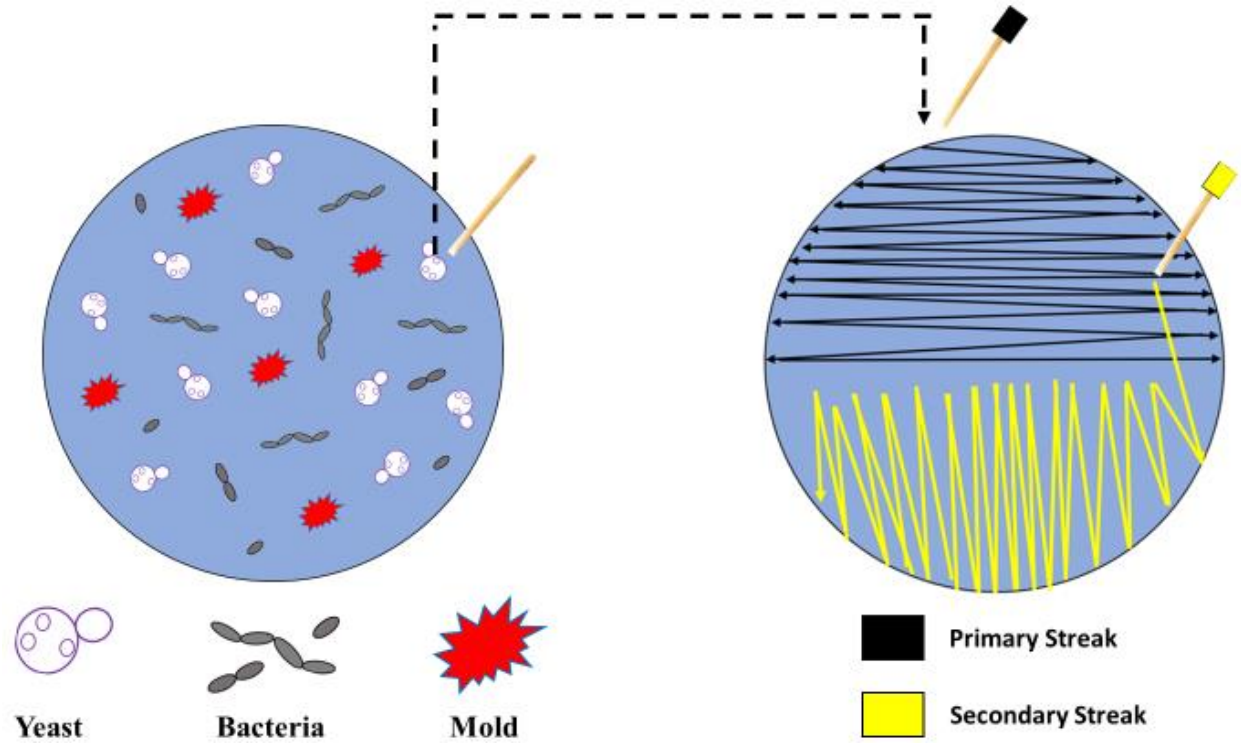

Figure 7. Isolate a Single Yeast Colony from Unwanted Microbes and Re-Streak: This picture illustrates a yeast isolation technique used to separate yeast colonies from unwanted microbes, such as bacteria and mold. A flamed, and sterilized wooden toothpick is used to harvest a single yeast colony off the growth media. Then, the yeast colony is steaked out across new sterilized growth media using primary and secondary streak plate methods. If executed correctly, this yeast isolation method should yield a pure culture of the selected yeast colony.

After a few days, visual cell growth should appear. In the best-case scenario, all the yeast colonies growing on the growth media plate should look similar or identical in size, color and shape. If this is the case, a pure yeast culture has been obtained. If there appears to be multiple fungi or bacteria growing on the plate containing different morphology (see Glossary), repeat this isolation re-streak purification step, and isolate a pure yeast colony to obtain a pure culture (see Glossary).

At this point, streak purification has been completed and the isolated pure yeast colonies should appear to be separated away from other yeast, bacteria or mold. The growth media plate should now contain visual growing colonies of a single pure yeast culture. (Figure 8).

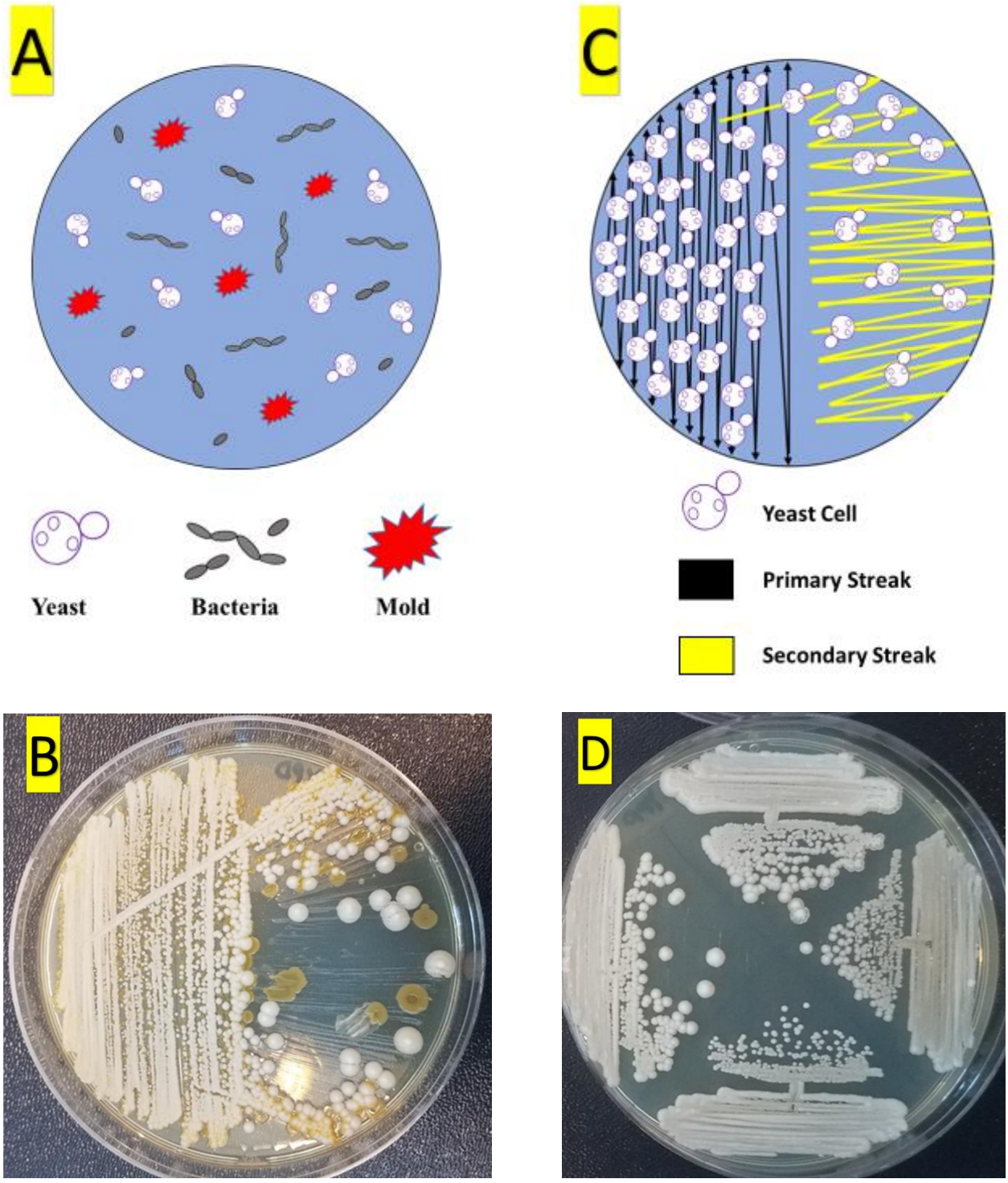

Figure 8. Example Pictures of Contaminated and Pure Yeast Cultures: Cartoon picture illustrating a contaminated growth media plate by unwanted microbes (A). Real picture illustrating bacteria contaminants on growth media plate (B). Cartoon picture illustrating a selected yeast strain growing as a pure yeast culture (C). Real picture of a growth media plate sectored into 4 pure yeast cultures (D).

5. Microscopy

Using a sterilized wood toothpick, a single yeast colony can be harvested from the growth media and analyzed using microscopy to confirm that it is indeed a yeast cell (Figure 9). The yeast cell is harvested using a sterilized toothpick and placed onto a glass slide containing one drop of deionized water (important that water is sterile). Then, a glass cover slide is placed on top of the drop sample, to suspend the yeast specimen in between the two glass slides. The glass cover slide also serves to protect the objective lens from interfering with the sample drop. The glass slide is then placed into the stage deck of the compound microscope for viewing. 40x magnification is typically suited to see yeast. For 100x magnification, one drop of optical Immersion oil may be placed in between the glass slide containing the specimen and the optical lens (see Glossary). This will help increase magnification. Objective lenses specifically designed for this purpose are known as oil immersion objectives.

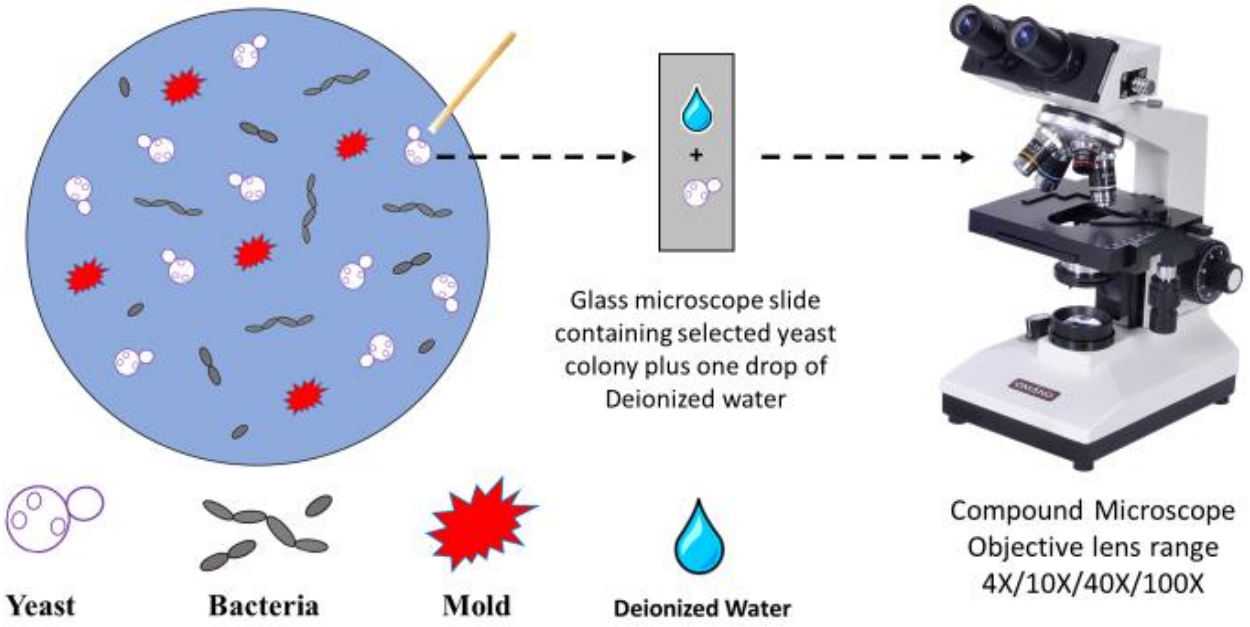

Figure 9. Microscopy: Using a sterilized toothpick, a single yeast colony is harvested off the growth media and placed onto a glass slide with one drop of deionized water. Then, a glass cover slide is placed on top of the yeast specimen, securing the sample in between the two pieces of glass. The glass slide containing the yeast specimen is then placed in the stage of the compound microscope for viewing (40x / 100x).

6. Grow Yeast Starter Culture

After microscopy analysis confirms that the organism is indeed a yeast, a yeast starter culture should be grown. Using a sterilized toothpick, scrape or extract a single yeast colony off the pure yeast culture growth media, and insert the colony into a pasteurized organic sulfite-free grape juice medium (see Table 6). This step enables the growth of the selected pure yeast strain and builds the yeast starter culture (Figure 10).

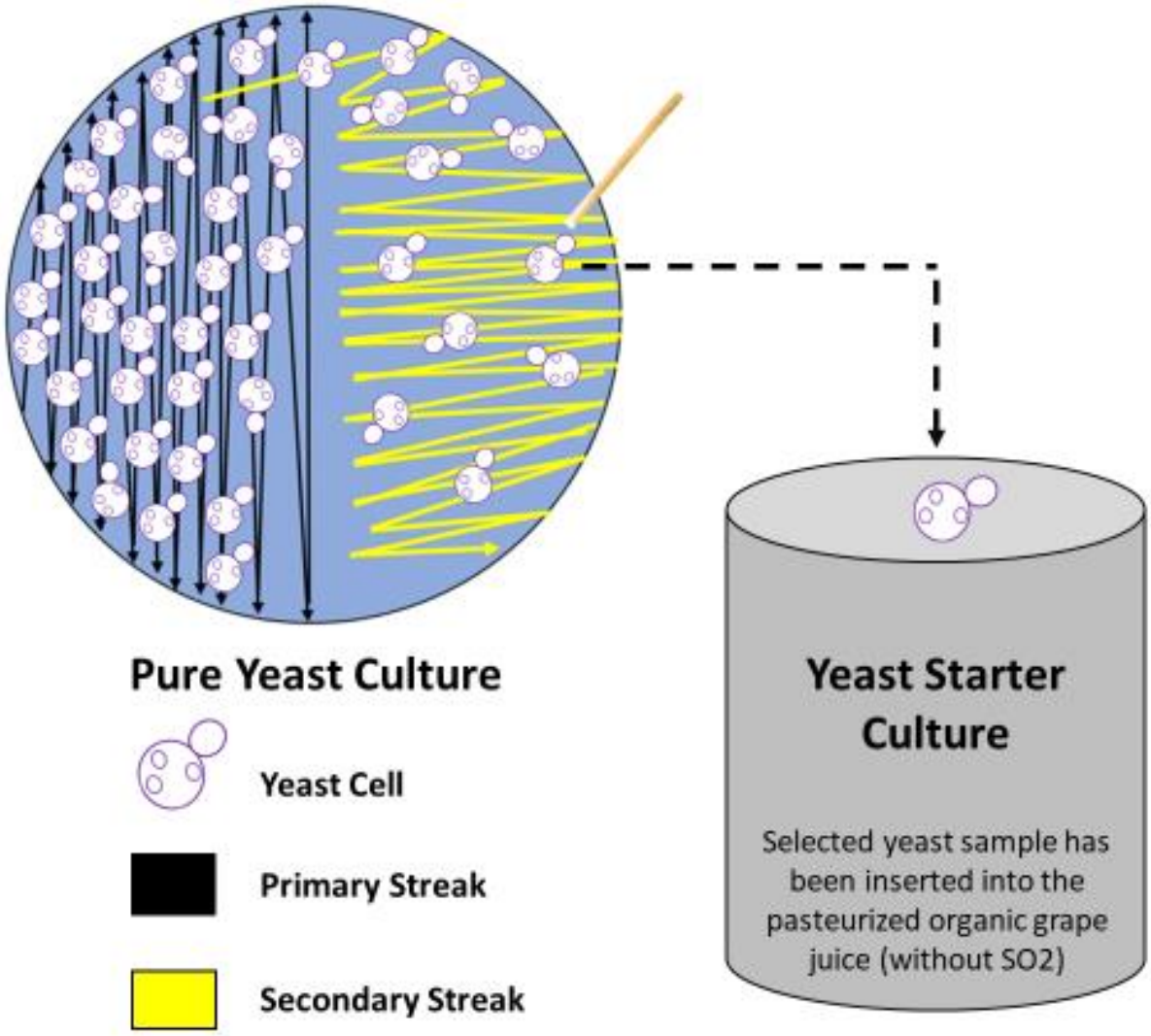

Figure 10. Grow Yeast Starter Culture: This picture illustrates the process of building a yeast starter culture. A pure yeast colony is scraped off the growth media and inserted into pasteurized organic grape juice (without SO_2). This step allows the yeast cells to grow and build biomass in the grape juice medium.

7. Model Fermentation Bench Top Trials

Once the yeast starter culture starts to build cell biomass and is visually fermenting, the next step in this procedure is to inoculate a grape must/juice model fermentation, with the developed pure yeast starter culture (Figure 11). Performing bench-top trial fermentations are very important when trying to determine if the selected yeast strain has desirable flavor/aroma characteristics and reliable fermentation kinetics. It is important to note, that many non-*Saccharomyces cerevisiae* yeast strains may produce off-odors or contribute poor fermentation kinetics, due to their low ethanol tolerance. The purpose of bench-top trials is to investigate and determine if the selected yeast strain can produce a unique, premium, healthy wine; containing interesting aromas and flavor characteristics. Another bench top trial option is to co-inoculate a small amount of commercialized *Saccharomyces cerevisiae* with the wild yeast starter culture. This will allow the wild strain to develop a unique aroma and flavor profile in the beginning of fermentation, while the commercialized strain added will complete the fermentation to dryness.

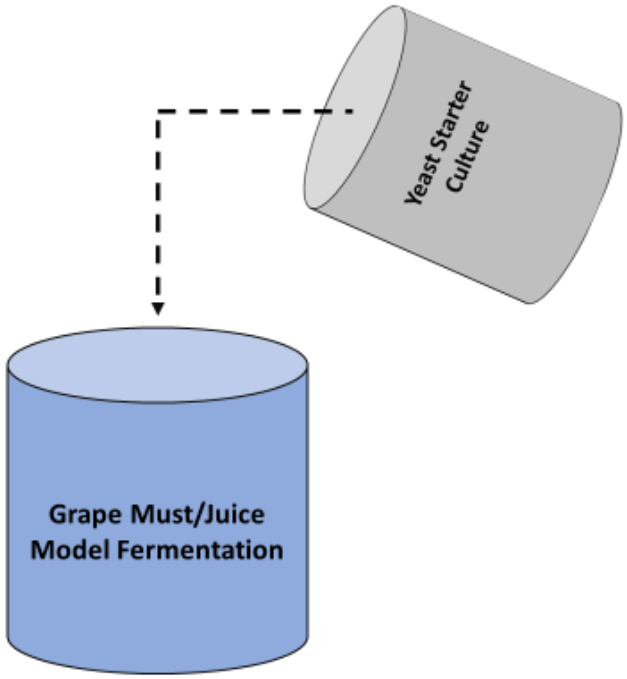

Figure 11. Model Fermentation Bench-Top Trials: Picture illustrating a pure yeast starter culture inoculated into a grape must/juice model fermentation.

8. Analyze Fermentation

Analyze the model fermentation and determine if the selected yeast strain has provided desirable fermentation kinetics and sensory characteristics such as aroma and flavor.

9. Yeast Strain Identification

If bench-top trial fermentations were successful and the yeast strain was found to possess desirable sensory characteristics and fermentation kinetics, the pure yeast colony should be harvested from the growth media plate and sent to a third-party identification service (Charles River Labs or Genewiz Labs among others) to identify the genus and species of the isolated vineyard yeast.

10. Inoculate Must/Juice

Build a starter culture from your isolated pure yeast strain to the recommended cell biomass, and initiate fermentation by inoculating a selected lot of grape must/juice. Scott Labs recommends an initial yeast starter cell concentration of 3–4 million (3×10^6) viable cells per ml of must or juice. A specialized counting chamber device called a hemocytometer can be used to measure cell concentrations under a microscope. Monitor the fermentation adhering to excellent winemaking practices and enjoy the sensory characteristics of your unique in-house yeast strain (Figure 12).

11. Freezer Storage

Once the genus and species of the isolated vineyard yeast is discovered through a third-party commercial identification service, the yeast strain can be sent to a strain bank where it will be stored at -80 degrees Celsius for future fermentations (see Figure 3 at the beginning of this chapter for Yeast Isolation Flow Chart Procedure).

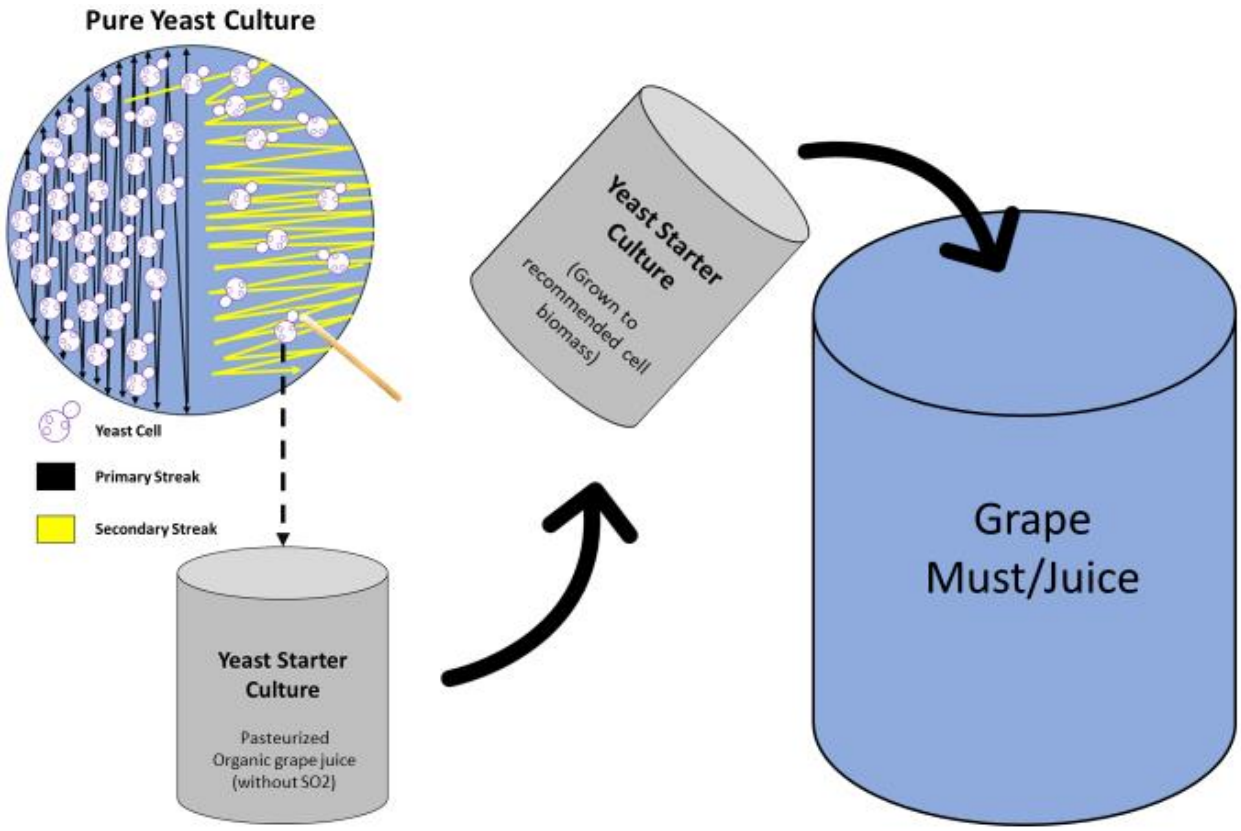

Figure 12. Inoculate Must/Juice: Using a sterilized toothpick, a strain is selected from the pure yeast culture, then grown to the recommended cell biomass and inoculated into a significant volume of grape must or juice. Scott Labs recommends an initial yeast starter cell concentration of 3–4 million (3×10^6) viable cells per ml of must or juice.

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GLOSSARY

Microbiome – All of the different microorganisms in a particular environment.
Microflora – Fungi, bacteria and all collective microorganisms living in a particular site, habitat or ecosystem.
Aseptic Technique - Using practices and procedures to prevent contamination from other unwanted microbes.
Morphology – Characterization of colony shape, size and color. For example, microbe identification by visual inspection of culture, such as colony shape/form and size (circular, irregular, rhizoid), color (white, cream, yellow, orange, pink, red), topography (flat, raised, convex, concave, umbonate), edge (entire, undulate, lobate, dentate, rhizoid) appearance (smooth, shiny, wrinkly, powdery) and texture (dry, fluid, mucoid, brittle).
Biomass - The total mass of organisms in a given area or volume.
Colony - A group of fungi grown from a single spore or cell on a culture medium.
Pure Culture – A culture medium that contains only one yeast strain growing in the absence of other species or types
Culture Medium – Otherwise known as growth medium, is a solid, liquid or semi-solid designed to culture and support the growth of microorganisms
Immersion Oils - Are transparent oils that have specific optical and viscosity characteristics necessary for use in high magnification light microscopy (greater than 60x magnification).