Project Number:

Apple Yeast Investigation

A Major Qualifying Project Report
Submitted to the Faculty of the:
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Degree of Bachelor of Science

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Abstract

This project investigated the microorganisms that naturally grow on apples, focusing specifically on yeast. The type of yeast cultivated were identified through DNA isolation, amplification, and sequencing. This report provides an overview of the methods that are used for yeast isolation and culture growth, which then allows for easier DNA identification. Upon yeast species identification, further research could then be conducted to provide insight on the potential benefits and uses in the cider brewing industry. The yeast was not found to have any harmful effects and may be further researched to determine potential uses downstream in cider brewing processes.

Table of Contents

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INTRODUCTION

The brewing industry experienced an increase in demand for various beer, wine, and cider products over the past decade. Alongside this increasing demand in product, there has also been a growing trend in consumer desire for variety. With the multitude of varieties, tastes, and flavors in beer, wine, and cider, local breweries are beginning to explore not only varied ingredients, but also varied yeast species to carry out the fermentation.

One of the major components of the brewing process is the fermentation stage, which requires yeast to convert sugars into ethanol. Yet, breweries have only begun to investigate the type and variety of yeasts in their fermentations. By using techniques such as DNA isolation and genome analysis, researchers can determine the different species that may alter the taste of certain products. This has been done for many domesticated yeast strains, but is not often done for the "wild yeast" fermentations that use yeast sourced from the local environment. It is possible that yeast identification can lead to improved processes and possibly domestication of wild yeast strains for industrial fermentation.

This report used several lab techniques to perform genomic analysis in order to identify yeast that naturally grows on apples that may also be involved in the brewing process of ciders. In order to accomplish this, apple samples were collected from a local apple orchard and were brought to the Young lab, located at Gateway Park, for further testing. Here, the apples were subjected to a series of yeast collection procedures to allow for yeast isolation and growth. Finally, the yeast cultures were purified and the genomic DNA was isolated and sent to a local company for sequencing. The sequence was then used to identify the species of yeast and further research was conducted in order to determine its potential uses in the brewing industry. Although there are potentially many different types of yeast that may find their way into the cider making process, this report will focus on the one species that was identified.

BACKGROUND

Hard Cider Brewing

The production of hard cider has been around for thousands of years and has become an increasingly desired product amongst other alcoholic beverages. The process of making this cider was originally very unpredictable, however, breweries, with the support of chemistry labs, have made advances in refining the fermentation process. As a result, many flavor signatures are being produced and refined every day.

The Apples

The general process of creating hard ciders first starts with collecting quality apples. Apples that are suitable for further processing usually vary from a diameter of eight inches to even less than two inches. However, in general, the size does not play a large role in the quality of the ciders but instead relies heavily on the ripeness of the apples. With high quality, ripe apples there is also a high level of juiciness and flavor that can be attributed to the natural acids within the apple. The skin of the apples is also carefully evaluated to ensure there are no defects such as rot or early fermentation.

Certain aspects of the growing process are also closely monitored in order to produce a high yield of quality apples. Many of these factors relate directly to growing conditions such as proper growing temperatures, adequate water and soil, and low levels of nitrogen, as it causes limitations in the fermentation process. If provided with proficient conditions, the apples are then able to grow adequately ripe and glucose rich.

In order for a cider to achieve a full-bodied flavor and taste, breweries often use a variety of apples in a single batch of cider, ultimately resulting in a range of flavor characteristics that work together to create one signature flavor. There are countless species of apples that may be used in order to achieve this signature brew but there are generally four types of apple juices used in the process, including aromatic, astringent, acid-tart, and neutral tasting juices. It is with these different juices and flavors that the breweries may then experiment with in order to achieve a multitude of flavors for their ciders.

The Chemistry and Fermentation

The general process of producing hard cider involves the fermentation of the natural sugars, acids and juices found in the varieties of apples chosen. The large amounts of glucose found in these juices are then further broken down into ethanol and carbon dioxide with the support of the yeast fermentation process. The chemical process is as shown below in equation 1.

$$C_6H_{12}O_6\ (Glucose) \rightarrow 2C_2H_5OH\ (Ethanol) + \ 2CO_2\ (Carbon\ Dioxide)$$

Equation 1: The Breakdown of Glucose

This fermentation process shown above is a two-step process in which the glucose is first broken down by the yeast present in the system. Following, lactic acid bacteria then break down the natural malic acid in the apples to form carbon dioxide. The yeast used in this process often naturally grows on the apples but can be extremely slow if relying solely on this natural yeast. Therefore many breweries resort to adding additional yeast to the process in order to speed up the fermentation and breakdown of glucose –called "pitching." When pitching yeast, as mentioned above, it is very important to classify and identify which yeast will have different effects on the flavors and tastes of the resulting ciders. Some breweries choose to add specific growth aids in order to promote yeast growth and speed up the fermentation. Some growth aids that are commonly added are yeast nutrients such as ammonium sulfate and thiamine along with other additives such as extra sugar, honey, or other sweeteners. While also increasing the fermentation rates, these additives produce a higher resulting alcohol content.

The Process

The cider making process consists of several steps beginning with the collection and ultimately ending in a final cider product.

A. First the apples are harvested when they have reached their prime level of ripeness, usually during the fall in the United States. During this season, workers are tasked to picked and gathering the apples to later be processed. They are all eventually loaded up and stored for about a week prior to processing in order to allow the apples to soften and the sugars and juices to build inside.

B. After softening, the apples are then washed and stripped clean of bacteria or additional chemical residue. The apples are then sent down a conveyor belt to further be examined and washed so to reassure that all the apples are thoroughly cleaned. Finally all of the apples are inspected and all rotten or damaged apples are removed from the belt, as these will have a negative effect on the cider taste.

C. The apples are then sent to a large mill and ground into a fine pulp. By doing this, the juices are completed extracted from the apples. Some of this pulp is then used and sent further into the cider making process while some is stored in a refrigerator for future processes.

D. Next, the newly ground up pulp is sent to a presser. Here, the pulp is sifted and then transferred to a large tray designed to hold the pulp in place for pressing. A press operator then puts the stack underneath the press, which then delivers a large amount of pressure using a hydraulic system.

E. The cider is then expelled from the press and pumped through plastic tubing into a cooling tank. While traveling to this tank, the pulp passes though wire mesh in order to further sift out any pulp pieces from the liquid product. The cooling tank then stores the cider and protects it from any contamination.

F. Fermentation is then ready to occur. Various fermentation-aiding chemicals are then added to speed up the process and allow for a more efficient fermentation. Depending on the brewer or manufacturer, the cider is then stored in either large individual tanks or smaller bottles. During fermentation, a yeast containing sediment is formed and is eventually filtered or siphoned out before reaching a final product. In bulk fermentation, the cider is siphoned off after the yeast has died.

G. Finally the cider is ready for filling and packaging. It is filtered once more and pumped towards the proper filling unit. A conveyor belt of sterile empty bottles passes under a filling machine, where the machine

pumps cider into the bottles to the desired volume. The caps are then put on the bottles and then labeled. These bottles are then packed and prepared for mass distribution.

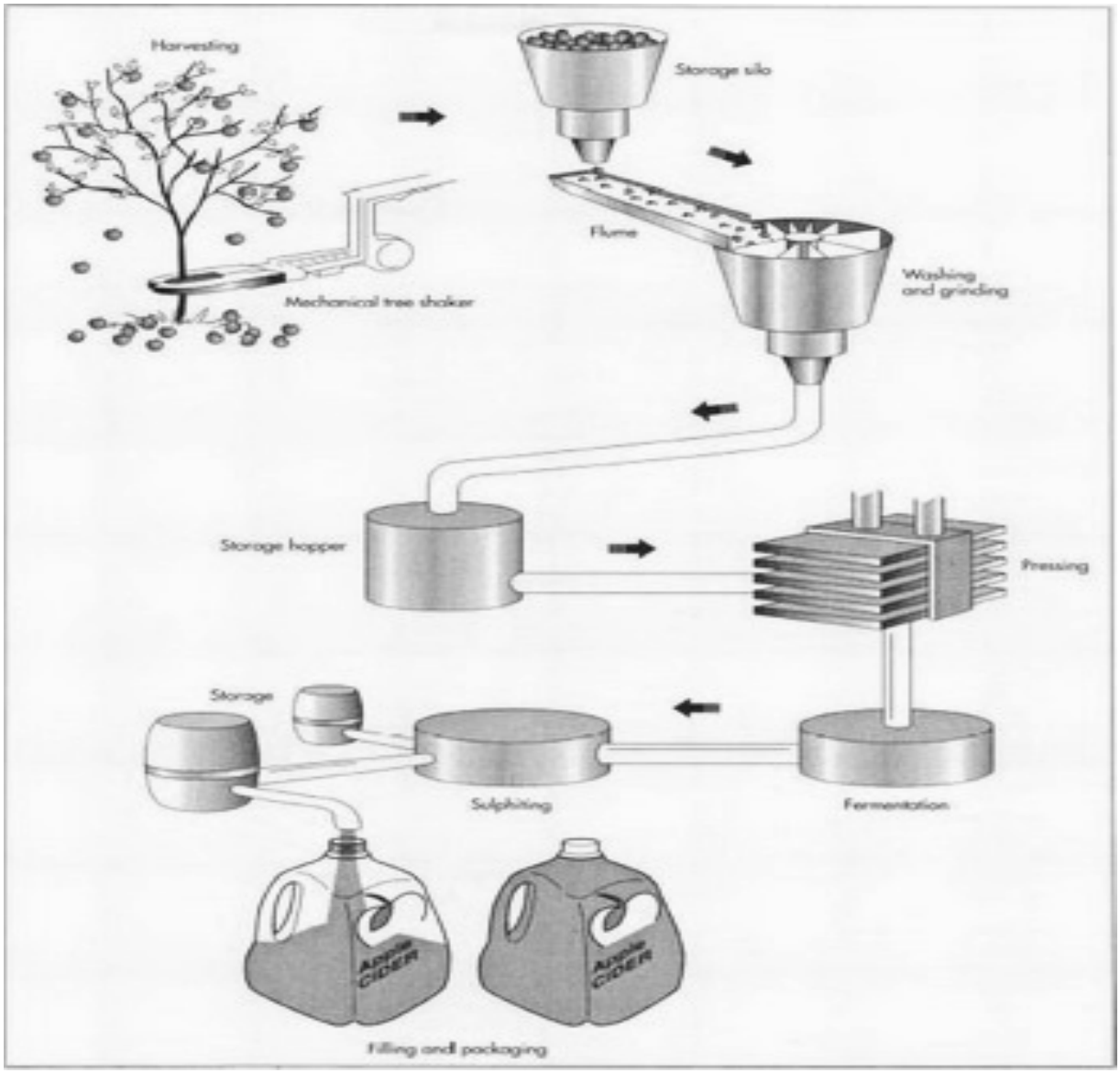

Figure 1: Hard Cider Production Process[1]

[1] "How Products Are Made: Cider". 2018. Made How: Vol. 4. http://www.madehow.com/Volume-4/Cider.html

Yeast

Yeast is a single celled microorganism that acts as a catalyst to breakdown sugars such as glucose, sucrose, fructose, and maltose. Using this very common biochemical reaction, brewers and scientists alike have used yeast to breakdown sugars found in fruits in order to create an alcohol content in their ciders or beers. Yeast is also very commonly found in bakeries, as bakers use the carbon dioxide to allow for dough to rise. Yeast also have other important medicinal uses and are continuously examined to determine further applications.

Types of Yeast

In general there are three individual types of yeast that can be found in our everyday environment, of which fall into one of the following categories: bakers yeast, nutritional yeast, and brewers yeast.

Bakers Yeast

Bakers yeast is generally the type of yeast that is used at home or commercially for bread, cakes, or pastry making. It can often be found in various forms and can be used for a range of baking and cooking methods. There are three types of yeast that typically fall into this category which consist of cake yeast, active dry yeast, and instant yeast. Cake yeast is found to be a form of "wet yeast" and is characterized to have high moisture content, allowing for cakes and other products to remain moist throughout baking. Alternatively, active dry yeast is processed one step farther than cake yeast, instead is providing with low moisture content, typically used for bread and other dryer products. Instant yeast is similar and is also used for baking breads, as it has a much more rapid "rising time" and allows for a much faster product. The species of yeast that fall into this category are generally the various forms of *Saccharomyces cerevisiae.*

Nutritional Yeast

Nutritional yeast, like bakers yeast, is a strain of *Saccharomyces cerevisiae* that has been refined and is usually in the form of a power or pills. This yeast is marketed and sold due to its high protein count along with its various vitamin contents. In fact, this nutritional yeast contains about 50% protein by weight and is a rich source of amino acids and B-complex vitamins. In order to produce and distribute this yeast, the strains of *Saccharomyces cerevisiae* are taken and grown on mixtures of cane and beet molasses. Once the fermentation is complete, the

leftover yeast, or “cream yeast”, is heated and held at pasteurizing temperatures. During this time, nutrients are pumped into the vessel to allow for further yeast growth by providing select nutrients corresponding to the species being grown. The resulting yeast is then dried and ground up for customers to be used as nutritional supplements.

Brewers Yeast

Brewers yeast has a multitude of species that are frequently experimented with in order to produce various flavors or signature tastes. However of the various types of yeast that are used, many of them belong to the *Saccharomyces* genus. However, the species does make considerable differences in the fermentation process. For example, *Saccharomyces cerevisiae* is a “top fermenting yeast”. This means that the yeast in fact floats at the top of the have the liquid product and ferments from the surface. This type of fermentation is often used for products likes ales, porters, stouts a, and wheat beers. On the other hand, there are also “bottom fermenting” yeasts such as *Saccharomyces uvvarum*, in which the fermentation occurs within the mixture instead. This process is often used for products like malt liquors and lagers.

Fermentation

Fermentation creates ATP, or adenosine triphosphate, which is used by yeast cells as an energy source. This process is anaerobic which means that it does not need the presence of oxygen. The entire process is illustrated below in figure 2.

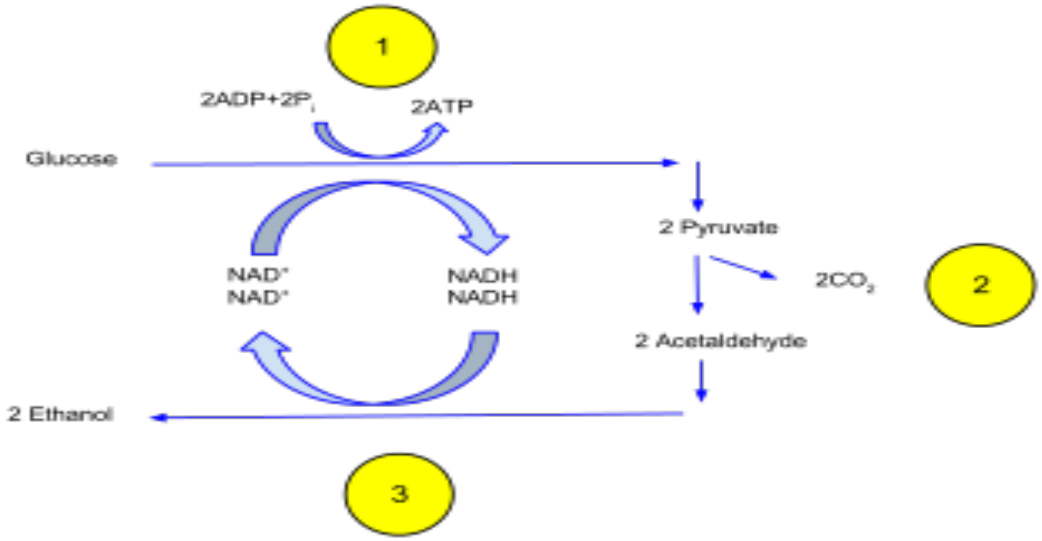

Figure 2: Breakdown of Glucose to Form Ethanol[2]

Overall, the first step of converting a single glucose to pyruvate will result in two pyruvate molecules, two ATP molecules, and two ADP molecules. During this same conversion, NAD+ (oxidized nicotinamide adenine dinucleotide) is reduced to form NADH. The pyruvate that is formed then releases a carbon dioxide molecule and becomes acetaldehyde. This molecule then oxidizes the NADH back to NAD+, which then allows for the process to repeat and another glucose molecule to be broken down. The final product of this process is ethanol, providing the alcoholic content to the ciders and beers.

Yeast Identification

Plating and Yeast Growth

For the purposes of researching this "wild yeast", it is grown grown on a media that contains the necessary nutrients to promote growth and survival. There are two forms of the media that are used, one of which is a liquid broth and the other is a solid agar plate. When using the liquid broth, it is used to wash the sample, while also washing many of the microorganisms with it. When using the solid agar, it is normally applied to a petri dish base. The type of media that is chosen typically corresponds to the microorganism that is being grown, as some medias perform better than others for specific species growth.

[2] "BioCoach Activity: Fermentation" Pearson, concept 5. 2018. http://www.phschool.com/science/biology_place/biocoach/cellresp/fermentation.html

YPD is the media type that was used when collecting the yeast for this report. It stands for yeast extract, peptide, and dextrose, which are the three key nutrients to promote further yeast growth. Other medias that also may be used for plating but for the purposed of this report, YPD will be a sufficient growth media. This assumption is made due to the lack of understanding of the various yeasts that may grow on the apple samples. As YPD will promote growth amongst a variety of yeasts and is non-selective, this assumption was justified and remained the ideal media.

DNA Isolation and Purification

Prior to DNA amplification, the DNA must first be removed from the cells and isolated from other materials. The steps taken in order to achieve this may vary, but overall consists generally of the ones being used in this report. First, the cell walls of the cells need to be broken up through chemical or physical means. To do so, there are several steps involving centrifuging, incubation, and various chemical additions that will allow for the breakage of the any materials that are not DNA.

Upon separating the DNA through various steps, it is imperative to use the proper section of DNA during the identification process. Therefore, in the case of yeast identification, the section of DNA that is used to identify the species and strains is the section known as Internal Transcribed Spacer (ITS) 1 to segment 4, when ready to use PCR.

PCR – Polymerase Chain Reaction

PCR is used for the overall amplification of specific DNA collected from a sample. PCR works by first using heat to separate DNA into single strands. Each separated strand is then replicated to form double strands of each using a heat resistant enzyme. The PCR machine will then continue this cycle, creating double strands from every separated strand of DNA. This process allows for the duplication of a very small amount of DNA and allows for easier identification.

METHODOLOGY

In this section of the report all of the procedures and equipment used to identify the yeast strains that were found in the different samples collected. All samples were collected from live apples at one of the local apple orchards of Worcester, Massachusetts using a variety of collections methods.

Apple Sample Collection

In order to have a more extensive range of data, two methods of collection were used for this experiment.

1. Samples were initially picked by hand from an apple orchard local to Worcester, Massachusetts. The apples were then immediately brought to the lab and stored at room temperature until ready for plating procedure.
2. Samples were picked using sterile bags, allowing for the apples to remain isolated from further contaminants. The apples were then stored at room temperature until ready for plating procedure.

Yeast Sample Collection

Three methods of sample collections were used for gathering yeast samples for this experiment.

1. A piece of apple skin was peeled and directly wiped onto a YPD agar plate.
2. The apple was subjected to the plating procedure below after swabbing a portion of the apples using an inoculation loop.
3. The apples were rinsed in a 10x YPD solution containing a small percentage of ethanol to eliminate the other microorganisms in the sample. The beaker being used for the apple is autoclaved and sealed prior, allowing for sterile experimentation. After YPD rinse, the apple was allowed to incubate at room temperature for 72 hours or until visible white substance (yeast clusters) was seen floating in the solution. A sample was taken and then subjected to the plating procedure below.

Plating Procedure

Materials:

- Inoculation Loops
- YPD Plates
- Pipetter
- Pipette: tips
- YPD Solution

An inoculation loop was used to remove a small sample from both the apple skin and from the floating cultures in the YPD media from the submerged apple. The pre-prepared YPD agar plates where then opened only enough to streak the plate according to the pattern shown in the illustration below. The initial inoculation, area A, should yield the heaviest yeast growth, followed by dense growth in area B, weak growth in area C, and single colony growth in area D. Both of these samples along with the sample that was directly wiped on the agar plate were then subjected to incubation for at least 24 hours.

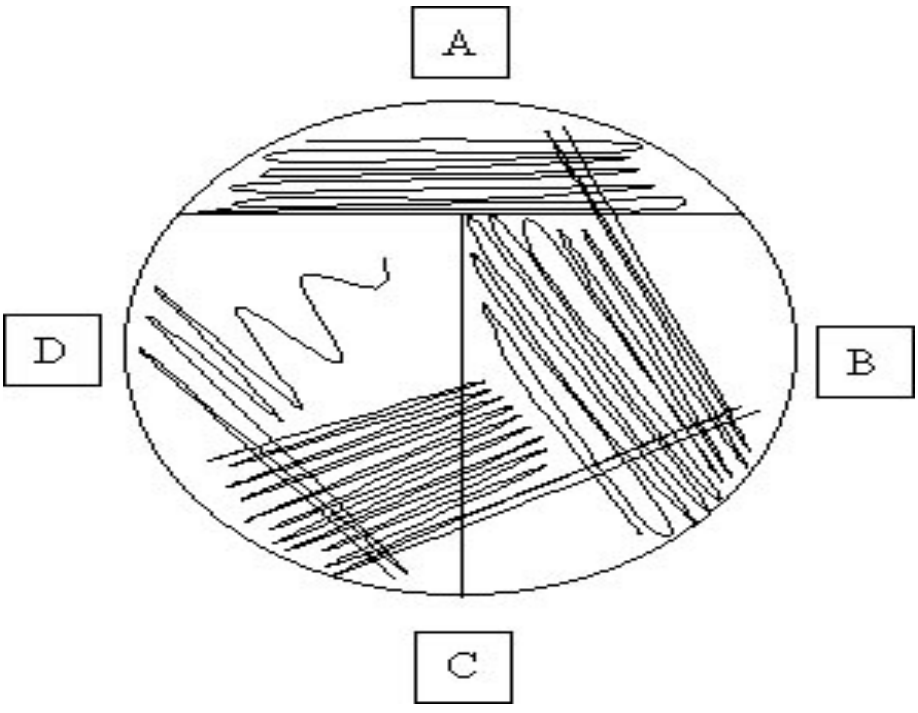

Figure 3: Agar Plate Streaking Pattern[3]

[3] "DIYeast: Isolating Yeast". *Bootleg Biology.*2018
https://bootlegbiology.com/diy/isolating-yeast/

In cases where individual colonies were not successfully isolated, the culture dilution procedure was used.

Culture Dilution Procedure

Materials:

- Falcon Tubes
- YPD Plates
- Pipetter
- Pipette: tips
- YPD Solution

1. Obtained Falcon Tubes and picked samples from the growth cultures on the agar plates using a pipette tip.
2. Transferred the pipette tips with yeast to the Falcon Tubes.
3. Pipetted 5 ml of YPD into each falcon tube.
4. Placed samples inside the incubator, distribute evenly along the wheel, and retrieve after 24 hours.
5. Re-plate each solution evenly using an inoculation loop and the procedure shown above.
6. Incubate plates for minimum 24 hours.

Contaminant Transplant Process

During the growing process, several other colonies or cultures also grew in the media. In order to isolate the yeast, these cultures were removed from the plate using a clean pipette tip and disposed of properly in the biohazard waste containers.

Colony Picking

Materials:

- YEP
- Dextrose
- DI Water
- 14 mL polypropylene round bottom tubes
- YPD broth
- Pipetter with tips

1. Make YPD broth by mixing 15g YEP, 10g Dextrose, and 500 mL deionized

water in a glass bottle and autoclave it to sterilize.

2. Fill 14 mL polypropylene round-bottom tubes with 5 mL YPD.
3. Pull single colonies from plates using a pipet tip and mix them into the tube.
4. Culture colony in a tube of YPD broth for 24 hours.

DNA Isolation

Materials:

- Micropipettes (2 - 20 µL and 200 - 1000 µL)
- 1.5 mL Microcentrifuge Tubes
- Eppendoff Minispin Centrifuge
- YPD Liquid Broth
- 50 mM EDTA
- Zymolase, resuspended with Nuclease-Free Water to a final concentration of 75 units/µl.
- (What is the name of the kit we used?)
- Ice Bath
- Room Temperature Isopropanol
- Room Temperature 70% Ethanol
- 31.1 nM ITS1 Primer
- 33.7 nM ITS4 Primer
- OneTaq® 2X Master Mix with Standard Buffer

1. Add 1 mL of the cultures grown in YPD solution to a 1.5 mL microcentrifuge tube.
2. Centrifuge at 14,000 rpm for 2 minds to pellet the cells and remove the supernatant.
3. Resuspend cells in 293 µl of 50 mM EDTA.
4. Add 7.5 µl of 75 units/µl zymolyase, and gently pipette 4 times to mix.
5. Incubate the sample at 37°C for 60 minutes to digest the cell wall. Cool to room temperature.
6. Centrifuge at 14,000 rpm for 2 minutes and remove the supernatant.

7. Add 300µl of Nuclei Lysis Solution to the cell pellet and gently pipette to mix.

8. Add 100µl of Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds.

9. Let the sample sit on ice for 5 minutes.

10. Centrifuge at 14,000 rpm for 3 minutes.

11. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube filled with 300µl of room temperature isopropanol.

12. Gently mix by inversion until the thread-like strands of DNA form a visible mass.

13. Centrifuge at 14,000 rpm for 2 minutes.

14. Decant the supernatant and drain the tube on clean absorbent paper.

15. Add 300 µl of room temperature 70% ethanol and gently inverted the tube several times to wash the DNA pellet.

16. Centrifuge at 13,400 rpm for 2 minutes. Carefully aspirate all of the ethanol.

17. Drain the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.

18. Add 50 µl of DNA Rehydration Solution.

19. Add 1.5µl of RNase Solution to the purified DNA sample. Vortex the sample for 1 second.

20. Centrifuge briefly in a microcentrifuge for 5 seconds to collect the liquid and incubate at 37°C for 15 minutes.

21. After incubating at 65 C for 1 hour, use the DNA Nanodrop to ensure there is at least 200 nanograms of DNA.

22. Store the DNA at 2–8°C until prepared for PCR.

PCR

These procedures were adapted from Promega.

1. Prepare primers through rehydration.

2. Add 311µl nuclease-free water to the 16S vial, making 100 mM solution. This primer is used to isolate the base sequence (5'-3') TCCGTAGGTGAACCTGCGG.

3. Add 337µl nuclease-free water to the 16S, making 100 mM solution. This primer is used to isolate the base sequence (5'-3') TCCTCCGCTTATTGATATGC.

4. Make a 10 mM solution of each primer by combining 10 µl in 90 µl of nuclease-free water.

5. Add 25 µl Master Mix, 2.5 µl of each primer, 2 µl of DNA sample, and 18 µl of nuclease free water to a sterile, thin-walled PCR tube to create a 50 µl reaction and gently mix.

6. Transfer PCR tubes to a PCR machine and thermocycle using the conditions below:

Step	Temperature (°C)	Time (s)
Initial Denaturation	95	30
Melting (30 cycles)	95	15
Annealing (30 cycles)	50	15
Extension (30 cyles)	53	120
Final Extension	53	300
Hold	10	When samples are removed

Table 1: Thermocycle Conditions

7. DPN1 digestion 1 uL at 37C for 1 hour.

8. Clean DNA.

9. Send samples and primers to Eton BioSciences DNA Sequencing service for DNA sequencing.

Genotyping the Yeast

The Eton BioScience genotyping results were sent back to the lab including a DNA sequence (.seq file) and the chromatogram file (.ab1 file) of the results for each sample (reverse and forward). In order to then determine the species, these sequences needed to be further analyzed using a set of programs and procedures. These steps are as follows:

1. Downloaded A Plasmid Editor program created by M. Wayne Davis using the following link: http://biologylabs.utah.edu/jorgensen/wayned/ape/2.
2. Opened the .ab1 files.
3. Removed part of the sequence that did not have high accuracy.
4. Copied the shorter sequence from the .seq file.
5. Pasted this sequence into the U.S National Library of Medicine's Basic Local Alignment Nucleotide BLAST Tool

(https://blast.ncbi.nlm.nih.gov/Blast.cgi)

6. Determined the species of the sequence, if results were found, using the query cover, E value, and identification percent. An example of sequencing results is shown below in figure 4.

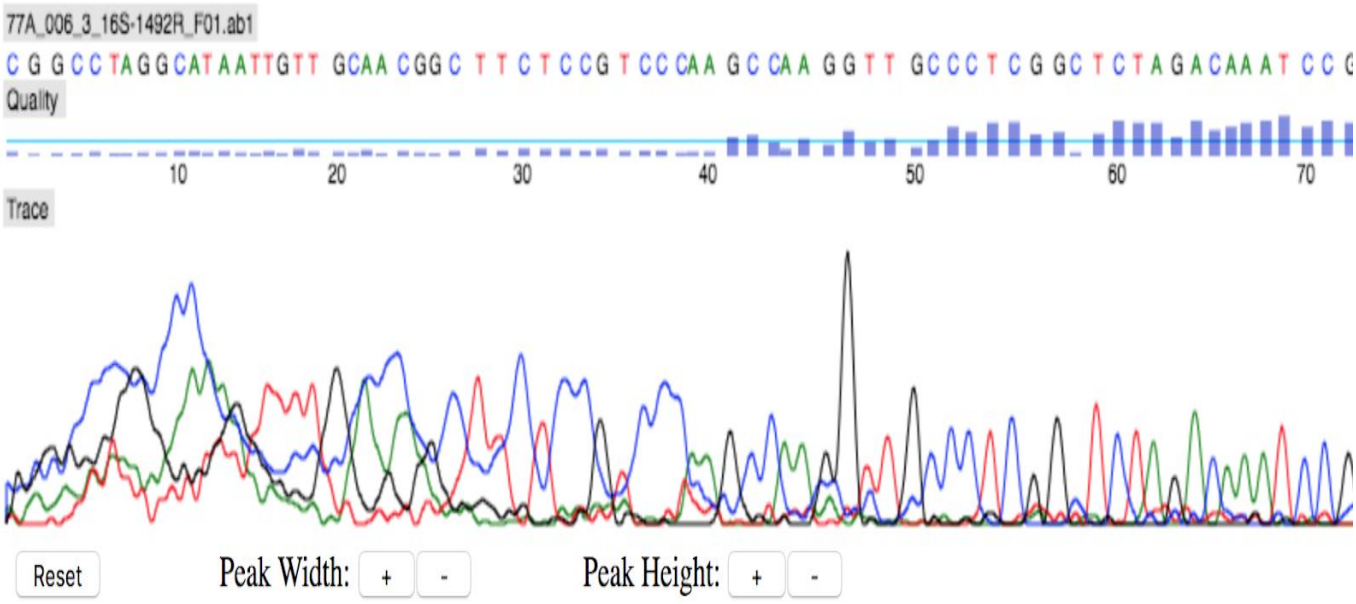

Figure 4: Sample Sequencing Results[4]

[4] Brogan, Garrett, Lucas Chico, Alexandra Rozen, and Ellen Thompson. 2017. North Country Hard Cider Yeast Investigation . Worcester Polytechnic Institute.

RESULTS AND DISCUSSION

Onsite Collection - Open Agar Plate

For the first collection of data, several pre-prepared agar plates were taken to the apple orchard. The agar plate was opened and allowed about a minute to collect any microorganisms in the air. The assumption of this test was that there would be a vast majority of yeast in the surrounding air and that much of the yeast would be attracted to the YPD media on the plate. However, this assumption was incorrect and instead there was small amount of microorganisms that were cultivated on the plate. Due to time constraints, the varieties of microorganisms were not isolated and identified. However, many of these species were assumed to be of the bacteria and fungi variety by simply testing using sight and smell. The colonies that did show similarities to yeast cultures were observed and picked for testing. Upon testing however, there were no signs of any DNA after multiple trials of the yeast isolation procedure and it was then assumed that the species was a form of bacteria and instead died during the procedure. An example of one of the plates is shown below in figure 5, illustrating the contamination of the plate.

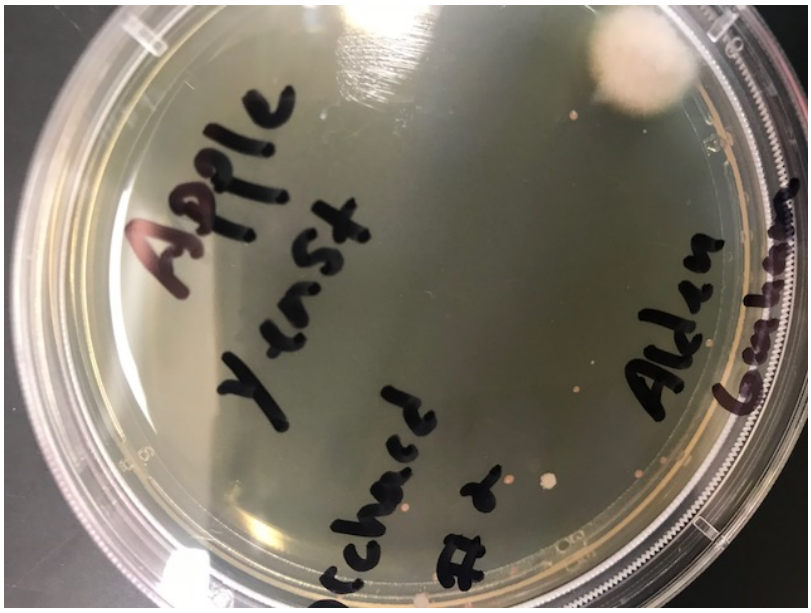

Figure 5: Plate Using Open Agar Plate Collection Method

Direct Skin Contact

The next collection method was to remove part of the skin and allow for direct contact with the agar plates. The agar plates were incubated for 24 hours in order to allow for culture growth. The resulting growth was very similar to that of the open agar plate results, as many other microorganisms contaminated the YPD media. However, the contamination of this plate was significantly greater to that of the open plate. Many of the cultures that formed had a very strong scent and appeared to resemble fungi or bacteria. As a result, the plate was only left with small cultures that appeared to resemble yeast. These cultures were picked and tested but again resulted in a lack of data. This again could have resulted from the false assumption that this species was yeast or the contamination of the yeast. Resulting agar plates can be seen below in figure 6.

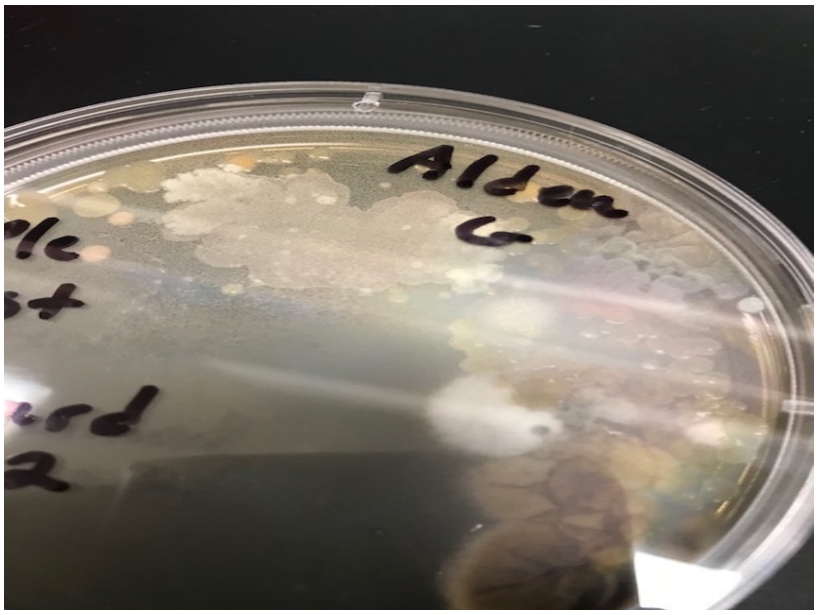

Figure 6: Direct Skin Contact Agar Plate

Swabbing

Another form of collection used was the swabbing of the collected apples. Also relating closely to the apple skin and open late methods, the swabbing resulted in a significant growth of other microorganism contaminants. However, the contamination for this method was much less overpowering and there were several cultures that appeared to resemble yeast. The DNA isolation however proved to lack in results and the different species tested were assumed to be bacteria instead. As a result, the cultures were tested once more but yet again turned out to be negative. Figure 7 below shows the resulting agar plate.

Figure 7: Swabbing Method Plate Results

YPD Wash

The final form of collection the use of a YPD wash that was used to rinse the apples while the runoff was collected and used as a growth media. The media was then allowed for 72 hours of culture growth and with the assistance of a small dose of ethanol, the contaminant microorganisms would theoretically be eliminated from the apples, as many of which are not as ethanol tolerant as yeast. This assumption proved to be correct and resulted and resulted in a large quantity of yeast growth. In fact, once the first batch of colonies was swabbed and streaked, it took three more attempts to re-streak the yeast, as it was extremely difficult to isolate a single colony for testing due to the massive amount of cells that were grown. Finally once the colony was isolated and picked, it was subjected to the purification procedure and identified. Figure 8 shows the successful results below.

Figure 8: YPD Wash Method

Yeast Profile

The resulting profile of the sequencing was naturally growing wild yeast called *Bettanomyces buxellensis*. Not as common as the *Saccharomyces* yeast involved in beer fermentation, *Brettanomyces* yeasts have, however, very recently become increasingly popular in many breweries in order to provide more variety to their beer selection. These yeasts, particularly *Brettanomyces bruxellensis*, are most commonly used in lambic-style beers, which are beers that are subjected to these wild yeast naturally, as opposed to the use of other cultivated strains of yeast. This style of brewing is known to contribute flavors that are not normally produced by *Saccharomyces* yeasts, in particular flavor signatures such as smoky, barnyard, spicy and medicinal. Additionally, the *Brettanomyces* yeast also has the ability to reveal and emphasize masked flavors through their production of β-glucosidases. The primary function of these enzymes is hydrolysis of cellobiose, which also then allows this yeast to stay in their fermentation containers for much long durations of time. Finally, this yeast, as it is naturally growing, generally has a long fermentation time. As a result the yeast often dies off during the aging process and the resulting beer solely maintains the flavor signature that the yeast provided. If any yeast has survived, it is usually processed out with a final product resulting.

CONCLUSIONS AND RECCOMENDATIONS

The yeast identification procedure yielded definitive results for the yeast that was found growing naturally on the collected apples. This species was found to be non-harmful and is commonly used in the beer brewing industry to provide a signature taste to their products. However, when collecting samples to be processed, there were several limitations and complications that intervened with data collection and measure should be taken in order to prevent future happenings. For the open agar plates, there were major contamination issues caused by a multitude of microorganism that seemed to overpower the yeast growth. In order to make adjustments to this process in the future, agar plates could be held open in a more controlled environment, allowing for a decreased chance in contamination. Similar issues were seen when using both the peel and the swab to collect cultures. The most promising results were found with the YPD rinsing of the apples. By rinsing the apples in an YPD-ethanol solution, the contaminants were eliminated almost entirely and the media was left with liquid suspended yeast cultures. However, although these results were the most promising, there was overall a lack of DNA when subjected to the DNA isolation. Therefore in order to gather more data in the future, experimentation should be done with the concentrations of ethanol in order to determine which concentrations results in solely yeast growth while also preserving larger amounts of DNA. Other experiments should also be conducted to further understand the affects of this yeast strand on both fermentation and overall flavor signatures of the ciders.

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APPENDICES

Appendix I: DNA Sequencing Data

Sample #	Species	Query Cover
1	*Bettanomyces buxellensis*	99.1%
2	*Bettanomyces buxellensis*	99%

Appendix II: Images- Contaminated/Inconclusive