**Yeast Growth and Analysis on Strawberries**

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**Abstract**

The goal of this experiment was to determine whether strawberries or raspberries grew the more yeast and have the most diversity. The fruits were crushed down and then plated to grow yeast. Those were then taken to be run through a PCR and Gel Electrophoresis and biochemical assays to find out what type of yeast was grown. The same type of yeast was found on both the raspberries and strawberries, showing the hypothesis was disproven. This makes sense since both organisms are fruits and grown similarly.

**Introduction**

Yeast is a eukaryotic organism, although it is more complex than bacteria, it is often a good organism to do studies on because it shares the same characteristics of bacteria that allow it to grow quickly (Sherman 2004).

This organism can be found everywhere, from water to bark, but where main focus in this experiment is on fruits, specifically strawberries and raspberries. On strawberries, there is a specific disease that is actually destroying them, called *Botrytis cinerea,* or commonly known as grey mold. In a study done by Pei-Hua Chen, and others, they stated that there were actually reports saying that yeasts are a good suppressor/preventer of grey mold on strawberries. The scientists went on to create an experiment that actually tests this and whether these yeasts inhibit grey mold growth, which was successful with certain strands of yeast, which around 900 strands were tested. In raspberries, there was also a study done that determined that yeasts are good organisms to bring about raspberry ketones, which are major impact sensory molecules in fruits, not just raspberries. This experiment also determined that yeast would be good organism to have biologically engineered to bring out fermentation properties of different fruits (Lee 2016).

To isolate the yeast from the original host, strawberries and raspberries, is to mash the fruits into a liquid and plated to grow the colonies to determine the morphological aspects of the yeast. Tests are then run to determine the different types of yeast that were collected. One of tests performed is a polymerase chain reaction, or PCR. What this test does is amplify small fragments of DNA of what was collected, in this case yeast DNA. Once this is done, a basic local alignment search tool, or BLAST, is run. What this tool does is compare different regions of DNA sequences or amino acids that are similar with each other. This also helps to find the DNA sequence to determine what kind of organism was collected.

When comparing strawberries and raspberries, strawberries would have more yeast species because of the bigger surface area compared to raspberries that have less.

**Materials and Methods**

Sampling collection, Dilutions and Plating of Yeast

Strawberries were collected and crushed using a mortar and pestle until a liquid solution is created. A clean 50 mL conical tube was collected and filled with the solution up to 5 mL, then 45 mL of distilled water was added. The tube was then shaken, to mix the water and the solution, and the pH of the solution was tested. This test tube was considered 100 dilution, or the original sample. Three sterile tubes were then collected, labeling them 101, 102, and 103. In each tube, 900 mL of sterile water was added. Once the water was added to each tube, including 100, 100 µL was extracted of the original sample, or stock solution, and transferred to the 101 tube. That tube was then vortexed, or mixed with the micropipette, and the tip of the micropipette was changed out. Extracted from the 101 tube was 100 µL that was transferred into the 102 tube, being vortexed by the micropipette. The tip was changed once again and 100 µL was then taken from the 102 tube and transferred into the 103 tube, which was vortexed with the micropipette again. Agar was then made called YPD, containing the antibiotics streptomycin and penicillin. Four plates of YPD were collected and each was labeled for each sample as original (100), 101, 102, and 103 with the date, the group’s initials, and the course section number. On the plate labeled 100, 100 µL were pipetted onto the middle of the plate. The sample was then spread with a glass ‘hockey stick’ until the liquid was absorbed by the media. This was repeated for each dilution on the rest of the plates that were prepared. All four plates were then incubated at room temperature (18ᵒ-20ᵒC) for two days.

Once the plates grew for the period of time, the samples needed to have isolated colonies grown for future tests. Looking at the different plates that had growth, they were observed to find which colonies, two for each sample as a whole, had good, separate colonies to test for future assays. Once those colonies were determined, another YPD plate with antibiotics was collected and the plates will be split into two sections, one for two different colonies from that particular sample. In each half, it will be divided into 3 different sections, labeled 1, 2, and 3 from top to bottom. A colony that is separated from the rest of the yeast that was grown is collected and streaked onto one half of the plate in the first section. The loop that was used was then burned by a Bunsen burner, to get rid of any cells that were on it, and then cooled for the next step. From the first section, two streaks were made from that and streaked into section two, the loop was burned again, and from the second section, two streaks were made into the third section, streaking throughout that entire section. This was repeated with a different colony of that same sample. The plate was then labeled, after all the streaking was completed, with the date, the student’s initials, and the course section and incubated at room temperature until colonies were formed. Once the colonies were formed, the plates were then placed in an incubator to prevent further growth until the students were to come in once again and observe them.

Counting yeast cells and Morphology

The students came back to class and the colonies that were on the plates were observed, counted, and recorded. To determine the number of “colony forming units” (CFU), the number of colonies were multiplied by the dilution factor (100, 101, 102, and 103) and that number was the number of bacteria in and 100 µL sample. That number was then multiplied by 10 to convert the units to CFU/mL and that number was recorded. After that, the cells were then observed for their morphology, or their shape, margins, elevation, size, texture, appearance, pigmentation, and optical property. A chart was provided in order to determine what determine the different characteristics. These morphological characteristics were then recorded and saved for future use.

Colony PCR and Gel Electrophoresis

An autoclaved microcentrifuge tube was collected and 30 µL of water was added to the tube. A colony, from the second set of plates, was chosen to extract DNA and using a sterile toothpick, was collected and swirled in the water. This was done for 20 seconds to allow the yeast cells to come off the toothpick and was disposed properly. An ice bucket was collected containing DNA template, IST4 primer, IST5 primer, 2X Master Mix, and water. A 200 µL PCR tube was collected and labeled with a group number, that was assigned at a different time. Into this tube, 10 µL of DNA template, 10 µL of primer IST4, 10 µL of primer IST5, 20 µL of water, and 50 µL of the Master Mix were added. The total volume should add up to 100 µL of liquid and a new micropipette tip was changed between the reagents. If bubbles were formed while adding these reagents to the tube, centrifuge the tube for 15 seconds to get rid of the bubbles. The tube was then placed into the PCR machine, which heats and cools the samples according to a pre-programmed profile. During the temperature changes, the number of DNA molecules doubles. The DNA will go through 30 cycles of going between 95ᵒC and 72ᵒC to melt the DNA, which exposes the nucleotides, allowing the primers to anneal at specific sites, and then replicate the DNA. Once the DNA has gone through this process 20 times, the DNA will sit at 4ᵒC indefinitely, until ready for use.

Once the PCR reaction is complete, gel electrophoresis was performed. The agar used was 1% agarose gel with 1X TAE buffer. The DNA solution from the PCR needed to be diluted, which allowed the DNA to sink to the bottom of the well in the gel. Provided, 7 µL of a loading buffer, which contains 2 µL of 6X loading buffer and 5 µL of water, was transferred to a centrifuge tube while 3 µL of the PCR reaction was added to it. Pipette the solution gently, trying not to create any bubbles, if bubbles are created, the tube needed to be centrifuged for about 20 seconds to get rid of them. The gel for electrophoresis was placed on the platform of the machine and then the machine was filled with 1X TAE buffer until the gel was covered. The wells of the gel should be on the negative side of the machine since DNA also has a negative charge, so when the assay is run, the DNA moves towards the positive side. That solution was then injected into a well in the gel and where it was placed was recorded so that the different DNA solutions that were also being tested would not be confused with another. The top of the chamber was then placed, careful to connect the red cables to the red power supply and the black ones to the black power supply. The test was then run for 30 minutes, or until the dye is a little over half way down the gel. The power was then turned off when those details were met, and the gel was then viewed. There was a control well, that was injected by the professor, that contained known base pairs, and the DNA that was being tested from the yeast was compared to the known base pairs.

Amplicon purification and DNA Quantification

A 1.5 mL tube was collected and labeled with the number that was given during the PCR reaction. To the tube, 450 µL were added of binding buffer and 90 µL of the PCR reaction sample was also added. They were mixed carefully by pipetting up and down. The mixture was then transferred, through pipetting, to a spin filter column, that is provided. That tube is then moved to the centrifuge, but careful to make sure the centrifuge is balanced when placing the tube. The tube was centrifuged for one minute, causing the DNA to stick to the white part of the spin column while the binding buffer and PCR reaction components pass through into the tube below. That liquid can be discarded into the sink. Into the spin column, 200 µL of DNA wash buffer were added and spun for one minute in the centrifuge as well. The liquid at the bottom, which carried proteins, salts, and other contaminants, of the tube can again be discarded into the sink. This was repeated one more time. A sterile 1.7 mL microcentrifuge was collected and labeled with initials, the date, and the PCR group number. The spin column was then transferred to the labeled tube, but the tip of the spin column was observed with caution to not touch anything else and get contaminated. To the center of the column, or the white part, 30 µL of elution buffer was added without touching the center directly. This solution was then sitting for one minute, allowing the DNA to be rehydrated and suspended, and then it was centrifuged for one minutes at 13,000 rpm. The DNA moved to the bottom of the microcentrifuge tube and the nanodrop was used to measure the DNA concentration (ng/µL) and A260 (absorbance of light at 260 nm)/A280 of 2 µL of the purified product. The nanodrop needed to be blanked using the loading buffer, not water. Those values were then recorded.

Biochemical assays

Temperature Assay:

Three YPD + antibiotics plates were given and, on each plate, a colony of yeast that were grown during the second round of plating, and the same one that the colony was used for the rest of the experiment, was plated onto the three plates. Each plate was labeled with initials, the date, and the temperature (RT-20ᵒC, 30ᵒC, and 37ᵒC) the yeast was incubated at. The room temperature plate was also the control for the salt assay, or 0M.

Salt Resistance Assay:

Two other YPD plates were also provided but were supplemented with 0.5M or 1.5M of salt (NaCl). The same cells that were used for the rest of the experiment were used and streaked onto each plate and place at room temperature to grow.

Amylase Assay:

A YPD containing 1% (w/v) soluble starch was provided and a colony of yeast, that was used in the rest of the experiment, was streaked onto the plate and left to incubate at room temperature. After colonies appeared, an iodine solution is added to the plate to detect any residual starch. The agar will naturally turn black/purple because of the iodine solution, but if there is no starch present, there will be clear portions around the colonies called a “Halo”.

DNA sequencing

The DNA was sent to a company called EurofinsGenomics to sequence the DNA to identify the type of yeast that was found of the different samples based on the rRNA gene. The company provided a limited number of sequencing primers, which is ITS1 or ITS4, which works with the samples that were created, so only the purified DNA was needed to be sent. 10 µL of the purified amplicon was transferred by the professor into a sequencing reaction tube. The bar-code and specific primer used for sequencing was told from the company and recorded.

Bioinformatics analysis

Basic Local Alignment Search Tool (BLAST) was used to compare the differences of DNA sequences between the yeasts that were grown.

**Results**

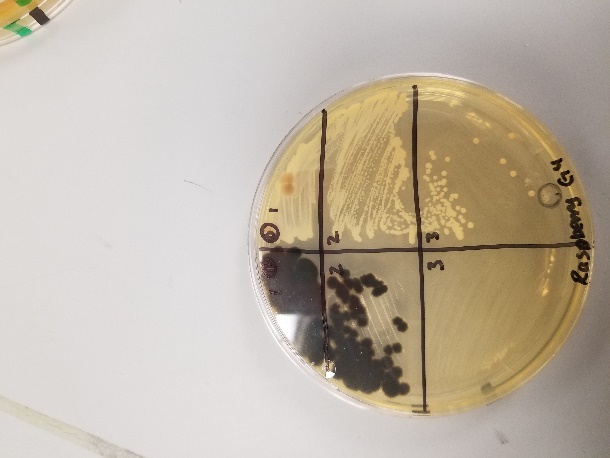
In **Table 1,** the morphology of the bacteria found on the Strawberries are shown. This table shows what the different yeast colonies looked like. The original solution, or 10ᵒ, had the most colonies out of all of the samples, and 10ᵒ2 and 10ᵒ3 did not grow anything. Two types of yeast that were plated, shown in **Figure 1,** which are two different species. One is much darker and more filamentous while the other is more of a cream/white color and circular. The one on the right is the one that was used for testing since it had more colonies and was easier to plate and gather colonies.

That colony of yeast, after being run through a Polymerase Chain Reaction (PCR), was observed doing multiple tests, the first being electrophoresis. In **Figure 2,** well 2, counting from bottom to top, contains the DNA of strawberries, well 3 is the marker, and well 6 is the positive control. This figure shows the amount nucleotides that are obtained in the strawberry DNA, which is close to the brightest band on the right of the marker, which is 500 nucleotides, so the strawberry DNA has between 600-800 nucleotides. In **Table 2,** a nanodrop was performed also on the DNA that was extracted, which is number 10. This table shows the nucleic acid concentration, in ng/µl, and the 260/280. The Nucleic Acid concentration in the DNA was 65 ng/µl and the 260/280 was 1.97, which are good numbers.

The colonies that were left over from the original plate were collected and plated into different environments. One of those environments being temperature difference, shown in **Figure 3,** which shows the growth of the yeast in the three temperatures, room temperature (20ᵒC), 30ᵒC, and 37ᵒC, side by side. The yeast grown in room temperature conditions grew the best, meaning having more separated colonies, the colonies found in 30ᵒC were a bit bigger that the room temperature, and the smallest colonies were found in the plate that was grown in 37ᵒC. One of the other environments was salinity. These plates are also shown side by side, as seen in **Figure 4,** and the same plate from the room temperature environment was also used in this comparison as the control for no salinity. The yeast grew on the plate with no salinity well, while the ones found on the 0.5 M plate were significantly small and there was no growth on the plate that had 1.5M salinity. There was another test done with Amylase, which is an enzyme that breaks down carbohydrates. **Figure 5** shows the results of the test, which were negative since none of the colonies had halos, or white spots, showing around them.

The DNA was sent to a company to determine the nucleotide sequence of the yeast that was sampled. **Figure 6** shows the nucleotide sequence that was obtained from the collected yeast. That nucleotide sequence was then fed through the BLAST system to find what yeasts would be most similar in nucleotides in the data of the BLAST website/company. **Figure 7** shows the top five hits that came up on the system when the nucleotides were run through. All the species that came up were from the same Genus, meaning they were all related, and the top hit, Uncultured *Hanseniaspora* clone CEgs1139 18S ribosomal RNA gene, partial sequence,was 94.88% similar to the yeast that was extracted. **Figure 8** shows the match up of nucleotides between the top five hits side by side. The gaps mean that the nucleotide didn’t match at all or there wasn’t enough data to support making a guess, which are the letters that are slightly less gray than the rest and lowercased letters. **Figure 9** shows a phylogenetic tree of different types of fruits that were tested as well. This figure compares that different yeast that were found on the fruits and puts them in a tree to show which ones are similar or in the same family, etc. PCR8\_Strawberry is the sample that was tested here. The closest similarity between the classes for DNA sequencing was yeast from a raspberry, and there was another yeast from raspberry far away from those two.

**Tables and Figures**



**Figure 1. The purification of yeast extracted from Strawberries.** The two yeasts depicted were two that were grown on the original plate and purified on another. The different sections were created for streaking purposes, starting from the top and pulling from the bottom, in order to isolate the colonies.

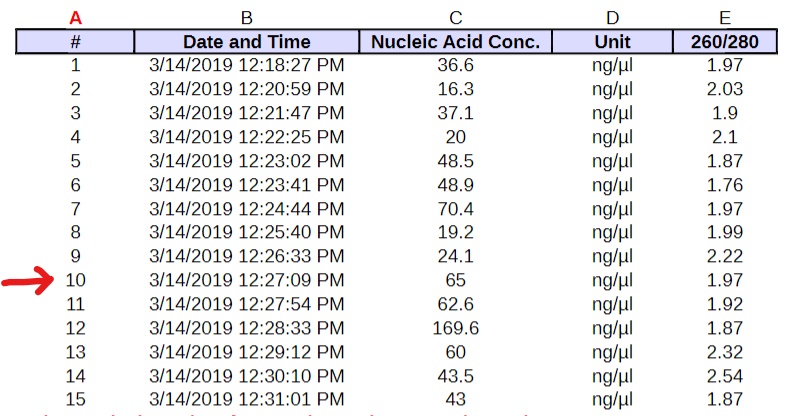
**Table 1. Morphology of Yeast Found on Strawberries.** The morphological aspects of yeast recovered from strawberries. The number of colonies, shape, margin, elevation, size, texture, appearance, and color were recorded from the original plates that were cultured.

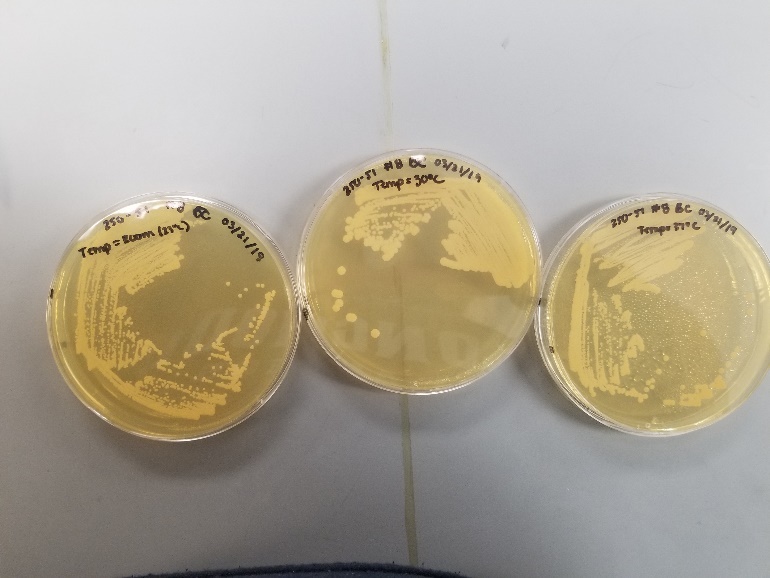
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strawberry | 10ᵒ | 10ᵒ1 | 10ᵒ2 | 10ᵒ3 |
| Colonies | 15 | 1 | 1 | 0 |
| Shape | Circular, irregular | Circular | circular |  |
| Margin | entire, filamentous | entire | entire |  |
| Elevation | convex, flat/raised | flat | convex |  |
| Size | all sizes | small | large |  |
| Texture | smooth, rough | smooth | rough |  |
| Appearance | shiny and dull | shiny | dull |  |
| Color | white, brown, cream | cream | white |  |



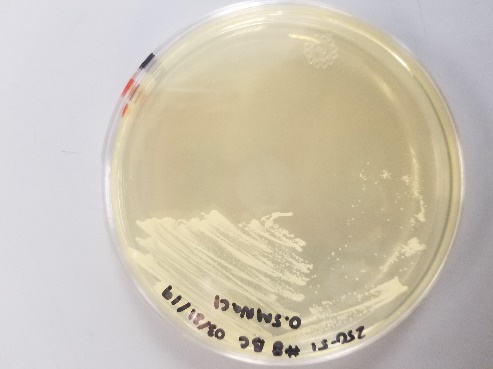
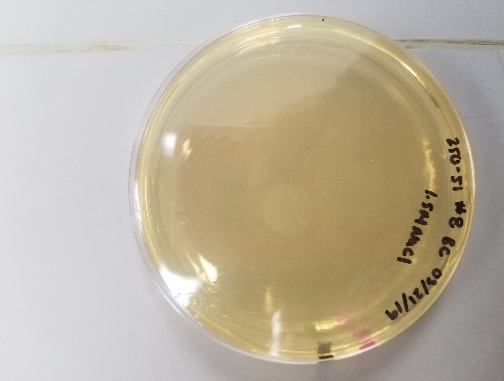
**Figure 2. Gel Electrophoresis on DNA extracted from yeast grown on Strawberries.** Gel electrophoresis was performed to determine the approximate number of nucleotides present in the DNA extracted from yeast that was grown on Strawberries.

**Table 2. Nanodrop of DNA extracted from yeast grown on Strawberries.** The nanodrop was performed to determine the nucleic acid concentration, with the unit, and the 260/280. The number 10 is the data that was collected during the experiment.





**Figure 3. Biochemical Assay: Temperature Change on Growth.** The yeast was grown at three different temperatures, room temperature (20ᵒC), 30ᵒC, and 37ᵒC, and colony growth was observed for change.

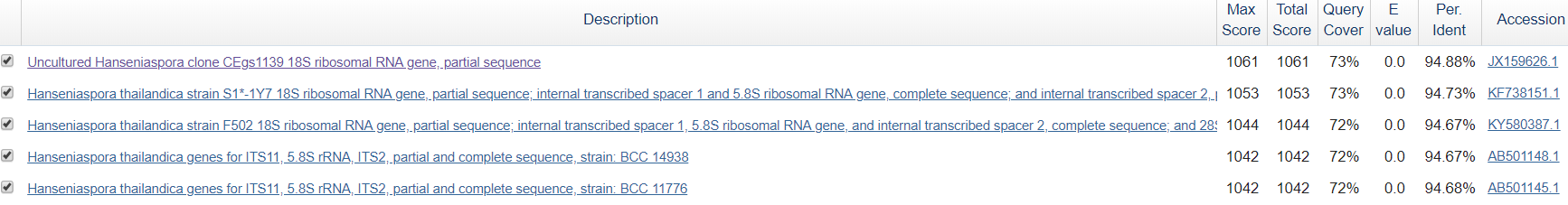


**Figure 5. Biochemical Assay: Amylase Presence in Yeast.** The yeast was grown at a normal temperature and salinity with starch on the plates for the yeast to eat. If Amylase was present, there would be halos around the colonies, showing that they were eating the starch, which the yeast presented is negative for.

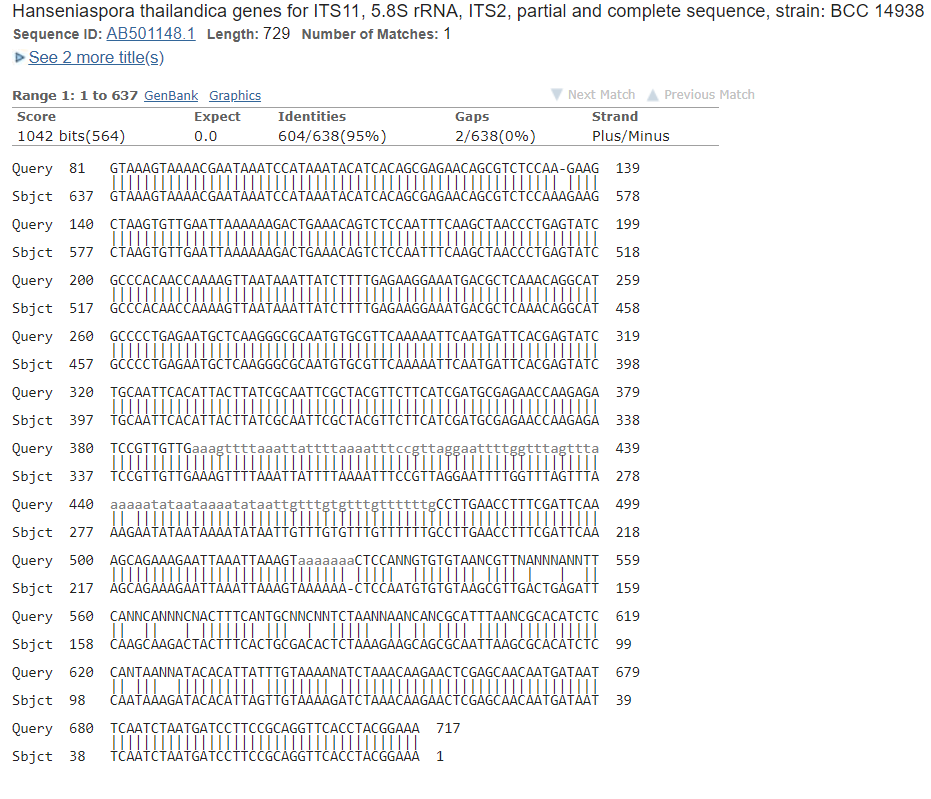
**Figure 4. Biochemical Assay: Salinity on Growth of Yeast.** The yeast was grown at three different salinities, 0M, 0.5M, and 1.5 M (left to right). The colonies were observed for changes in growth when exposed to these conditions.

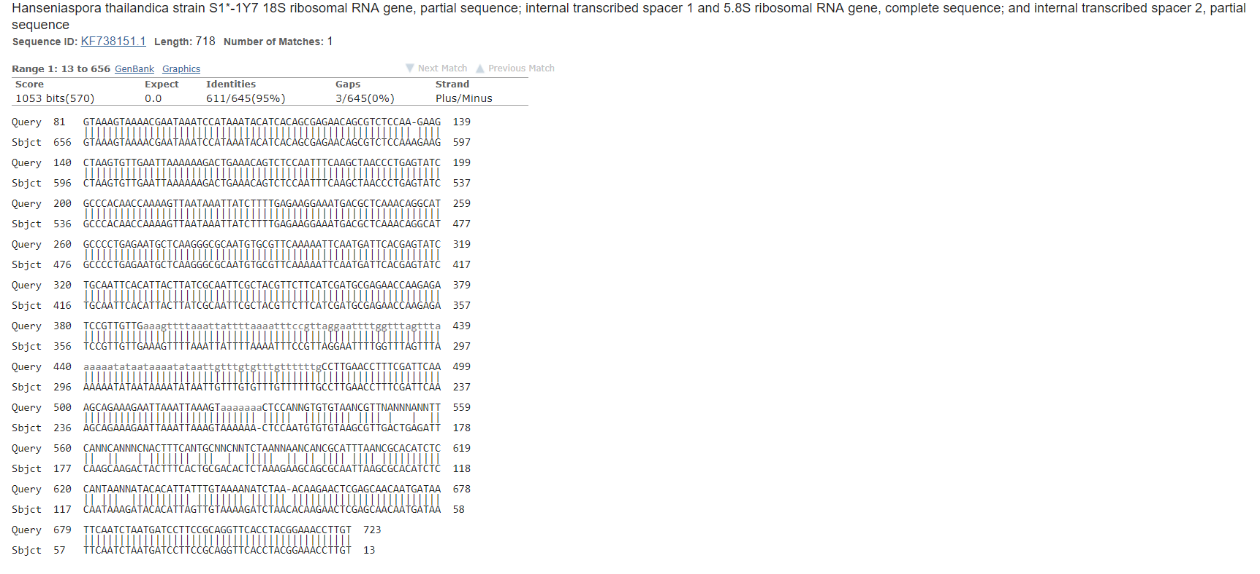
NNNNNNNNNNNNNNNNNNNNNGGNNAACTNNGANGANNNNAAAAGCAACCNNNNGCCTANNGNANNNNACCATNNNCCNNGTAAAGTAAAACGAATAAATCCATAAATACATCACAGCGAGAACAGCGTCTCCAAGAAGCTAAGTGTTGAATTAAAAAAGACTGAAACAGTCTCCAATTTCAAGCTAACCCTGAGTATCGCCCACAACCAAAAGTTAATAAATTATCTTTTGAGAAGGAAATGACGCTCAAACAGGCATGCCCCTGAGAATGCTCAAGGGCGCAATGTGCGTTCAAAAATTCAATGATTCACGAGTATCTGCAATTCACATTACTTATCGCAATTCGCTACGTTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTAAATTATTTTAAAATTTCCGTTAGGAATTTTGGTTTAGTTTAAAAAATATAATAAAATATAATTGTTTGTGTTTGTTTTTTGCCTTGAACCTTTCGATTCAAAGCAGAAAGAATTAAATTAAAGTAAAAAAACTCCANNGTGTGTAANCGTTNANNNANNTTCANNCANNNCNACTTTCANTGCNNCNNTCTAANNAANCANCGCATTTAANCGCACATCTCCANTAANNATACACATTATTTGTAAAANATCTAAACAAGAACTCGAGCAACAATGATAATTCAATCTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTNNNATTTTATCCCNNNCAAANNNAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNANNNAAAAANNNNNNNNNNNNNNNNNNNNNNA

**Figure 6. DNA Sequence of Nucleotides.** The DNA sequence of the yeast extracted from the Strawberries is shown.

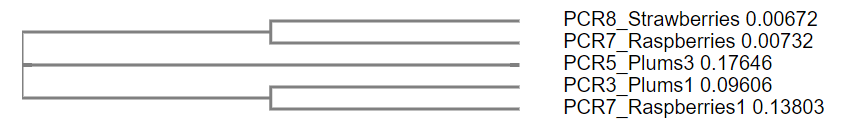


**Figure 7. Top Five Hits from BLAST.** Displayed are the top 5 hits from performing BLAST on the nucleotide sequence that was provided and the similarity between them.





**Figure 8. DNA Sequence Comparison.** Displayed are the top five yeasts and the comparison of their DNA sequences to the DNA sequence obtained from the Strawberries.



**Figure 8. Phylogenetic Tree Between Different Yeasts on Different Organisms.** Displayed is a phylogenetic tree of different types of yeasts that were found in separate experiments and how they are related to each other.

**Discussion**

The potential outcome of this experiment would be that strawberries have a larger surface area allowing for more variety of yeast found than compared to raspberries. This was rejected since for both the raspberries and strawberries, they were a part of the same Genus and the same ancestor based off the phylogenetic tree (the PCR7\_Raspberries was done by a colleague). The biochemical assays, in particular the salt assays, show that the yeast cannot grow in extreme temperatures. This shows that yeast do not have good responses to environments that they are not used to since they aren’t exposed to these environments on a daily basis. In the Amylase test, the test was testing to determine whether or not the yeast had the enzyme Amylase, which eats at starches. The yeast that was grown was negative for this, because Iodine, which was added to the plate, attaches to the starches, and if the enzyme was produced, there would be no starch surrounding the colonies, which causes a halo effect, and the yeast that was grown did not have this halo effect, meaning that the test was negative for amylase. When doing the DNA sequencing testing, the top five hits came up to show that they were a part of the same genus, which means that the yeast collected was also a part of that genus. The DNA nucleotides of all the types were very similar to the yeast that was collected, meaning the DNA that was created during the Polymerase Chain Reaction (PCR) was good DNA and had no broken pieces. The two types of yeasts that were purified at the beginning of the experiment showed the variation of yeast that was collected from the original sample.

Some limitations of the study were that the plating may have been done in a contaminated area, or not cleaned enough, the yeast didn’t have enough time to grow in normal temperature conditions after serial dilutions, and there is no knowledge how the berries were treated. What could be done in the future to better the experiment is to work in a lab that is clean, like a microbiology lab, allow the yeast to grow for more time, and grow the berries themselves without treating them so that there is more of an opportunity for yeast to grow on the berries.

**Works Cited**

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