The Analysis of Yeast in Soil of the High Bridge Trail in Farmville, Virginia

Biology 250

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**Abstract**

Yeast is a ubiquitous organism that can be found in a multitude of places on Earth, including soil. The goal of this project is to determine if multiple species of yeast can be found at the High Bridge Trail in Farmville, Virginia. Using a variety of both DNA analysis and biochemical analysis, soil samples from the trail were analyzed and sequenced. The yeast has traces of *Saccharomycetes,* and *Cryptococcus podzolicus,* so it can be concluded that the High Bridge Trail does have multiple species of yeast within it.

**Introduction**

Yeast is a common fungus that can be found in a variety of locations on the Earth. Yeast needs and uses sugars to grow. It produces ethyl alcohol and carbon dioxide (Renad *et. al.,* 2018). Some of the most usual places to find and isolate yeast from are soil, water, fruits, and even beer (Menna *et. al.,* 1957). Yeast has many different species which can result in some samples from the environment having multiple species. Some studies that included isolating yeast from soil had investigated the types of yeast that could be found in different locations (Paul *et. al.,* 2014). An example of this is a study conducted in the Alpine Forest in Italy (França *et. al.,* 2016). The environment where the sample was taken for this experiment was similar to this study because it was collected from the High Bridge Trail in Farmville, Virginia.

Yeast is important in different ecosystems because it helps with plant growth and even aids in breaking down dead plant roots and tissues. This is extremely important in soil and therefore makes the presence of yeast a positive thing. It helps with decomposition, as well as with nutrient cycling in soil heavy environments (Menna *et. al.,* 1957).

Several studies have concluded that using an environment similar to forests would show the most amounts of yeast. In other studies, this method of isolating yeast from forest soil results in obtaining yeast called *Dothideomycetes* and *Saccharomycetes* (França *et. al.,* 2016). In an experiment collecting yeast from a similar soil type, it is expected that similar strands of yeast would also be obtained from an equivalent sample. In another study, the effect of weather on the presence of yeast within the soil was conducted. The researchers performed many similar methods of isolation and found *Cryptococcus podzolicus* in wet cold environments (Mestre *et. al.,* 2011).

If soil is collected from the High Bridge Trail in Farmville Virginia, then it will contain several different types of yeast, including but not exclusively: *Dothideomycetes, Saccharomycetes,* and *Cryptococcus podzolicus*.

**Materials and Methods**

**Sample collection, Dilutions and Plating of Yeast**

This sample was collected from several soil beds in Farmville, Virginia on the High Bridge Trail during February of 2019. Three small plastic containers were used to transport the soil. A serial dilution was used to prepare the sample for plating. To a clean test tube, 5 milliliters of soil was added. The soil was removed, pressed firmly with a mortar and pestle, then placed back into the tube with a spatula. To the same tube, 45 milliliters of distilled water were added. This was repeated in triplicate. The pH of each tube was tested using pH paper and was recorded. For each sample, 3 centrifuge tubes were labeled 1-3. Then 900 microliters of the stock solution were added into the number 1 tube for each sample. Then 900 microliters of the diluted soil were added to the first tube for each sample. The stock solution and diluted soil were vortexed using a micropipette. Then 100 microliters of the mixture were pipetted into the second tube containing 900 microliters of the stock solution and vortexed for each of the three samples. This was repeated for the third tube for each sample. Then 12 plates containing Yeast Peptone Dextrose with Streptomycin and penicillin as antibiotics were prepared. A new sterile loop was used to streak each sample onto the plates. This was repeated for each plate. The plates were labeled with the date and which dilution they contained. The plates were left to grow and room temperature for three days.

**Counting yeast cells and Morphology (note that Morphology includes COLONY and CELL)**

The plates were then analyzed by counting the number of cells on each plate. This number was recorded. The plates were examined for the color, shape, and size of each of the suspected yeast colonies. To look at the individual cells, a microscope was used to examine parts of the samples. A wet mount slide was prepared to look at under a microscope using specific colonies from two plates. The qualities of the cells were recorded after examination under the microscope.

**Colony PCR, and Gel electrophoresis**

A polymerase Chain Reaction was used to amplify a short nucleotide sequence. To an autoclaved microcentrifuge tube, 30 microliters of water was added. A specific colony was chosen, and a toothpick was used to place some of it into the water. The colony was swirled into the water for 20 seconds. An IST4(5’ TCCTCCGCTTATTGATATGC 3’) and IST5 primer (5’ GGAAGTAAAAGTCGTAACAAGG 3’), or a fungal specific primer along with a master mix and water were placed into an ice bucket. To a 100-microliter tube, 10 microliters of the DNA template(yeast suspension),10 microliters of the IST4 primer, 10 microliters of the IST5 primer, 20 microliters of water, and 50 microliters of the master mix were added. The tube was placed into a PCR machine, a Thermal Cycling Device, and was stored in refrigerator for 1 week afterwards.

A small volume of the PCR solution was used for gel electrophoresis. A 1% agarose gel with 1X TAE buffer was prepared to run the electrophoresis. A loading buffer containing 2 microliters of 6X loading buffer and 5 microliters of water was used in addition to the DNA solution( from the PCR). The DNA had to be dyed with ethidium bromide in order to be seen on the gel before it ran as well. To the tube containing the loading buffer, 3 microliters of the DNA solution was added and gently mixed. This was then loaded into a single well on the gel. The gel was run at 120 volts for 30 minutes before being stopped. The ethidium bromide made the DNA visible under UV light which then was able to be quantified using the ladder image to approximate base pairs, and this was recorded.

**Amplicon purification and DNA Quantification**

In a labeled tube, 450 microliters of the binding buffer were added. Then 90 microliters of the PCR reaction were added to the tube containing the binding buffer. This was mixed slightly. This mixture was then transferred to a spin filter column that would bind to the DNA. This mixture was then placed into the centrifuged for 1 minute on 13,000 rpm. Then 200 microliters of DNA wash buffer were added to the filter and centrifuged again. This was repeated again. The filter column was transferred to a new tube. To the new tube, 30 microliters of the elution buffer was added. This mixture was then placed in the centrifuge one more time.

Then using a nanodrop, the DNA concentration of 2 (ng/ul) was measured and recorded as well as the A260 and A280 values.

**Biochemical assays**

A temperature sensitive assay was completed by using 3 plates containing YPD as well as the aforementioned antibiotics. Using a new sterile loop on each plate, a single colony was transferred and streaked onto separate plates for 1 week. The plates were labeled room temperature(RT), 30°, and 37°C. The room temperature plate served as a control for the other assays.

A salt resistance assay was completed using 2 YPD plates that had either 0.5M or 1.5M Salt (NaCl). Using a new sterile loop on each plate, a single colony was transferred and streaked onto separate plates. The plates were labeled and left at room temperature for 1 week.

An amylase assay was completed using 1 YPD containing 1% (w/v) soluble starch. Using a new sterile loop on each plate, a single colony was transferred onto the plate and incubated for three days at room temperature.

**DNA sequencing**

The sample was sent to an outside company, Eurofins Genomics, to be analyzed for the sequence using the primer IST4 and IST5 as specified before. They sent back the DNA sequence from the sample.

**Bioinformatics analysis**

Basic Local Alignment Search Tool (BLAST) was used to test for similarities between our DNA sequence and other known DNA sequences within the database.

**Results**

To determine the types of yeast that can be found on the High Bridge Trail in Farmville, Virginia, three samples of soil were collected in February of 2019. The collection site was muddy and mostly shaded. The soil had to be diluted in order to obtain the yeast. For sample 1 no dilution, there was yeast present and the colonies were white, round and convex. For sample 2, there was sufficient yeast and the colonies were white, round, and convex. For sample 3 and 4, there was not any growth. (Table 1).

In order to obtain more of the sample, polymerase chain reactions were conducted and then gel electrophoresis was performed. For the desired soil sample, the approximate number of base pairs was about 600 (Figure 1). The gels were fully loaded so it was important to label each one specifically (Figure 2). The sample was put through a nanodrop to test for the nucleic acid concentration and the 260/280. The desired soil sample used had 43.5 ng/l nucleic acid concentration. The 260/280 was determined to be 2.54 (Table 2).

Two colonies were chosen to perform chemical assays on. They are circled on the plate for clarity (Figure 2). The temperature assay was successful at each increment of temperature, growth was recorded on each plate (Figure 3). The amylase assay would form ‘halos’ around did not form around each colony after the iodine was added (Figure 4). The salt resistance assays the 0.5 NaCl moles plate had more growth than the 1.5 NaCl moles (Figure 5).

Using the Basic Local Alignment Tool, or BLAST, an alignment of several similar sequences is shown in Figure 8. The most similar strands are at the top of the figure in all red (Figure 7).

The top 5 matches to the sample are shown within Table 3. The percent identity for the number one match was 100%, while the number 5 match was 85.82%. The e-value for each of the matches was not significant (Table 3). For each match, the sequence that was similar was recorded in order to compare the base pairs for each (Table 4).

Figure 8 shows the phylogenetic tree between several High Bridge Trail sequences. The most similar sources branch off together from the original source (Figure 8).

**Table 1.** The morphology of the first plate is shown below. Diluted samples were streaked onto YPD agar growth plates and left to grow and were then examined for yeast abundance, shape, and color.

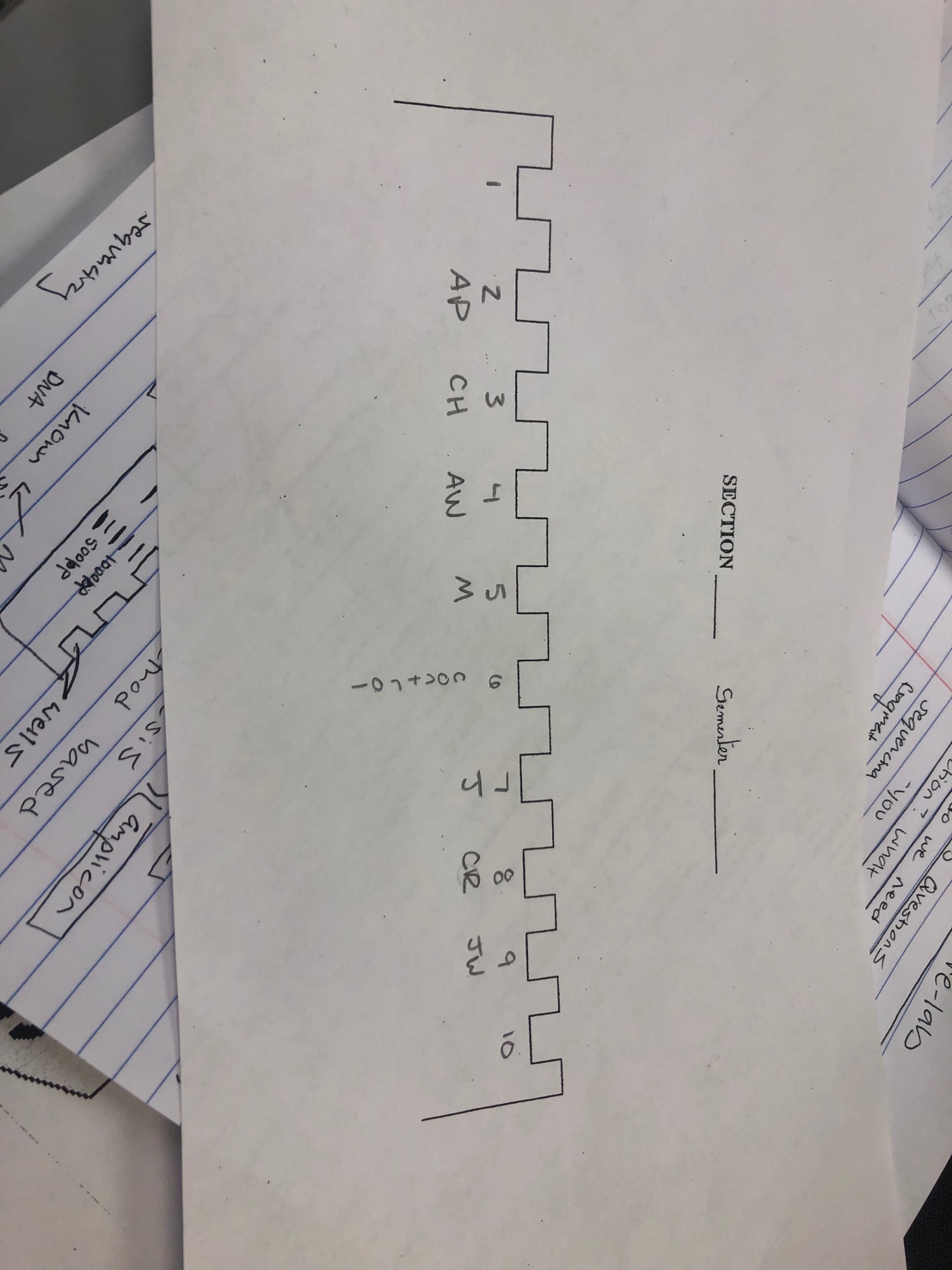
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | Dilution | Yeast | Circular | Convex | Color |
| 1 | 100 | Yes | yes | Yes | White |
| 2 | 101 | Yes | yes | Yes | White |
| 3 | 102 | No | no | No | No |
| 4 | 103 | No | no | No | No |



**Figure 1. Colony Polymerase Chain Reaction on potential yeast samples.**

The gel electrophoresis shows approximately how many base pairs that a sample may contain.

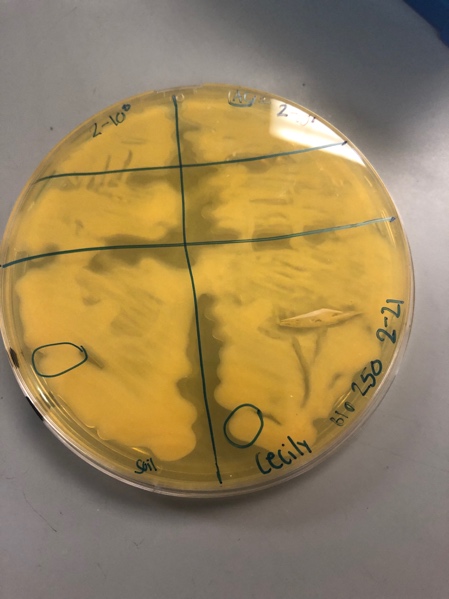
The well with the most marks, number 6, had the control which shows common markers.

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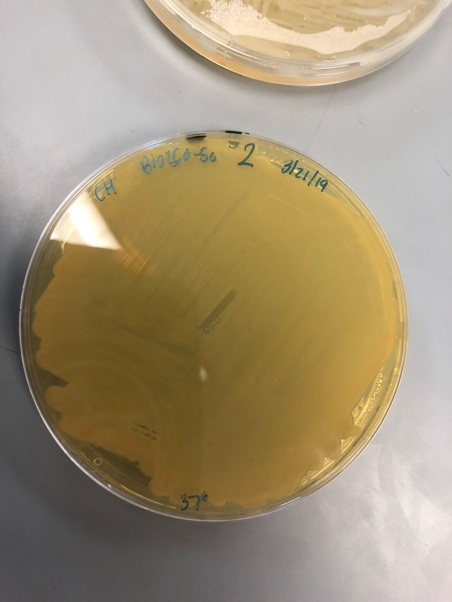
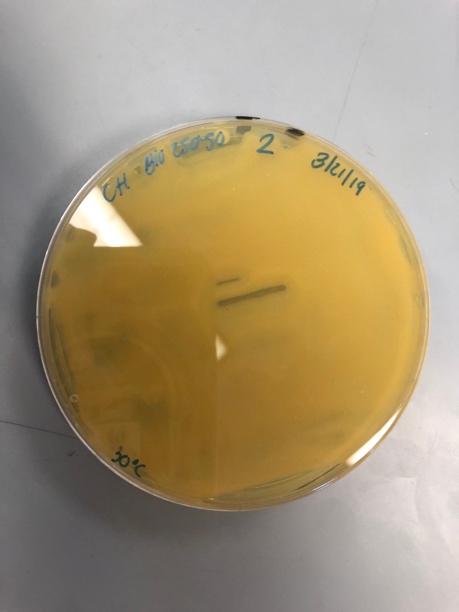
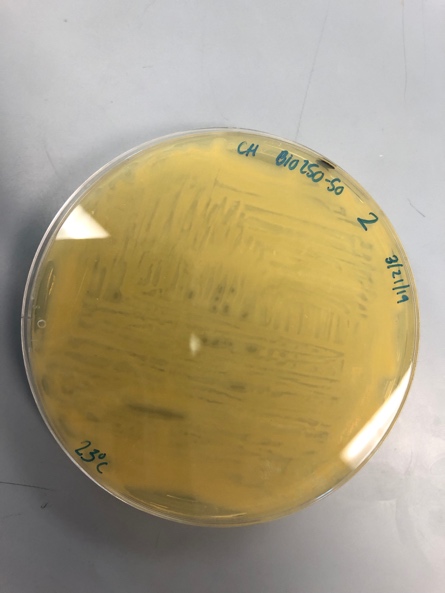
**Figure 2. Content of each well for Gel Electrophoresis.** Multiple samples of yeast were run on each gel, so the gel that contained the desired soil sample was well 3. The control was located in well 6.

**Table 2. The Nucleic Acid Concentration of the Sample via a Nanodrop.** The DNA sample was placed onto a nanodrop machine to read the acid concentration and the 260/280 percentage. The 260/280 shows how much light is reflected. This test was only run once.

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Nucleic Acid Concentration | Unit | 260/280 |
| Soil Sample | 43.5 | ng/l | 2.54 |

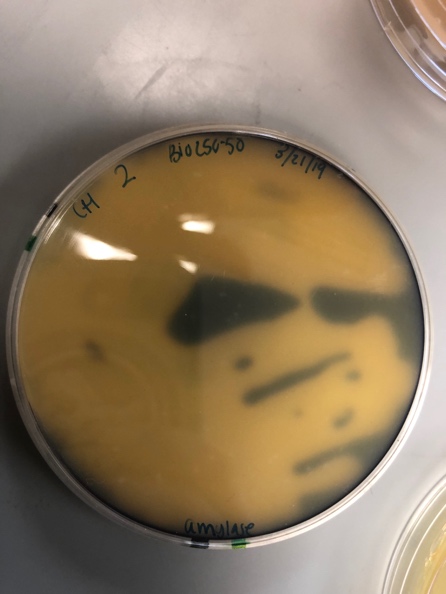


**Figure 3. Colonies that were selected to perform biochemical assays on.** More secluded colonies were chosen to perform the assays on from the growth plates.

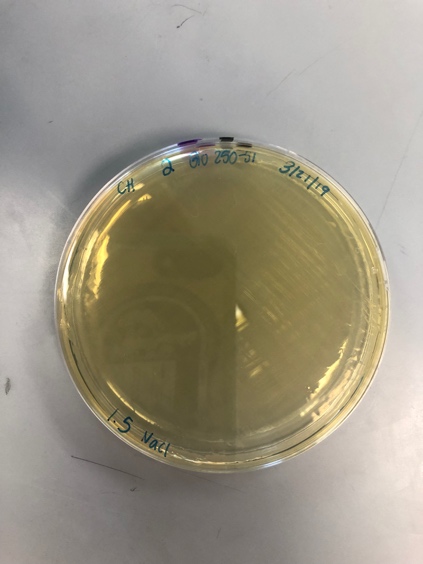
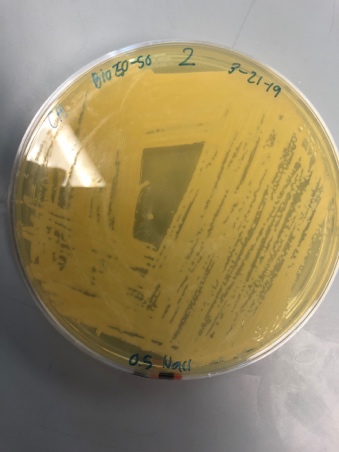


**Figure 4. Temperature Assays on yeast soil samples.** Temperature assays were performed at 25 °C, 30 °C

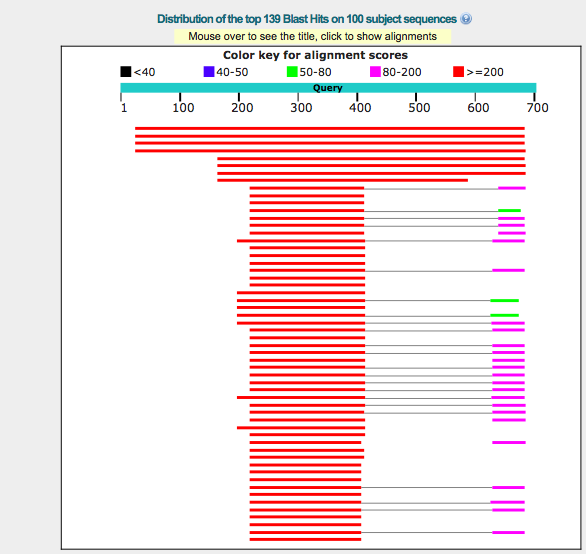
, and 37 °C.



**Figure 5. The amylase Assay on the yeast soil sample.** Iodine was added to the plate after less than a week of growth to test for consumption of starch.

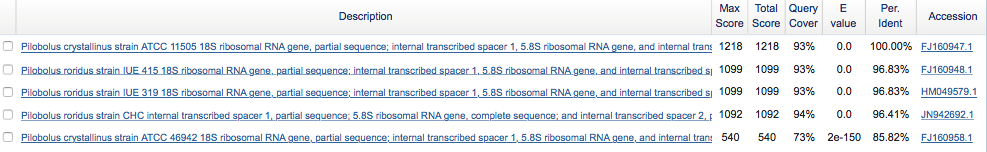


**Figure 6. The Salt resistant assay on the yeast soil sample.** Growth was recorded for both plates containing 0.5 NaCl and 1.5 NaCl.



**Figure 7. The alignment of the yeast soil sample against similar strands of yeast.** Using the Basic Local Alignment Tool, BLAST, the sequence from the PCR was compared to other similar strands and scored accordingly.

**Table 3. Top Five hits on BLAST compared to the original sample.** Percent identity and the E-value were recorded from BLAST for the top 5 matches.



**Table 4. The top 5 matches known sequences compared to the desired sample.** Using BLAST, matching sequences were identified and recorded.

|  |  |
| --- | --- |
| Sample Name | FASTA Sequence |
| High Bridge Trail sample | Nnnnnnnnnnnactgnngannancnttaattaaaacattccaactacaaactggctcggccggtctttcctgctgtaatgggggaaaggttgtccttgtcagggaatcctttactgtgaaatcttttttgaaatctttttgaaccttgccgtaatagcgaggcgagaaaagatgcccaaaaaattatactgaatacctattagtccttataaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataactattgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattaacccaacgccggccaatacttgttattgacctgggcgggacttttacgtcattgacgggaacagggcaaattagtttttgcttaacccgtttaaatactatttcttctttggccgacaataagtggggttcactgacagatataaaatcattagcgggtgcttttattagcctattttatattctggccttcgattctctcatggtttttggcgtgtccattgaacgaaacacattttttgatctgaaatcaagtgggattacccgctgaacttaagcatatcnnnnnnnngaaagaaaa |
| *Pilobolus crytallinus* Strain ATCC | Aacaaggtttccgtaggtgaacctgcggaaggatcattaattaaaacattccaactacaaactggctcggccggtctttcctgctgtaatgggggaaaggttgtccttgtcagggaatcctttactgtgaaatcttttttgaaatctttttgaaccttgccgtaatagcgaggcgagaaaagatgcccaaaaaattatactgaatacctattagtccttataaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataactattgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattaacccaacgccggccaatacttgttattgacctgggcgggacttttacgtcattgacgggaacagggcaaattagtttttgcttaacccgtttaaatactatttcttctttggccgacaataagtggggttcactgacagatataaaatcattagcgggtgcttttattagcctattttatattctggccttcgattctctcatggtttttggcgtgtccattgaacgaaacacattttttgatctgaaatcaagtgggattacccgctgaacttaagcat |
| *Pilobus Roridus* Strain | Aacaaggtttccgtaggtgaacctgcggaaggatcattaattaaaacattccaaatacaaactggctcggccggttttctctactgtaatggggggaaggctgtccttgtcagggaatcctttactgtgaaatcttttttgagtctttttgagtcttgccgtaatagcgaggcgagaaaagatgcccaaaaaattatactgaatactattagtccttaaaaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataactattgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattcacccaacgccggccaatatttatttattgacctgggcgggactttacgtcattggcgggaacagggcaaattaggttttgcttaacccgtttaaatactatttcttctttggccgacaataagtggggttcactgacagatataaaatcattagcgggtgcttttattagcctattttatattctgcccttcgattctctcatggtttttggcgtgtccattgaatgaaacacaattttttgatctgaaatcaagtgggattacccgctgaacttaagcat |
| *Pilobus Roridus* Strain IUE 319 | Aatgatccttccgtaggtgaacctgcggaaggatcattaattaaaacattccaaatacaaactggctcggccggttttctctactgtaatggggggaaggctgtccttgtcagggaatcctttactgtgaaatcttttttgagtctttttgagtcttgccgtaatagcgaggcgagaaaagatgcccaaaaaattatactgaatactattagtccttaaaaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataactattgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattcacccaacgccggccaatatttatttattgacctgggcgggactttacgtcattggcgggaacagggcaaattaggttttgcttaacccgtttaaatactatttcttctttggccgacaataagtggggttcactgacagatataaaatcattagcgggtgcttttattagcctattttatattctggccttcgattctctcatggtttttggcgtgtccattgaatcaaacacaattttttgatctgaaatcaagtgggattacccgctgaacttaagcat |
| *Pilobus Roridus* Strain  CHC | cgtaacaaggtttccgtaggtgaacctagcggatgacggatggcattaattaaaacattccaaatacaaactggctcggccggttttctctactgtaatggggggaaggc tgtccttgtcagggaatcctttactgtgaaatcttttttgagtctttttgagtcttgccgtaatagcgaggcgagaaaagatgcccaaaaaattatactgaatactattagtccttaaaaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataacta ttgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattcacccaacgccggcaatatttatttattgacctgggcgggactttacgtcattggcgggaacagggcaaattaggttttgcttaacccgtttaaatactatttcttctttggccgacaataagtggggttcactgacagatataaaatcattagcgggtgcttttattagcctattttatattctggccttcgattctctcatggtttttggcgtgtaccacttgtaacgaaacacaattttttgagtctgaaatcaagtgggattacccgctgaacttaagcatatcaa |
| *Pilobolus crytallinus*  *ATCC* | Aacaaggtttccgtaggtgaacctgcggaaggatcattaattaaaaacattctacccaaccggttttaaggtggttttcttctgtaatggttgaaaattccttttgccggggaatcctttactgtgaaatccggatttgatttctttttcggccatttatttgactggaaaagaaagcccaaacaatattataccgaatatattagaccttttaaatgttataaacaatcagggtaaaagctgattaaaacaacttttaacaacggatctctaggctctcgcatcgatgaagaacgtagcaaattgcgataactattgtgaattgcatttcgtgaatcatcaagtttttgaacgcatcttgcactctgtggtattccgcagagtacacttgtttcagtatcatattcaccccaacgccggtcaattttttttgttgacatgggcgggacactagtcattgatgggaacagggcaaattccgttttgtttaacccattgaaattctaatgctttattctttggccgaaaaacttttggttcattgatagtaataaaattattaaatgctttttactaagcccattttatttctatctaatgtttctcatggtttggaggattcatttaagaataacaaaacaaattttttgatctgaaatcaagtgggattacccgctgaacttaagcat |



**Figure 8. The phylogenetic tree for the soil sample versus other similar samples.** By using BLAST, this tree was generated which shows the similarities between different soil samples.

**Discussion**

The hypothesis was supported because multiple species of yeast were found within the samples that were tested. The *Saccharomycetes,* and *Cryptococcus podzolicus* were both found within the sample which supports the hypothesis. There were some possible limitations in this study that may have altered the results.

For example, the dilutions of the soil may have caused the last two plates to not contain any yeast . Too big of a dilution might have caused no yeast to be plated on the higher solution plates (Table 1) (Menna *et. al.,* 1957).

A second example could be that the PCR reaction may have been hindered because the samples of yeast might have been the same or contaminated (Figure 1) (Mestre *et. al.,* 2011).

A third example would be the nanodrop test only had one trial so it is possible that the given reading is inaccurate and would most likely change if run again (Table 2). The chosen colonies for the assays may have been too close to other colonies and thus might not have been the best choice to use. It is also possible that even though the colonies were separate enough to get accurate readings. (Figure 3) (Paul *et. al.,* 2014).

The biochemical assays were quite successful in determining the abundance of yeast in the soil sample [1]. The temperature assay showed that the yeast was capable of surviving in both normal and somewhat extreme temperatures, which begins to support the hypothesis in question (Figure 4). The amylase test was less successful because the colonies that grew were very close together and were unable to produce the halo needed to determine if the starch was gone (Figure 5). The salt resistance assays were successful because the yeast grew better on the lower concentration (Figure 6) (Yurkov *et. al.,* 2017).

The BLAST tool allowed for further identification of the yeast found [6]. The alignment allowed for a visual representation of how similar the matches were to the original DNA sequence (Figure 7). The top 5 hits had many similar qualities, like being in the Pilobolus family[2]. They also almost all had 100 percent identity which provides some good insight as to how close this is to the original sample (Table 3) (Thapa et. al., 2015).

The number of matching base pairs is best shown by comparing each by using a set up similar to FASTA, or the Federal Acquisition Streamlining Act (Table 4). But this approach may lead to minor errors in reading base pairs. The phylogenetic tree could be improved with more samples and would need more data to conclude if these findings are truly correct (Figure 8).

References:

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