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BIOL 250

Yeast Project Research Proposal

The aim of this research is to isolate and identify different species of yeast from three distinct water sources in the Farmville area. Observing differences in the abundance and composition of yeast in these water sources may give insight into the types of environmental and human influences acting upon our public water sources. Since all three water sources are used regularly for public recreation, identifying some of the microbial communities present in the water has implications for public health. We are interested in answering the question of how the diversity and abundance of yeast differ across our water sources. We hypothesize that since each water source is subject to different human and environmental impacts, the composition of yeast will differ between each water source.

The methodology for this project includes sample collection, sample isolation, plating, incubation, and sample analysis. Samples were collected from the shores of each water source using three sterile containers. Samples were collected during the afternoon on the same day at Wilke’s Lake, Sandy River, and Briery Creek. Sample isolation techniques will include dilution and filtration of each water sample followed by a streak-plate procedure (Van Wyk et al, 2012) onto an YPD + Streptomycin + Penicillin-G media. Each sample will have four replicates, all being incubated at 25°C for seven days (Svagzdiene et al, 2010). Samples will be analyzed by characterization of morphological traits, a genetic assay, and biochemical assay. Morphology will be characterized by color, shape, texture, and other physical features (Oshea & Walsh, 2006). A genetic assay will be performed using a PCR reaction to extract the yeast DNA followed by a BLAST analysis as a means of DNA sequencing (Gadanho & Sampaio, 2005). The biochemical assay will involve a colorimetric test, test for substrates, and tests for susceptibility. All biochemical tests will be compared to a negative control for assessment.

Although all three types of analysis will prove valuable, performing a biochemical test on the yeasts collected from different water sources will provide insight into the water sources’ possible ecological or biochemical differences (Gadanho & Sampaio, 2005). For example, if a certain location(s) produces yeasts that exhibit high tolerance to different biochemical stresses (changes in pH, temperature, salinity, etc.) then those yeast have probably evolved high tolerance due to their environments. Although a morphological and genomic analysis will allow us to identify the species of yeast present in our samples, the biochemical assessment may give insight as to *why* certain types of yeasts may exist in their respective environments.

Bibliography

Gadanho, M. and Sampaio, J.P. 2005. Occurrence and Diversity of Yeasts in the Mid-Atlantic Ridge

Hydrothermal Fields Near the Azores Archipelago. *Microbial Ecology, 50*(3): 408-417.

The goal of this research was to survey yeast community associated with the deep-sea hydrothermal systems of the Mid-Atlantic Rift. Although the sampling methodology varied greatly compared to the procedure for our study, this study used very similar identification techniques compared to the ones we will employ in lab, including centrifugation and PCR testing. The researchers were able to isolated various species of yeast and distinguished between pigmented and non-pigmented yeasts. Non- pigmented yeasts included Candida atlantica, C. parapsilosis, C. atmosphaerica, C. lodderae,Exophiala dermatitidis, Pichia guilliermondii, and Trichosporon dermatis. Pigmented yeasts included R. sphaerocarpum, R. toruloides, Rhodosporidium diobovatum,, and Rhodotorula mucilaginosa. The researchers then performed a phylogenetic analysis on their identified yeasts, a test are not currently planning to perform during our sample analysis, yet one that may prove helpful in further categorization and identification of our yeast samples. Thirty three percent of the total yeast taxa found at the Mid-Atlantic Ridge hydrothermal fields was comprised of new phylotypes. This project proves useful because it also relates the evolved biochemical properties of these hydrothermal yeast to their extreme location, which gives evidence to the fact that yeast samples from different locations often exhibit different biochemical properties because of adaptations developed in response to environmental changes or stresses.

[Garrels,](https://search-proquest-com.proxy.longwood.edu/biologicalscience1/indexinglinkhandler/sng/au/Garrels,+James+I/$N?accountid=12144) J.I. 2002. Yeast genomic databases and the challenge of the post-genomic era

[*Functional & Integrative Genomics*](https://search-proquest-com.proxy.longwood.edu/biologicalscience1/pubidlinkhandler/sng/pubtitle/Functional+$26+Integrative+Genomics/$N/26871/PagePdf/219941826/fulltextPDF/54D75020CD4B4A63PQ/3?accountid=12144)*,* [*2*(4)](https://search-proquest-com.proxy.longwood.edu/biologicalscience1/indexingvolumeissuelinkhandler/26871/Functional+$26+Integrative+Genomics/02002Y09Y01$23Sep+2002$3b++Vol.+2+$284$29/2/4?accountid=12144): 212-37.

As a secondary source, this paper does not provide a methods or results section, but rather compiled a genomic database for yeast. The database presented in this paper includes an online component composed of three different databases, YPD, MIPS, and SGD. The paper distinguishes what type of information can be found in each database and gives example descriptions of yeasts and their properties. Having an online yeast genome resource which will prove helpful in the identification of various yeast samples during our study.

Oshea, D.G. and Walsh, P.K. 2006. Morphological Characterization of the Dimorphic Yeast

KIuyveromyces marxianus var. marxianus NRRLy2415 by Semi-Automated Image Analysis. *Biotechnology and Bioengineering, 50*: 679-690.

Using a newly developed semiautomatic imaging method, the researcher of this study qualitatively assessed yeast samples. Aqueous samples containing yeasts were filtered, prepared, and placed under a microscope with picture-taking capabilities. Yeasts were assessed by the imaging porgram by separating yeast cells into six classes, estimating the volume of individual cells, and the measurement of morphological properties including length, width, shape, and extent of branching. The main conclusion of this study was determining the error involved in using a semiautomatic imaging method, which was found to be less than 3%. The actual procedure and goal of this study is not particularly relevent to our research; however, this study included important morphological details and notable characteristics for identification, making this paper valuable for the morphological assessment of our yeast samples.

Svagzdiene, R., Lau, R. and Page, R.A. 2010. Microbiological quality of bottled water brands sold in

retail outlets in New Zealand. *Water Science & Technology: Water Supply, 10*(5): 689-699.

The aim of this study was to determine if retailed bottled water in New Zealand complied with the Australia and New Zealand Food Standards (ANZFS) Code and the New Zealand Microbiological Reference Criteria. This study employed an yeast isolation and incubation technique that is within the limitation of our class, making it an adequate model procedure. Additionally, the results of this study identified characteristics of specific yeasts (among other microbes) that were found The researchers determined that all thirty eight bottled water brands tested negative for various three types of hazardous microbial species; however, twenty four out of thirty eight brands tested positive for the presence of yeast. Although yeast is not regulated by the two aforementioned code and criteria, the researchers concluded that the presence of yeast in bottled water may be indicative of improper water processes and could pose potential health concerns for people of young or old age, immunocompromised individuals, and consumers with specific medical sensitivities to yeasts. This conclusion is important to our study since the water we are sampling is often used for public recreation and could potentially have health implications based on the species of yeasts found.

Van Wyk, D.A.B., Bezuidenhout, C.C. and Rhode, O.H.J. 2012. Diversity and characteristics of yeasts

from water sources in the North West Province, South Africa. W*ater Science & Technology: Water Supply, 12*(4): 422-430.

This study focused on identifying the distribution and diversity of yeasts in water sources of the North West Province, South Africa. The basic methodology, from sampling to analysis, of this study would be plausible and effective for our study, making it an appropriate model on which to base our procedure. Specifically, this paper outlines a filtration process, streak-plate method, and incubation procedure that it applicable to our study. The researchers identified the yeasts using morphology and biochemical assay involving ID 32C system (bioMerieux, France) composed of 29 assimilation tests, one susceptibility test, one colorimetric test, and a negative control. Candida, Cryptococcus, Pichia, Rhodotorula, Zygosaccharomyces, Aureobasidium spp., Clavispora spp., Cystofilobasidium spp., Hanseniaspora spp., Meyerozyma spp., Sporidiobolus spp., and Wickerhamomyces spp.were the genera of yeasts identifed. This paper proves relevant to our study because of its procedural applications, but also because the research question is very similar to that of our study. The researchers aimed to gain preliminary data on the yeast populations of the water sources of interest in order to determine potential health effects or environmental influences on their sample sit, which is essentially the goal of our study as well.