Soil Microbe Identification: An Innovative Approach To Teaching Microbiology Labs

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Introduction

Biology 2060 (Elementary Microbiology) is a prerequisite for many nursing, dental, and veterinary programs; thus, students in the course are typically pre-health majors that plan on applying to higher graduate or professional programs. However, the labs that are currently used in this course are "cookbook" labs in which the procedures are designed to yield expected results. This approach is suitable for some techniques such as Gram staining so that students can learn to master basic skills, but it leaves students with no appreciation for the scientific process. Moreover, many students lose sight of how the lab techniques they are learning work together so they can end up feeling lost or lose interest. Recent initiatives from the National Science Foundation and National Research Council are in favor of developing open-ended inquiry-based labs.

In the lab we are developing, students will collect soil from a location of their choice and try to isolate and identify bacteria from the samples. Working with bacteria from soil instead of from an individual's body (which is the approach currently being used in the course) will remove the threat of spreading a communicable disease and will allow students to work in groups. This will cut down the cost of supplies, but more importantly it will strengthen the students' teamwork skills - a major objective of the class. Students will then be responsible for keeping their cultures growing for several weeks as they run a series of tests (e.g., Gram staining, metabolic tests such as catalase, starch hydrolysis, and hemolysis tests) that will help them identify their unknown by using the dichotomous keys in Bergey's Manual. This lab will serve as not only a teaching experience as they run their own tests, but it allows there to be no biohazards as they will not pull from their own bodies. The idea is that students will learn these concepts more by doing.

Before this lab can be implemented, it must be tested to make sure the procedures are able to be completed each class period, and that students will eventually be able to find and identify their sample through the series of steps listed in the procedure. After testing is complete Autumn Kelsch, along with the help of Dr. Jessica Habshi will be writing the new lab procedure, introduction, and review questions for the students to use throughout the course.

Methods

Overview:

Over the span of five months (September 2019 - January 2020) we collected and isolated colonies, ran differential tests, and eventually compared our results with Bergey's Manual to try to identify the samples we isolated. We began with a serial dilution of soil suspended in water to form an inoculum. After deciding which dilutions to use, we made streak plates (see figure 1). These plates were recultured through the series of experiments to keep the samples fresh. We then characterized the colonies, performed gram stains (see figure 1) and ran a series of metabolic tests (see figure 2).

Recording Data:

After each session we spent in the lab running our tests, we wrote a conclusion and any observations about the lab itself. For example, we would record the amount of time it took us to complete the tests, any possible issues or errors that may occur during the procedure, and what topics would be best for students to review and learn throughout that lab session. We also took pictures of the plates each day, wrote down any errors we made, and took pictures of each test.

Analyzing Data:

In the end, if we did our work right, we would be able to find our control sample in Bergey's Manual (Pseudomonas fluorescens), and be able to identify our samples using the same dichotomous key. We input our data into a table (see figure 2) in order to help us organize our results better.

Gram Staining and Streak Plates

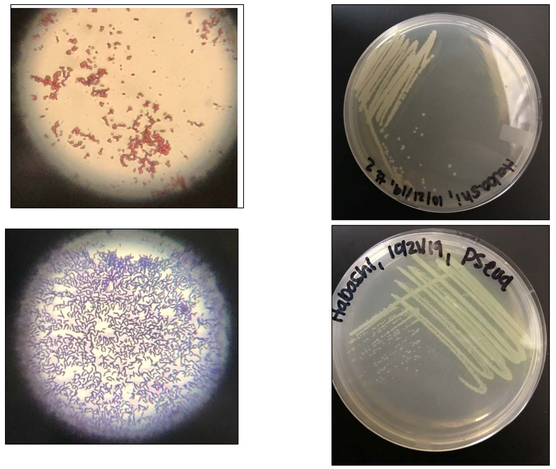

Figure 1

Results

Classroom Setting:

Over the course of the experiment, we kept a detailed record of where tests could fail or yield inconclusive results. We determined that the most difficult part of this lab was the basic procedures (ie: gram staining and streak plates). The streak plate technique works very well if followed exactly. However, if a student accidentally touches the plate with their gloved fingers, or does not heat the inoculation loop for the right amount of time, it can result in contamination of the sample. In addition, with the gram staining procedure too little or too much of any of the chemicals (crystal violet, ethanol, safranin) can yield a false result.

In The Lab:

Over the experiment, our results seemed very clear and conclusive. Our gram stains (with exception of one sample that we ended up discarding) were either very purple or very red. Our oxidase test either produced bubbles or did not. However, when we began to organize our data and begin using Bergey's Manual, we were unable to find our samples and our control within the key. All tests appeared to be very conclusive and if there were any discrepancies of opinion the test was run again (ie: oxidase and catalase metabolic tests). However, no clear identification was made regarding any of the samples leading us to think there was a large error made, or the key is simply outdated.

Metabolic Tests

Sample/Test	1	2	3	5	Pseudomonas
Bile Esculin	-	-	+	+	-
Catalase	+	+	+	+	+
Citrate	-	-	-	-	-
Dextrose	+	+	+	+	+
Gram	-	+	-	+	-
H2S (SIM)	-	-	-	-	-
Indole (SIM)	-	-	-	-	-
Lactose	+	+	+	+	+
Mannitol Salt	-	-	-	-	-
Motility (SIM)	-	-	-	-	-
MR-VP	-	-	-	-	-
Oxidase	-	-	-	-	+
Trehalose	+	+	+	+	+
UREA	-	-	-	+	+

Figure 2

Conclusions

Unfortunately, despite rigorous testing, we are unable to identify the microbes we isolated by using Bergey's Manual. This leads us to assume that either the differential tests were incorrect, or that Bergey's Manual is no longer a viable identification resource.

The problem may also come from the bacteria we were able to culture. Bergey's Manual appears to mostly contain bacteria that come from the order of the Enterobacterales. Since there is so little known about what makes up soil and what bacteria are able to be grown in that environment, it could be possible that Bergey's Manual simply does not have the bacteria listed.

However, since this was our first time learning and running through all the procedures as a student would in class, we want to try it again to ensure that we have reached the right conclusion. Our results seemed very clear, with no discrepancy between interpretations of tests by both researchers, so we remain optimistic that another try at this series of tests will yield more conclusive results.

Future directions

We have recently applied for the Undergraduate Research and Creative Opportunities Grant, and are hoping to run the series of experiments again in a few months. We want to establish a lab on the Utah State Brigham City campus that inspires discovery and expect to have a new lab developed by August 2020.

Upon decision of the URCO grant, we will begin testing again. If this procedure does not work, we will turn to other methods for identification of microbes (ie: genetic sequencing), however the majority of other methods can be very expensive defeating part of the purpose of this new lab.

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