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Yeast: The Gateway to Redefining and Improving Biology Labs

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A. Introduction:

The biology lab lesson plan presented below arose from my research with Dr. Jennifer Round at Ursinus College during the summer of 2018. I researched the synaptic adhesion protein, Slitrk2, and its interaction with the MAGUK scaffolding protein, PSD-95. The mapping of the Slitrk2/PSD-95 interaction was achieved via a yeast-two hybrid assay. While I enjoyed collegiate research like that above, the methods employed in such research are not feasible at the secondary education level, my career focus. Therefore, Dr. Round and myself looked to align my summer project more with the students I will teach as a secondary level biology teacher.

According to research, science education currently focuses heavily on fact learning rather than understanding the process of science, and how we use it (Willingale-Theune et al. 2009). This idea is supported by Pennsylvania's biology standards, which exclusively address biological content. There is no mention of general scientific practice, and how we conduct research properly. A way to remedy this fact-favoring approach though is through effective labs, where students can better understand science through hands-on experiences. Especially important in these labs is the task of instilling curiosity, which can further motivate students and put them on a track for more exploration (Moore 2003). It is argued that labs should push students to think and reflect more deeply about science while still achieving content (National Research Council 2006). We must move away from "cookbook labs" also, where students follow the same protocol and reach the same results. Instead, educators should allow students to formulate their own questions and hypotheses and reach their own conclusions. Therefore, to benefit my future students, I dedicated part of my summer to develop a lesson plan that begins to tackle these challenges.

Yeast, the organism I primarily worked with, provides a convenient and effective model for young biology students to build their knowledge. Yeast is commonly used in secondary education today; however, its use is quite basic with teachers focusing on its fermentation ability. In my background research, a common yeast lab involves using yeast to blow up a balloon as it produces carbon dioxide through fermentation. Contrary to this typical application, yeast is way more diverse and can provide students with more guided exploration. Focusing on establishing worthwhile lab experiences, we can culture, grow, and experiment with yeast under safe and feasible conditions including low costs. Essentially, students will carry out their own experiments with yeast to improve their biological knowledge as well as scientific practice. Along the way, we will begin to dissect primary scientific literature also, a topic rarely addressed in secondary school curriculum yet enhances students' critical thinking skills greatly. I also intend to enact this lab no matter the level of biology; every student deserves the opportunity to gain this information. At the heart of this lab, I desire for students to gain fundamental scientific skills and insights they can carry with them into their future education.

B. Experiment Description:

We refer to *Saccharomyces cerevisiae*, commonly known as yeast, as a model organism because of its diverse application in research and insight into the biological world. Yeast cells are classified under the broad term – eukaryote, meaning they possess a nucleus. Conveniently, human cells are also eukaryotes; therefore, studying yeast allows us to better understand the essential cellular processes we share. Another great benefit of yeast is that its growth and maintenance are affordable. This allows secondary level biology teachers, like myself, to perform innovative and engaging labs with yeast while not drastically exceeding our budgets. A science department, as of 2000, has on average $2500 to spend on lab equipment, which would

stretch even less today because of inflation (National Research Council 2006). Despite this, I have discovered some potential grants to increase and supplement my spending amount.

In this lab, students will look to better answer the question: what happens to yeast growth if I expose it to (blank)? Students will begin their experiments by reading background on yeast, including their proteins, cell membranes, and cell division. This information can then inform them as they develop their hypotheses. Students (in groups of 2-4) will then receive a liquid yeast culture that they will need to determine the optical density (OD) of at 600 nm. This will be achieved via a spectrophotometer. A dilution will then need to be performed to reach an OD of 0.2. Students will expose the yeast to certain household items to then observe the effect they possess on growth. Does the substance inhibit, improve, or do nothing to growth? Listed below are substances I have brainstormed, but students will be allowed to brainstorm other options they may want to test.

- Caffeine (soda or coffee)
- Aspirin
- Spices (pepper, cinnamon, etc.)
- Vinegar
- Baking Soda
- Laundry Detergent
- Vitamins

Students will plate some of their exposed yeast culture onto agar plates, which will then be incubated for 3 days at 30 degrees Celsius. They will also continue to grow their liquid culture. After the growth period, students can then collect their data, which will take two forms. It is important that they learn to quantify data because reputable science does not rely on observation alone. We must ensure that our quantified data supports our observations on yeast growth. To quantify plates, students will simply count the number of yeast colonies on their exposed plates and compare that to control plates with yeast grown under no exposure. To

quantify liquid cultures, students will once again obtain the OD at 600 nm. Based on how much light can pass through the sample, students can determine how growth was affected. This will also be compared to control yeast liquid cultures.

To present their findings, students will write scientific papers just as scientists do. Students will communicate to me their understanding of the project by including: an introduction, methods, results, a discussion, and references. In order to place their findings in context, I will teach students how to find primary scientific literature, read it, and use it properly. They will need to use 2 primary articles in their papers (distributed through the introduction and discussion). While this lab certainly reinforces biology content covered in the classroom, it also places an emphasis on practical skills, which builds the basis of science and research. Students learn culturing yeast in YPD broth, sterile technique, taking ODs, dilutions, plating yeast on agar plates, data collection and quantification, reading primary literature, and scientific writing. These skills will certainly help any of my students pursuing a future career in the sciences; however, they will also expose my other students to critical reading skills and hands-on experiences.

In addition to this main project, there are two supplementary parts; one of which is skill-based, and the other of which is informative. The skill-based one involves students looking at their yeast plates (after data collection) under a light microscope. Microscope skills such as focusing correctly are essential to any research work in biology. Students will select a sample from their plates and mount it onto a glass slide along with a coverslip. Hopefully, they will be able to magnify and focus on their yeast, so they are able to see some features such as the cell membrane/cell wall and nucleus. In addition, they may be able to see certain differences in yeast morphology based on the group it came from (experimental or control). The other supplementary assignment involves some independent research. Yeast, as mentioned, is a model organism and is

involved in some interesting research. Students will investigate yeast in research today and present what they found briefly to the class. The idea is to expose students to the research that labs around the world perform currently, and how that might impact the future in areas such as medicine.

C. Alignment with PA Biology Learning Standards:

3.1.B.A1 Compare and contrast the cellular structures and degrees of complexity of prokaryotic and eukaryotic organisms.

*3.1.B.A7 Explain the consequences of extreme changes in pH and temperature on cell proteins (*including the cell as a whole).*

3.1.B.B4 Explain how genetic technologies have impacted the fields of medicine, forensics, and agriculture.

The standards listed above have been pulled from the Pennsylvania Department of Education and are addressed through the lab I have developed. However, many topics the lab addresses are still underrepresented in the PA standards. As mentioned, we tend to portray science at the secondary level as a concrete set of facts students must master. It is not until students reach postsecondary institutions really that they realize that science is ever-evolving. I believe we need to do a better job of communicating to all students what science really is, which is active discovery. I attempt to fulfill this "standard" by allowing them to explore the growth of yeast based on their innate curiosity. What substances do they want to expose yeast to? Which are they interested in? What do they hypothesize will happen to the growth?

Another realization we must address is that science is rooted in skills and techniques just as any other discipline is. A scientist does not find ground-breaking information suddenly but

works for years with many repetitions involved. I do keep in mind that not every one of my students wants to research postsecondary or become involved in the sciences; however, it is important to teach students discipline. Furthermore, most colleges require every student to take a basic lab science, and my lab exposes them to many skills they would encounter there, providing them a leg up from the start. From these realizations, I have created two additional learning goals for my lab:

1. Students will be able to acknowledge and understand that science is active discovery and more than just content-based.
2. Students will gain fundamental biological lab skills.

D. Lab Protocols:

***Protocols used/adapted from the lab of Dr. Jennifer Round at Ursinus College**

I. Starting a Liquid Yeast Culture (5 mL)

**I will start 2, 5 mL cultures for every section I teach; this will be more than plenty.*

1. Obtain a sterile culture tube, 5 mL pipette, sterile inoculation loop, starter plate of yeast, and a bottle of YPD broth.
2. Flame the outside of the YPD bottle using a Bunsen burner. Remove the cap and flame the lip of the bottle. Make sure to place the cap and bottle in the sterile field (area right around the burner).
3. Pipette 5 mL of YPD broth into the sterile culture tube. Flame the culture tube, place the cap on, and flame the cap as well.
4. Using an inoculation loop, pick 1 good size colony of yeast from the starter plate.
5. Open the culture tube, put the inoculation loop in the YPD broth, and swirl around to mix the yeast in. Flame the outside of the tube, apply the cap, and flame the cap.
6. Place the culture tube in a rotator in a 30 degree Celsius incubator overnight.

II. Obtaining Overnight Optical Density

**Each group will take the optical density of an overnight culture.*

1. Turn on the spectrophotometer and allow it to warm up.
2. Remove cultures (in culture tubes) from the incubator and perform a 1:10 dilution in 2 mL cuvettes.

   a. To do so…
      i. Pipette 1800 microliters of YPD broth into a cuvette under sterile technique.
      ii. Make sure to pipette 1800 microliters of YPD broth into an additional cuvette to act as a blank.
      iii. Shake culture tube gently to stir up settled yeast and pipette 200 microliters of culture into one of the cuvettes.
      iv. Pipette up and down gently to mix.
3. At the spectrophotometer, set the wavelength to 600 nanometers (nm). Blank it first by using the cuvette with only broth in it. Place it in the chamber and hit the blank button. *Note: When handling cuvettes, only touch the upper rim to prevent hand greases from skewing measurements.
4. After blanking, put the sample into the spectrophotometer and record the absorbance.

III. Diluting Overnight Cultures to the Correct Optical Density

**Based on the absorbance obtained in the procedure above, students will calculate the amount of overnight culture needed to inoculate 10 mL YPD broth to an absorbance of 0.2.*

1. Take the optical density obtained above and multiply it by 10 since the sample read was 10 times diluted.
2. Now, yeast substance exposure will take place at an absorbance of 0.2, so we need to make a culture at that absorbance.
   a. To do so…
      i. Take the current absorbance and divide it by 0.2.
      ii. Take that number and divide 10 by it. The resulting number is the milliliters of original, overnight culture needed to inoculate a new 10 mL culture. This is the culture students will experiment with from here on out.
      iii. Take the amount of overnight culture and subtract it from 10 to obtain the amount of YPD broth needed.
3. In a fresh sterile culture tube, pipette the YPD broth amount calculated above under sterile technique. Then, pipette the amount of overnight culture into the broth under sterile technique as well.

<u>Sample Calculation:</u>

1:10 Dilution Absorbance: 0.52

Undiluted Culture Absorbance (10X): 5.2

We need to dilute the 5.2 culture to a 10 mL, 0.2 culture.

5.2/0.2 = 26

10/26 = 0.38 mL culture needed

9.62 mL YPD broth + 38 microliters (0.38 mL) culture = 10 mL culture with an absorbance of 0.2

Students have now successfully made 10 mL of liquid yeast culture with an absorbance of 0.2. They will then expose half of it (5 mL) to their selected substance of choice. The other half will be dedicated to creating controls.

IV. Plating Yeast on Agar Plates

**Students will plate some of their exposed yeast onto 3 agar plates. Students plate multiple plates to replicate and provide more legitimacy to their data. 3 control plates will be streaked as well.*

1. Pipette 100 microliters of exposed yeast culture in a circle onto a pre-warmed agar plate.
2. Using a cell spreader (looks like a hockey stick), spread the liquid around the plate.
3. Allow the plate to sit (lid facing up) for 10 minutes, so the liquid can soak in.
4. After 10 minutes, place the plate (lid facing down) in a 30 degree Celsius incubator for 3 days.
5. Repeat, so there are 3 experimental plates and 3 control plates (use non-exposed yeast for these). *Note: Make sure to label plates with name, date, and condition (experimental or control). Label on the outside edge of the plate to prevent disruption when photographing.

Students will divide the remaining culture (4.7 mL exposed culture and 4.7 mL control culture) into 3 culture tubes each (3 experimental and 3 control). These will be allowed to grow overnight.

V. Collecting Data

**Students will collect data from their plates as well as liquid cultures.*

24 hours after the liquid cultures are put in the incubator…

1. Students will obtain the optical densities of their 6 liquid cultures (3 experimental and 3 control). This will follow the same procedure as obtaining the OD of the overnight culture – a 1:10 dilution followed by measurement on the spectrophotometer. Once again, students will multiply these ODs by 10 to collect the original ones.
2. To present this data, students will graph the mean absorbance with error bars of the experimental and control groups and analyze for a difference.

3 days after the plates are put in the incubator…

1. Students will count the number of colonies on each of the six plates (3 experimental and 3 control).

2. To present this data, students will graph the mean number of colonies along with error bars for each condition and analyze for a difference between the experimental and control groups. They will also photograph their plates and present them in their papers.

Yeast Peptone Dextrose (YPD) Broth Recipe:

1000 mL Water

10 g Yeast Extract

20 g Peptone

20 g Dextrose

1. Dissolve all powders in the 1000 mL of water in a 2000 mL flask using a stir bar.
2. Aliquot into media bottles, loosen caps, and autoclave on a liquid 15 cycle. *If an autoclave is unavailable, a microwave can be used to sterilize the liquid. Just bring the liquid to a boil multiple times to ensure sterility.

YPD Agar Plates Recipe:

1000 mL Water

10 g Yeast Extract

20 g Peptone

20 g Glucose

20 g Agar

1. Dissolve all powders in the 1000 mL of water in a 2000 mL flask using a stir bar.
2. Put aluminum foil and autoclave tape over the lip of the flask and autoclave on a liquid 15 cycle. *If an autoclave is unavailable, the mixture can be microwaved. Microwave multiple times to ensure sterility and that the agar is dissolved.
3. Allow it to cool for 20 minutes while stirring gently on a stir plate and with the foil cracked.
4. When cool, pour into sterile petri dishes. Pour enough to just cover the bottom.
5. Allow to cool overnight and package in the morning.

E. Funding Opportunities:

While I have tried to make this lab as cost efficient as possible, there is still equipment required that not every secondary school has at its disposal. Much of the equipment can be used for multiple labs though, so it is a worthy investment. Yeast, as mentioned, are certainly easier to

grow than other cell types; however, they still require a 30 degree Celsius incubator. Along with an incubator, other, costly items would include: sets of micropipettes, an autoclave sterilizer, and a spectrophotometer. I would also need to routinely purchase other lab supplies and make them such as yeast growth powders, petri dishes, inoculation loops, etc. While this is certainly not cheap, I believe the students will benefit greatly from the experience. I understand that in today's times school budgets are tight; therefore, I have listed some potential funding opportunities available to secondary biology teachers. My hope is that through these resources I can fund this beneficial lab.

1. Toshiba American Foundation (Grants for Grades 6-12)
   http://www.toshiba.com/taf/612.jsp
2. Motorola Solutions Foundation
   https://www.motorolasolutions.com/en_us/about/company-overview/corporate-responsibility/motorola-solutions-foundation.html
3. The National Science Foundation
   https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=5487&org=NSF

The Toshiba American Foundation awards grants both under and over $5,000 to allow teachers to bring more exciting and interactive learning to their students. Similarly, Motorola awarded over $9 million in grants in 2017, allowing teachers to engage students and prepare the next generation in STEM areas. Finally, the National Science Foundation provides grants to K-12 educators, looking to revise and create new curriculum. These are just a few of the resources I have found in my research. A lot of money is allocated to improving secondary STEM programs, and I, as an educator, want to utilize them to my greatest advantage.

F. Closing Remarks:

This lab, at its most basic level, allows students the opportunity to investigate the effects of certain substances on yeast, a model organism. Along the way, students work toward mastering crucial skills such as cell culturing, pipetting, data collection, and data analysis. The lab is meant to be more than an experiment but an experience, where students receive a taste of how actual science works. Furthermore, I think it is important for students to learn to communicate their ideas; thus, they present their findings in a shortened version of an actual publication-style scientific paper. This includes beginning to work with primary scientific literature. At the core, this lab breaks away from the typical, regimented high school biology labs and encourages students to think like scientists. It is possible to cover content while also allowing students the excitement of active discovery. Overall, students will progress as biologists, but they will also develop their thinking and ability to tackle information.

G. Future Directions

As I continue to study to be a teacher, I hope to develop more labs of the same quality and with the same learning goals in mind. I want to work toward reaching that balance of content and actual scientific practice. My faculty mentor, Dr. Jennifer Round, and I have discussed expanding this summer's work into an Honors project. I can envision myself continuing to look at the disparities in science education standards in Pennsylvania, and how I can correct them through labs I have developed. I am also interested in the benefits that engaging and interactive labs have on students and their ability to comprehend information. An ultimate culmination of this work, for me, would be enacting these labs in my classroom one day and analyzing their advantages as well as improving them. I desire to improve the labs biology teachers use because currently they are not used to their fullest potential. With a little creativity and work, we can

provide experiences that reinforce content while also allowing students to think and reflect on their own. Science is an extremely complicated practice, and I want to help students better understand its nuances and benefits.

H. References:

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