Name_____ Date_____ Hour_____

Student Procedure: Bioprospecting for Cellulose-Degrading Microbes — Filter Paper Assay Method

Overview: In this lab you will take on the role of a biofuels researcher bioprospecting for cellulose-degrading microbes in your local environment. The goal of this lab is to collect samples from your environment that contain microbes that can rapidly break down cellulose. To determine whether your microbial samples can degrade cellulose, you will observe whether or not your sample breaks down filter paper in a test tube. The methods that you use are very similar to those used by scientists at the Great Lakes Bioenergy Research Center (GLBRC) and the results could be useful for discovering new enzymes that can efficiently break down cellulose to produce sustainable biofuels.

Part 1: Planning and Bioprospecting

Set-Up Procedure:

1. In groups of 3-4, acquire each of the following:
    a. 4 test tube and caps (aluminum foil or parafilm can be used in lieu of caps)
    b. 25 mL of minimal media solution
    c. 4 pieces of filter paper cut into 1x10 cm strips
    d. Labels or masking tape and pen
    e. Protective gear - gloves, aprons, goggles
2. Serilize your work area and hands.
3. Add one strip of 1x10 cm filter paper to each tube.
4. Using a pipette or eyedropper, add 5 mL of minimal media solution to each tube.
    a. Be careful not to hit the filter paper when adding the media solution.
5. Using a sterilized glass rod, pipette, or other sterile object, push the filter paper flat against the side of the tube. Raise or lower the filter paper so that it is straight and not folded.
    a. If the filter paper strip is not flat against the side of the tube, it may fall into the solution and make the results difficult to interpret.
6. Securely cap the test tube and add a label. On the label, write your group name and date. Leave room so that you can later write the contents of the test tube.
7. Place your group's test tubes in the space designated by your instructor.

The Purpose of Media Solution & Filter Paper: The media solution is necessary to provide non-energy related nutrients for microbial cell growth. This media mainly provides three key nutrients essential for any cell growth – nitrogen, phosphorus, and potassium. Note that these nutrients by themselves cannot be used as energy – they only provide the raw materials for building a cell. The only source of energy in the test tube is the cellulose in the filter paper. If the community of microbes cannot break down cellulose, its members will die.

Controls: Your instructor will set-up test tubes that will serve as the "control" in this experiment. No environmental sample will be added to the control tubes. The control should not show any signs of filter paper degradation and will serve as a baseline for comparing any changes you see in your samples.

Bioprospecting: Planning and Bioprospecting

Planning: In your group, discuss some potential locations to collect environmental samples with cellulose-degrading microbes. In deciding where you want to collect samples, think about the environmental characteristics that would support large, diverse populations of these microbes. Brainstorm a list of potential locations to collect samples.

Location:	Why this is a good place to find cellulose-degrading microbes:

Collecting Samples:

1. In your group, acquire the following:
   4 plastic sealable sandwich bags
2. To collect your samples:
   a. Invert the bag so that the outside is on the inside (i.e. so that the bag is inside out)
   b. Reach your hand inside the bag and grab the sample through the bag
   c. Re-invert the bag so that the sample is inside the bag. (Your hands should never actually come in contact with the sample.)
   d. Seal the bag and date and label it.
   e. Collect four samples and bring them to the classroom.

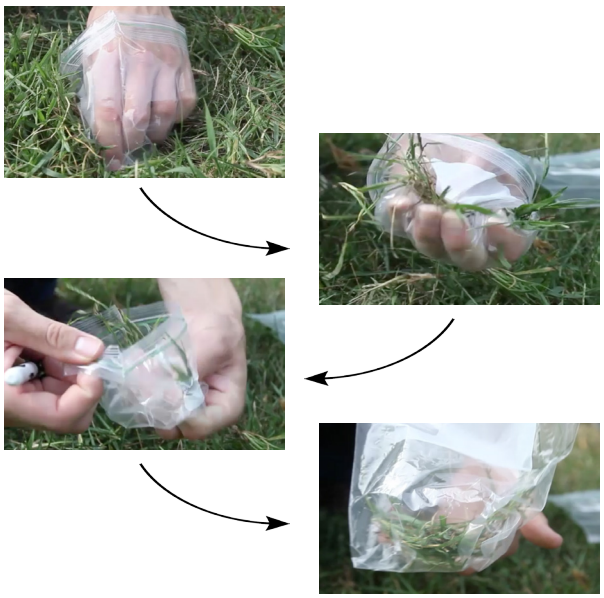

Figure 1: a proper collection method—the bag is flipped inside-out so the hand never comes in contact with the sample.

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Planning and Comprehension Questions:

You will need to select two of the four samples that you collected to test for cellulase activity. As a group, discuss which sample you predict will have the highest activity.

1. List the four samples that your group collected:

2. Why did you choose each of your two samples? What made you think that the microbes in these samples would have the most cellulase activity? For each sample, describe it and defend your choice with an explanation.

Sample Description	**Why was it chosen?**
1.	
2.	

3. How will you know if the microbes in your bioprospected samples are effective in producing cellulase enzymes? How will your test tubes change if your samples are effective?

4. If the microbes in your samples are not effective at producing cellulase enzymes or at breaking down cellulose, how will you be able to tell?

5. Could a microbe that does not produce cellulase enzymes survive in these test tubes? Why or why not?

Name_____ Date_____ Hour_____

Planning and Comprehension Questions (cont'd)

6. What role does the liquid media solution play? Why was it necessary?

7. What role does the filter paper play? Why was it necessary?

Predictions and Explanations:

8. What differences do you think you will see between your control and your test tubes with your bioprospected samples? *I predict that...*

9. Explain your reasonsing for all predictions made above: *I think these predictions will be proven correct because…*

10. What differences, if any, do you think you will see between two test tubes with your bioprospected samples? *I predict that...*

11. Explain your reasonsing for all predictions made above: *I think these predictions will be proven correct because…*

PART 2: Sample Inoculation and Observation

Innoculation:

1. As a group, acquire the following: your samples, your test tubes (that you prepared earlier).
    a. Check to make sure the filter paper is still flat against the tube; if it is not, use a glass rod or other sterile long object to re-flatten it agast the side of the test tube.
2. As a group, select two of the samples that you wish to add to your test tubes. Select your two samples based on which you think will have the most cellulose-degrading activity.
    a. Be prepared to defend your choice of samples.
3. With a gloved hand, acquire a pea-sized amout of one of the samples you chose from its bag.
4. Take one of the test tubes you prepared; slightly tip it so that the filter paper is on top.
    a. With a tweezers (or a gloved hand) add your pea-sized sample so that it does not hit the sides or the filter paper.
    b. If you do hit the side of the tube, use a sterile glass rod or similar object to push it all the way down to the media solution.
5. Cap the tube loosely and add the inoculated sample to the label.
6. Repeat this once more for the same sample in a second test tube.
7. Repeat this for the second sample using the remaining two tubes you prepared. For replicates of the same sample use "A" or "B" to distinguish them, ex: "Soil A," "Soil B."
8. When you have inoculated all four tubes, make sure they are labeled correctly with the substance and place them in a lab shaker as specified by your instructor.
9. Make sure tubes are not completely sealed. Without oxygen many microbes will not be able to survive.

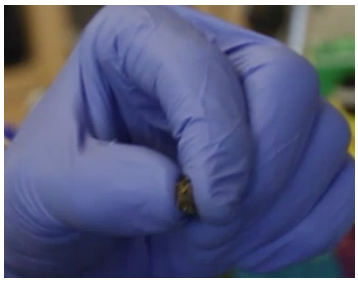
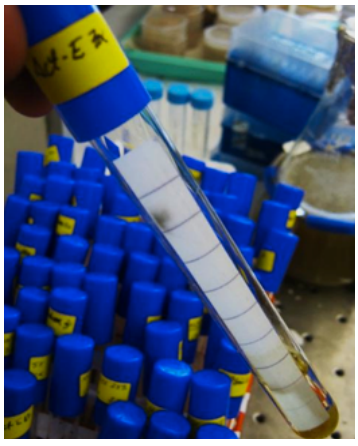


Figure 2 (above): an appropriately sized sample to be placed into the media with a sterile, gloved hand.

Figure 3 (below): a properly inoculated sample after 6 days of growth. Some growth has occurred in the media and the paper has a noticeable yellowed line indicating possible growth.



Observations:

9. Check your tubes periodically (if not daily) for any signs of growth and cellulose degradation.
    a. If your tubes have the same consistency and cloudiness as the day you added the sample, you likely do not have a sample that can degrade cellulose.
    b. If you see any increased cloudiness, yellowing or definite color change on the filter paper strip (ignoring the color change caused by the color of the sample itself), or microbial growth on the filter paper, you have possible cellulose degradation.
    c. If you observe any ripping, tearing, or dissolving of the filter paper, you have definite cellulose degradation, an indication of cellulase activity.
10. Record each observation on the accompanying table and describe, draw and/or photograph your visual observations for each tube (see attached table to record observations). When the experiment is finished, complete the attached questions.

Table 1: Filter Paper Test Tube Results

	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4
Description of Sample				
Observations (circle which description best fits each sample for each date)				
Date:	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth
Observations (you may write a description or draw it)				
Date:	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth
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Date:	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth
Observations (you may write a description or draw it)				

"No Growth" = sample remains largely clear and mostly unchanged. Little or no indication of microbial growth.

"Possible Growth" = media solution is cloudier than when the sample was added. Visible color change has occurred on the filter paper (often with a clear line on the paper). Signs of microbial growth and reproduction are observable.

"Definite Growth" = the filter paper has ripped, torn, or dissolved.

Table 2: Filter Paper Test Tube Results (second page, if needed)

	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4
Description of Sample				
Observations (circle which description best fits each sample for each date)				
Date:	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth
Observations (you may write a description or draw it)				
Date:	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth	No Growth Possible Growth Definite Growth
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Observations (you may write a description or draw it)				

"No Growth" = sample remains largely clear and mostly unchanged. Little or no indication of microbial growth.

"Possible Growth" = media solution is cloudier than when the sample was added. Visible color change has occurred on the filter paper (often with a clear line on the paper). Signs of microbial growth and reproduction are observable.

"Definite Growth" = the filter paper has ripped, torn, or dissolved.

Name: _____ Hour: _____ Date: _____

Lab Analysis and Comprehension:

For each sample, describe how it changed from the first day of incubation to your final day of results:

Test Tube	Observed Changes

1. Which tubes showed evidence of cellulase enzymes? Explain what happened to the microbes and filter paper in these tubes.

2. Which tubes showed little or no evidence of cellulase enzymes? Explain what happened to the microbes and filter paper in these tubes.

Lab Analysis and Comprehension (cont'd)

3. Review your intial predictions about the differences you expected to observe between the control and the samples you collected. Did your observations match your predictions? Propose an explanation for differences between what you predicted and what you observed.

4. Review your intial predictions about the differences you expected to observe between the two samples you collected. Did your observations match your predictions? Propose an explanation for differences between what you predicted and what you observed.

5. Compare your results with the samples collected by the rest of the class. Which samples showed the most cellulase activity? Which showed the least?

6. Based upon the class results, what generalizations can you make about the patterns you observed regarding environmental characteristics that support more cellulose degrading microbes?

Lab Analysis and Comprehension (cont'd)

7. Propose an explanation for any general patterns you observed in samples in your class's samples.

8. What additional information would you need to determine whether your explaination is accurate? Propose a new investigation that could provide information to evaluate your explanation.