Bacterial Communities Grow as Biofilms

Resource Type:

Curriculum: Labo biofilms and common locations where they grow. Students observed the organization of the cells in the biofiln identified the general types of organisms (eucaryotes versus procaryotes) that grew in their biofilms. They designed experiments for possible further study of biofilm growth.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Time.
Three consecutive class periods are required for this activity. The ideal intervals between class periods should be between 2 and 7 days. This lab should be done near the middle or end of the course when students are familiar with the cellular morphology of a variety of microbes.

Learning Objectives.
At the completion of this activity, students will:

1. understand what a biofilm is.
2. recognize that biofilms grow in many common household settings.
3. demonstrate that when cells are removed from a biofilm and suspended in water, they can attach to a new surface to produce a new biofilm even if the new growth conditions are very different from conditions at the original biofilm site.
4. recognize that microbes often grow in complex, diverse aggregates that contain more than one type of organism.
5. recognize that biofilm growth is very different from the growth of pure cultures of floating cells in broth or colonies on agar plates.
6. work in groups to discuss their results and to design possible future experiments to investigate environmental factors that influence biofilm growth.

Background.
Students should know how to use a microscope.

Microorganisms Used.
The microorganisms used were unidentified environmental microbes sampled from biofilms that grow on moist surfaces such as dish drains, shower curtains, bird baths, pond stones, etc.

PROCEDURE

Materials.
(For a class of 24 students arranged in six groups of four students)

Lab or Class Session 1 (This session can be conducted in lecture or lab.)

24 plastic zip-top sandwich bags
24 sterile packaged swabs
24 collection tubes: sterile screw cap tubes containing 5 ml of sterile distilled water

Lab Session 2

24 sterile glass slides: each slide wrapped separately in aluminum foil and autoclaved
24 sterile petri plates
24 sterile screw cap tubes containing 10 ml of sterile dilute trypticase soy broth:

5% trypticase soy broth (BBL Becton Dickinson Co.) in distilled water (5 g of powder/100 ml or 5% of "normal" medium concentration)

2 trays to incubate the petri plates
12 black wax pencil markers

Lab Session 3

12 wash bottles containing tap water
6 beakers (400 ml or larger) containing a small amount of disinfectant
24 pairs of latex or vinyl disposable gloves
Gram crystal violet stain
12 black wax pencil markers
Paper towels
Biohazard waste container
Microscopes
Immersion oil
Lens tissue
24 Biofilm Discussion sheets (authored by the instructor)
Microscope connected to a monitor (optional)
Digital camera (optional)

Student Version.

Lab Session 1

1. Introduction to Biofilms
Biofilms are complex aggregates of microbes that grow on surfaces. They attach to diverse substrates such as soil particles, pipes, and contact lenses. Biofilms perform beneficial activities such as water purification and nutrient cycling, but they als o cause problems. For example, they can foul plumbing systems and cause stubborn infections. Scientists are just beginning to understand the nature of biofilms because microbes have been traditionally studied as pure cultures. However, this is not the normal mode of growth for many microbes. This exercise will demonstrate that biofilms are very common and easy to cultivate, and that they have distinctive appearances.

2. You will receive a zip-top bag containing a sterile swab and a collection tube. Collect a biofilm sample from your body or household within 24 hours of your next lab session. Good collection sites that are likely to have biofilms include teeth

FIG. 1. Petri plates after staining and removal of the biofilm slides.

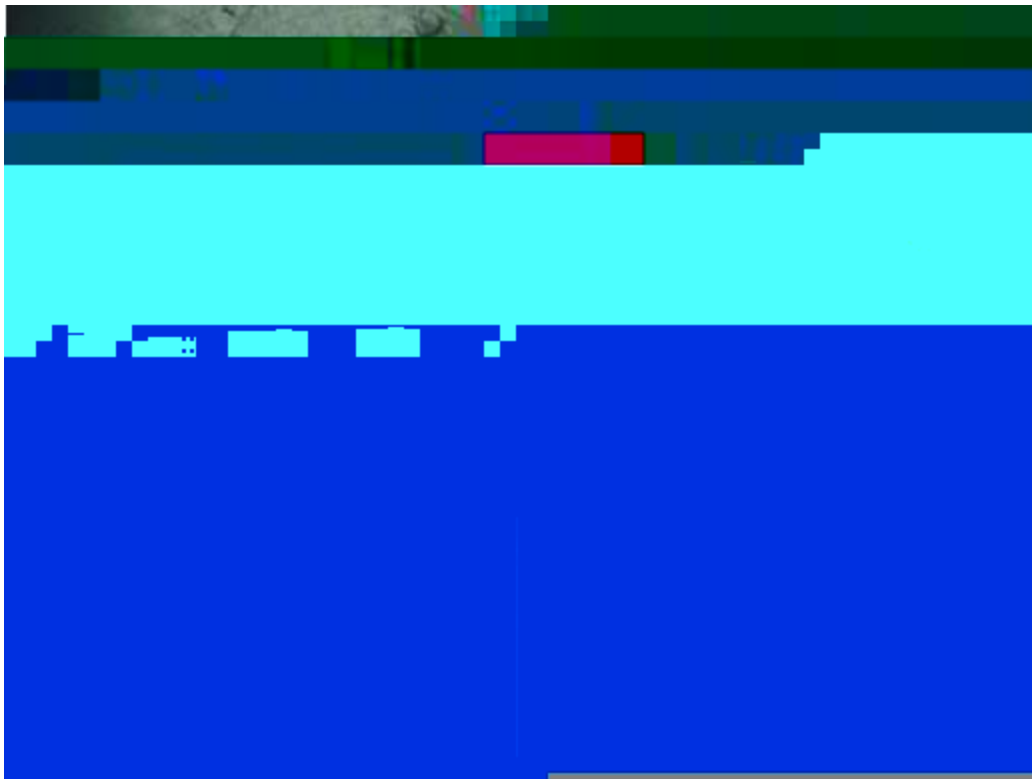

FIG. 2. Stained biofilm slides.

FIG. 3. Biofilm with heavy growth.

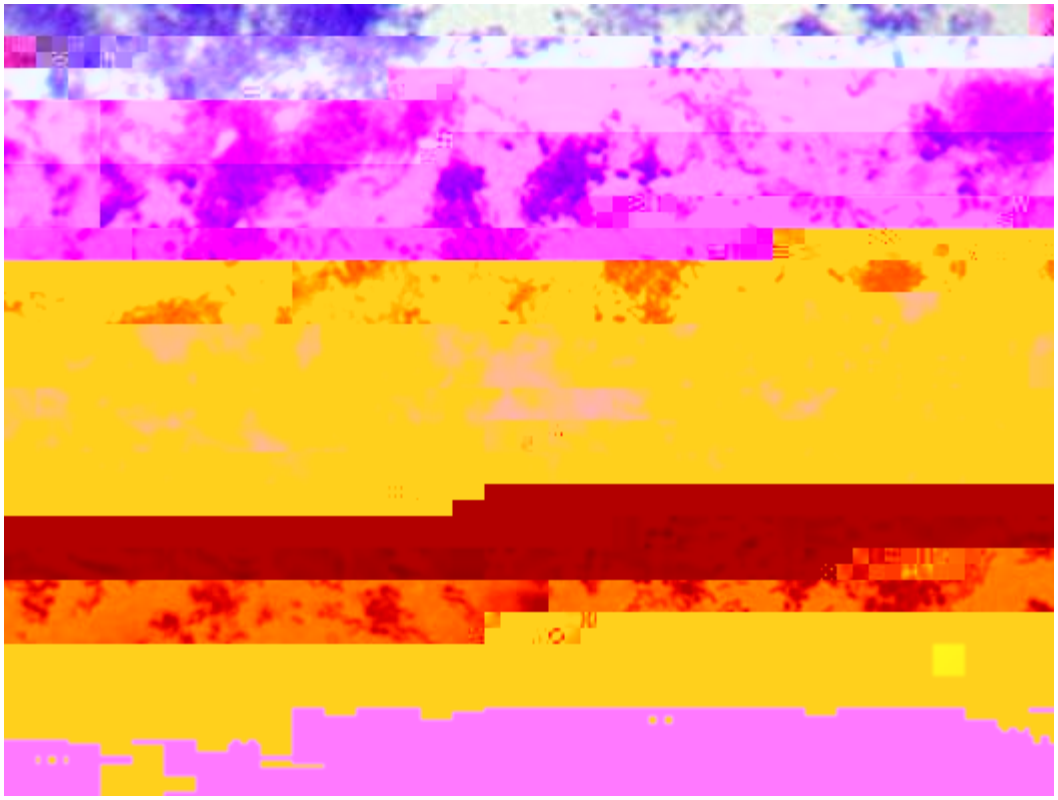

FIG. 4. Biofilm with moderate growth and microcolonies.

from each other.

5. Life in a biofilm community provides what advantages and disadvantages to a microbe?
a. Advantages of living in a biofilm community:

b. Disadvantages of living in a biofilm community:

6. Do you think that the biofilm you sampled is beneficial, harmful, a nuisance, or of no consequence for humans? Explain your answer.

7. What are some likely differences between bacteria that grow in biofilms and bacteria that grow as free-floating independent cells. For example, what differences might there be in growth rates, ease of exchanging DNA, resistance to antibiotics, etc.?

8. How could you modify this exercise to obtain additional information about your biofilm? (For example, you could determine whether a bacterium that was isolated from your biofilm can propagate a pure culture biofilm.) Think of some additional possibilities.

9. Devise an experiment that would provide information on how an environmental factor affects the amount of growth in a biofilm. For example, describe an experiment to determine the effect of temperature on biofilm growth.

10. Did you enjoy this lab exercise? Please explain your answer.

(End of Biofilm Discussion sheet) _____

Note: students may have difficulty determining whether there are eucaryotes in their biofilms. Briefly review the microscopic appearance of eucaryotic microbes versus procaryotes before the students view their slides.

6. After the slides have dried, ask the students to label their slides and observe them with the microscope at each power of magnification. Remind them to rotate the fine focus knob to observe the three-dimensional properties of their biofilms. Most biofilms will show several growth patterns such as scattered microcolonies (small isolated clumps that sometimes grow to considerable thickness) and smaller cell clusters. Ask students to look for these various growth patterns. They should

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
The Biofilm Discussion sheet is the basis for individual reflection as well as group and class discussions. I graded students on the thoroughness of their answers and their participation in the discussions.

Suggested rubric:
Completion of Biofilm Discussion sheet: 50 points
Preparation of stained biofilm slide: 25 points
Participation in class discussion: 25 points

Field Testing.
This lab has been tested with two separate classes of 24 students each. Most of these students had no prior science background before taking microbiology. Most of these students were studying microbiology as a prerequisite for admittance to an allied health program.

Student Data.
I asked my students whether or not they enjoyed this lab. The response from a poll of 20 students was 100% positive. Here are a few of the comments:

- "I didn't have a clue what a biofilm was before this lab."
- "This lab brought microbiology into my life."
- "It was interesting to see things that we collected rather than the usual cultures provided in lab."
- "I didn't know that my drain has that many bacteria."
- "I liked being able to choose what to collect."
- "This lab built on what we learned earlier in the quarter—I feel comfortable with lab techniques now."
- "It was cool to see how many organisms there are in common places."
- "This lab summed up many things that I learned earlier in class."
- "A fun lab—but we need more time to investigate the slides."
- "I was very aware of the three-dimensional character of the films by using the fine focus knob."
- "I was delighted to be able to grow my own biofilm after reading about them."
- "The biofilms formed with so little experimental effort that it seems very likely that bacteria really "want" to grow this way."
- "The big lesson is that these little guys (the microbes) are not only complex and subtle inside the microscopic world, but can exhibit macroscopic features of great subtlety and utility (for themselves)."

SUPPLEMENTARY MATERIALS

Possible Modifications.
The following four modifications were suggested by the students in their responses to Question 8 on the Biofilms Discussion sheet.

1. Isolate some of the biofilm bacteria and identify them using tests to characterize "unknowns." Determine whether similar organisms can be isolated from biofilms of diverse origins. Determine whether an isolated organism can form a biofilm when it is grown as a pure culture.

2. Compare a slide of the microbes that float in the broth above the biofilm slide to the biofilm slide to detect differences i n growth patterns. (We consistently found fewer and smaller aggregates among the floating microbes in comparison to the attached microbes in the biofilms.)

3. Prepare several biofilm slide cultures and harvest the slides over a period of days to observe changes as the biofilm matures.

4. Students collaborated to propose experiments to investigate how environmental factors influence biofilm growth in their responses to Question 9 on the Biofilms Discussion sheet. They suggested comparing the extent of growth when biofilms are grown at different pH levels, different temperatures, with different nutrient supplies, or on different subtrates. They also

Gilbert. 2003. Microbial characterization of biofilms in domestic drains and the establishment of stable biofim microcosms. Appl. Environ. Microbiol. 69: 177–185.

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Spring, 2004 Name_____

Biofilms Discussion Sheet

1. What is meant by the term, biofilm?

2. What site did you sample to obtain biofilm material?

3. Briefly describe your biofilm:
a. Describe each type of procaryote that you see based on cell shape and size.

b. Describe each type of eucaryote that you see based on cell shape and size.

4. Observe several of your classmates' biofilms.
a. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look similar.

b. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look different from each other.

5. Life in a biofilm community provides what advantages and disadvantages to a microbe?
a. Advantages of living in a biofilm community:

b. Disadvantages of living in a biofilm community:

6. Do you think that the biofilm that you sampled is beneficial, harmful, a nuisance, or of no consequence for humans? Explain your answer.

7. What are some likely differences between bacteria that grow in biofilms versus bacteria that grow as free floating independent cells. For example, what differences might there be in growth rates, ease of exchanging DNA, resistance to antibiotics, etc?

8. How could you modify this exercise to obtain additional information about your biofilm? (For example, you could determine whether a bacterium that was isolated from your biofilm can propagate a pure culture biofilm.) Think of some additional possibilities.

9. Devise an experiment that would provide information on how an environmental factor affects the amount of growth in a biofilm. For example, describe an experiment to determine the effect of temperature on biofilm growth.

10. Did you enjoy this lab exercise? Please explain your answer.