Chemotaxis: the Search for Food

Resource Type: Curriculum: Laboratory

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Abstract

Two bacteria, one motile and one nonmotile, were tested for their ability to seek out nutrients using sectioned petri dishes containing glucose yeast extract (GYE) agar and water agar. As hypothesized, only the motile bacterium was able to migrate from the nutrient-free environment (water agar) to the nutrient-rich one (GYE). Since there was no such migration from GYE to water agar, it can be concluded that chemotaxis, rather than random motion, propels motile bacteria toward nutrients.

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
It has been hypothesized that in many natural environments motile organisms have a competitive advantage over nonmotile strains because of their ability to actively "swim" toward nutrients and locations most favorable for their growth. They are also able to escape from potentially harmful conditions. This process is referred to as chemotaxis. In this exercise, the chemotactic response of a motile bacterium to an attractant will be compared to that of a nonmotile bacterium to the same attractant.

PROCEDURE

Materials.
(Quantities listed are per person.)
2 Trypticase soy agar (TSA) slants (24 h, 37ºC):

> Organism A: a motile organism (e.g., *Serratia marcescens*, *Escherichia coli*, or *Bacillus subtilis*);
> Organism B: a nonmotile organism (e.g., *Klebsiella pneumoniae*, *Micrococcus luteus*, or *Staphylococcus epidermidis*)

2 sectioned petri dishes each containing GYE agar in one half and water agar in the other half
2 sectioned petri dishes with GYE agar in each half (control)
Sterile Whatman filter strips (approximately 1/4- by 4-inches)
Beaker with 70% ethanol
Forceps

Student Version.
Methods.
1. Obtain one GYE-water agar sectioned petri dish.
2. Sterilize forceps by dipping into alcohol and passing them through the Bunsen burner flame. Use caution! Always point the forceps downward to prevent the burning alcohol from running down the arm.
3. Using the sterile forceps, center two sterile filter paper strips bridging the plastic ridge of a sectioned petri dish (Fig. 1). Press the paper strips gently into place.

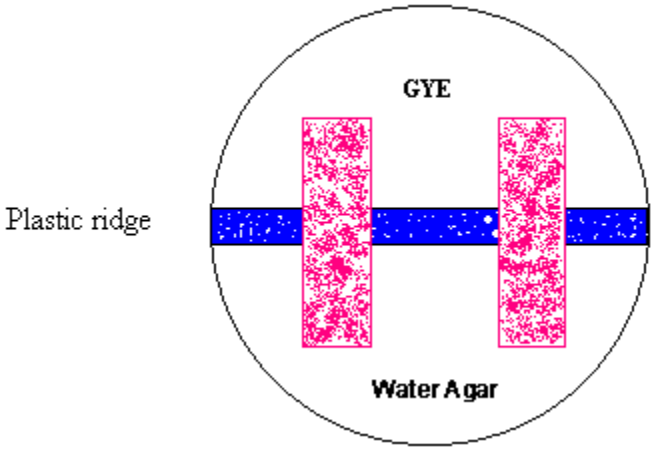

FIG. 1. Position of two sterile filter strips placed across the plastic ridge of a sectioned petri dish containing GYE agar in one half of the plate and water agar in the other half.

4. Using aseptic techniques and a loop, place a small amount of the motile Organism A (e.g., *Serratia marcescens*) from the slant onto **opposite ends** of the paper strips so that one strip is inoculated on the GYE side and one strip is inoculated on the water side (Fig. 2).

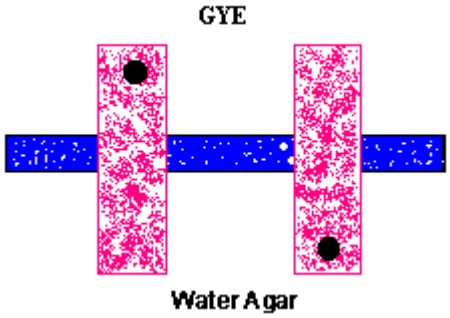

FIG. 2. Inoculate opposite ends of each of the paper strips with motile Organism A (indicated by black dots on the diagram).

5. Mark the sides which have received the inoculum on the underside of the plate and label which culture you have used.
6. Repeat the procedure using the nonmotile Organism B (e.g., *Klebsiella pneumonia*) to inoculate a second GYE-water agar sectioned petri dish.
7. Repeat steps 1 through 5 using the GYE-GYE sectioned petri dishes. These plates will serve as the control.
8. Invert the plates and incubate at 37ºC for 24 to 48 hours.

Results and Observations.
1. After incubation, observe the plates and compare results.
2. Record your observations by making correctly labeled diagrams (i.e., figures) indicating the type of growth for each of the bacterial species.

Discussion and Conclusions.
1. Write a short discussion explaining the results, including brief comments on each of the following questions:

a. Which of the bacterial species would appear to have a competitive advantage in finding nutrient sources?
b. How do you know that the bacteria moved across the bridge in search of food and not just "because it was there"?
c. How do bacteria detect the presence of nutrients (chemicals) in the environment?
d. Suggest any modifications to the experimental method used in this experiment.

Instructor Version.
1. Avoid using broth cultures for this experiment as it seems more difficult for the students to control the amount of inoculum added to the top of the filter strips. In addition, the extra moisture from the broth appears to have a wicking effect causing "movement" of nonmotile bacteria across the filter paper bridge to the nutrient side. This results in false positives and a great deal of student confusion.

2. The following observations can be expected when the initial inoculum is taken from a slant (Fig. 3).

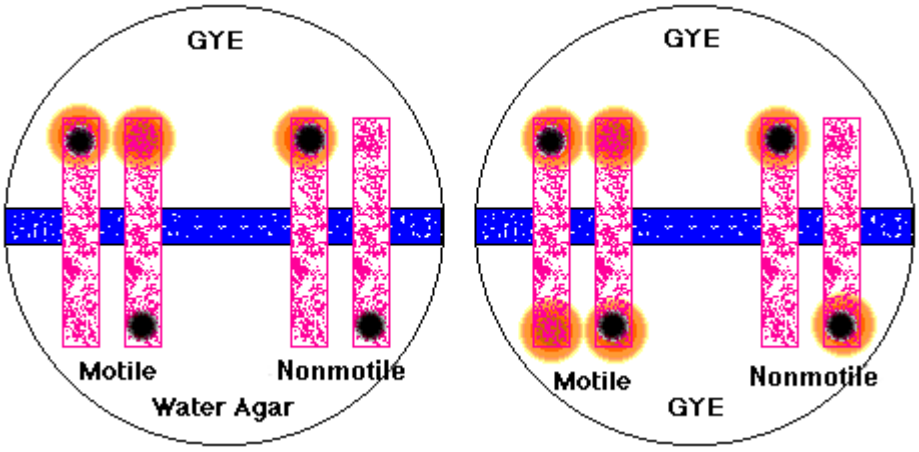

FIG. 3. Yellow circles indicate growth of organisms on and immediately around the end of the paper filter strips after 24 to 48 hours incubation. Black dots represent the original points of inoculation.

On the GYE-water agar plate, motile Organism A will demonstrate growth on and immediately surrounding the ends of both of the strips on the GYE agar side; there will be no growth on the water agar side. Nonmotile Organism B will only grow around the point of inoculation on the GYE side; there will be no growth on any portion of the strip where the inoculum was placed on the nutrient-lacking water agar side. This clearly indicates that, if the organism lacks motility, it will not be able to search out food and thus not be able to grow. Note: on this point, students can be asked how they would determine whether the organism has actually died or whether it is merely dormant.
On the GYE-GYE agar plate, motile Organism A will grow on both ends of the strips; however, nonmotile Organism B will only grow around the original point of inoculation.

3. An additional point of interest regarding the results is that if growth does occur, it is usually concentrated around the ends of the paper filter strips but rarely, if ever, on the middle portion of the strips (Fig. 3). We believe that the strip loses moisture during incubation and thus is unable to support growth. This is an excellent focus for student discussion.

4. Although we have experimented with more than one organism per plate (i.e., two organisms on four paper filter strips), the best results are obtained when only one organism per plate is used. We usually provide each student group with a different combination of Organisms A and B and then compile all of the results as class data at the end of the experiment. This enables us to examine a large number of different bacterial types and makes for a constructive session of debate and discussion.

5. We always include a GYE-GYE agar plate as a control. We also always use two filter paper strips in order to remove any experimental bias.

6. Sectioned petri dishes (Falcon 1003, Canlab) are standard petri plates divided into two halves by a plastic ridge so that different types of media can be poured into each half without the two mixing together. Note: the filter strips are more easily folded over the central plastic ridge if they are first allowed to absorb some of the moisture from the medium. Make sure that both ends of the filter strips fully adhere to the surface of the medium before the inoculation process.

7. The sectioned petri dishes are ideal, especially when different types of media (attractants, repellants) are used. When only one type of culture medium is used (e.g., GYE-GYE), standard petri dishes suffice and the student merely removes a strip of agar from the center creating a small "moat" which can then be gapped using the filter strips.

8. Instructors can use the basic methodology of this experiment and incorporate a wide variety of different modifications to suit their own purposes. For example, other combinations of nutrients (chemicals) can be used in order to investigate which ones act as repellants and which as attractants; combinations such as GYE-aspartate, GYE-serine, GYE-acetate, etc. Also, students may be asked to make suggestions and to design additional experiments.

SUPPLEMENTARY MATERIALS

References

1. **Adler, J.** 1976. The sensing of chemicals by bacteria. Sci. Am. **234**(4)**:**40-47.
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3. **Blair, D. H.** 1995. How bacteria sense and swim. Ann. Rev. Microbiol. **49:**489-522.
4. **DePamphilis, M. L., and J. Adler.** 1971. Attachment of flagellar basal bodies to the cell envelope: specific attachment to the outer, lipopolysaccharide membrane and the cytoplasmic membrane. J. Bacteriol. **105:**396-399.
5. **Silverman, M., and M. I. Simon.** 1977. Bacterial flagella. Ann. Rev. Microbiol. **31:**397-420.

Recipes.
For culture media (g/L distilled water):

Trypticase soy agar (Baltimore Biological Laboratory).
15.0 g of pancreatic digest of casein
5.0 g of papaic digest of soybean meal
5.0 g of sodium chloride (NaCl)
15.0 g of agar

pH 7.3

Heat to dissolve ingredients and autoclave at 121ºC for 15 min.

GYE agar.
10.0 g of tryptone
10.0 g of yeast extract
5.0 g of glucose
5.0 g of K_2HPO_4
15.0 g of agar
pH 7.0

Heat to dissolve ingredients and autoclave at 121ºC for 15 min.

Water agar.
15.0 g of agar
pH 7

Heat to dissolve agar and autoclave at 121ºC for 15 min.