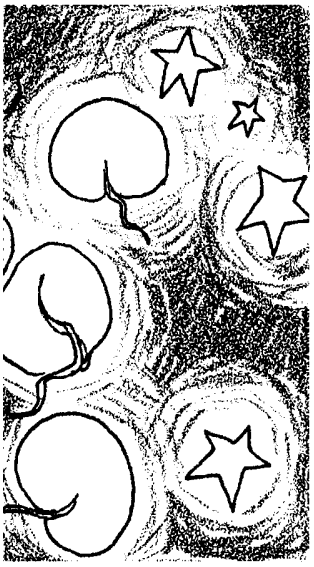
Glowing in the Dark: Bioluminescence

Key Concepts

1. Bioluminescence is the production of light by living organisms.

2. There are many marine plants and animals that bioluminesce.

3. Bioluminescent bacteria contain the light producing chemical caled luciferin that, with the aid of an enzyme called luciferase, combines with oxygen to produce oxyluciferin, water and light.



Background

Bioluminescence is an interesting and spectacular phenomena. Many sea animals bioluminesce, producing light through a series of complex chemical reactions mediated by the enzyme luciferase. The oxidation of the pigment luciferin to oxyluciferin releases light with virtually no waste heat.

Bioluminescence appears to play a variety of roles in the life of marine animals including: species recognition, mate recognition and location, a lure for food, a warning signal, and illumination. The determination of actual benefits awaits further study. Studies are also underway into the biochemistry of bioluminescence which may provide techniques for improving our own light producing capabilities.

Materials

I. Luminescent Bacteria from Marine Fish

For a each group of 2-3 students:

- 1 sterile scalpel
- 3 sterile petri dishes
- 1 sterile test tube
- 1 sterile stopper for the test tube
- 1 inoculating loop or cotton swab

For the class:

- 1 liter of sea water
- 1 fresh marine fish
- 1 pan and aluminum foil to cover the fish

- Marine Luminescent Bacterial Medium*
  20 grams peptone
  10 grams glycerine
  15 grams agar
- 1 liter sterile sea water
- 1 incubator
- 1 autoclave

II. Extension Activity-Bioluminescence in Sea Fireflies

- 1 gram of Sea Fireflies

Teaching Hints

In "Glowing in the Dark: Bioluminescence", your students will have an opportunity to culture and/or observe bioluminescent bacteria. The first part involves culturing bacteria from a marine fish or prepared culture. If the process of culturing bacteria is not possible, the extension activity involves observing a bacterium present in dehydrated bioluminescent "sea fireflies" that can be purchased from science supply companies.

I. Luminescent Bacteria from Marine Fish

Luminescent bacteria can be obtained by using the following method. It is important to note that this method is not completely foolproof. Unfortunately, the only way to determine if the method will work in a particular situation will work is to try it!

Directions for preparation:

Boil the sea water in order to sterilize it. Add the agar and other ingredients and stir until they dissolve. Pour the medium into one test tube for each lab group and autoclave at 15 lbs. of pressure or 120° Celsius for 20 minutes.

To culture the bioluminescent bacteria, half-immerse a fresh marine fish in a pan of sea water. Cover the pan with aluminum foil and incubate at 10-15°C for 2 to 3 days. This procedure is designed to induce maximum bacterial growth in the outer slime covering of the fish. If all has proceeded well, luminous spots should be visible to the dark adapted eye in a darkened room. You may elect to have your students take their scrapings from these spots only.

If problems occur in the isolation of the luminescent bacteria, a pure culture of the luminescent bacterium, *Vibrio fisheri*, can be obtained from the American Type Culture collection (12301 Parklawn Dr., Rockville, MD 20852). The catalog number is #25918. Mix with nutrient agar and 1% yeast extract for media. Incubate and follow the procedure beginning with item 6 in the student procedure.

Duplicate the activity pages. One page is recommended per lab group. Inventory the glassware and supplies required before you begin.

You will need to model the laboratory techniques used in culturing the bioluminescent bacteria. If your students are completely unfamiliar with bacteriology and sterile technique, you may want to have students practice the lab techniques by first completing the lab, "Marine Bacteriology" included as an addendum to this lesson.

It is very important to use sterile equipment. Once again, while it is unlikely that pathogenic organisms have established colonies on the agar plates, it is important to dispose of the bacteria colonies by autoclaving for 20 minutes at 15 pounds pressure (120°C). The bacterium *E. coli* doesn't survive sea water exposure for more than a few hours, but may affect culture growth in samples.

Circulate through the room as your students perform the activity. Remember to allow time for clean-up upon completion of the activity. Plan to provide a few minutes for a discussion of the results and to provide the answers for the analysis and interpretation questions.

Key Words

agar - a gel prepared from certain marine algae used as a base for bacterial culture media, as a laxative, and thickener in foods

autoclave - a pressurized, steam-heated vessel used for sterilization

bacteria - unicellar organisms that are free-living or parasitic

bioluminescence - the production of light by living organisms

catalyst - a substance that modifies the rate of a chemical reaction

colony - a visible aggregation of microorganisms on a nutrient medium caused by the reproduction of a single microorganism

detritus - in this case, decayed organic material

enzyme - a protein that functions as a catalyst

food chain - outline of who eats whom showing path of energy transfer in an ecological community

incubate - to maintain at optimal environmental conditions for development

inoculate - to implant microorganisms into a culture medium

luciferase - the enzyme involved in the process of bioluminescence

luciferin - a chemical involved in the process of bioluminescence

luminescence - the emission of light by processes that derive energy from nonthermal sources (e.g., phosphorescence, fluorescence, bioluminescence)

medium - a substance in which bacteria are cultivated

nutrients - in this case, minerals and other substances needed for phytoplankton growth

oxyluciferin - the chemical that is produced when luciferin and oxygen combine in the presence of luciferase.

Extension

Bioluminescence in Sea Fireflies

The "fireflies" are available from Carolina Biological Supply Company, item #20-3431. The one gram bottle contains several hundred dried bioluminescent ostracods called *Cypridina hilgendorfii*, so there are plenty for several classes to observe. The bottle comes with background information and procedures for observing the bioluminescence. The procedure can be as simple as darkening a room, placing a few of the "fireflies" on the palm of your hand, crushing their shells with a fingertip, and adding water to observe a blue glow. There are also directions for continued study of the enzyme system involving luciferin and luciferase. The studies require readily available equipment such as test tubes, warm water, watch glasses, and glass rods.

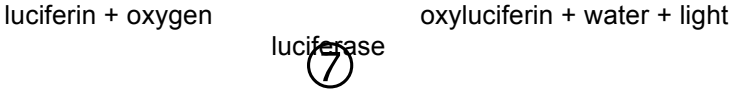
Bioluminescence in Marine Dinoflagellates

Living colonies of the bioluminescent dinoflagellate, *Pyrocystis lunula*, are available from Blue Moon Products, 350 West 800 North, Suite 218, Salt Lake City, UT 84103. Complete instructions for maintaining healthy colonies, as well as a variety of explorations which can be performed with these bioluminescent organisms, are also available.

Answer Key

Text questions

1. The term bioluminescence means the production of light by living organisms.
2. Luciferase is a catalyst that speeds the production of light by living organisms. As a catalyst, luciferase is not consumed by the reaction. Without luciferase the reaction proceeds at such a slow rate as to be considered insignificant.
3. We can write the light producing reaction as follows:



4. "Cold light" is light produced at near 100% efficiency; there is no waste heat produced.

Analysis and Interpretation

1. Answers depend upon the experimental results.
2. Answers depend upon the experimental results.
3. Petri dish number 3 serves as the control. It was not inoculated. If it does not grow bioluminescent bacteria, we can be more confident that the bacteria in the other dishes came from the fish slime with which we inoculated them.

4. Any colonies that appear in dish number three are due to contamination and poor sterile technique. Possible causes include unclean glassware, failure to flame mouth of test tube, contamination from air while the petri dish was open, etc.

5. The points when contamination by microorganisms from other sources is most likely to occur happen when the petri dish is open during the pouring and streaking procedures or if the dish is opened to observe or count the colonies.

6. The bacteria were most likely in the scrapings since the sea water used was sterile. The bacteria colonies may, however, have come from contamination in the ways mentioned above.

7. The refrigeration of the fish and the incubation at a cool temperature in a dark place seem to indicate that the bacteria prefer lower temperatures.

8. The relationship between fish and bioluminescent bacteria appears to be a symbiotic relationship in which both parties benefit. The fish gains a pattern of luminescence that allows it to be recognized. The bacteria gets a “home” and perhaps a food source in the dead outer slime layer. At any rate, neither party seems to be harmed by the relationship.

9. It is important to point out that the benefits subscribed to bioluminescence are based largely on speculation. The actual benefits to a particular organism may not be understood at this particular time. Some of the benefits tentatively assigned include: species recognition, mate recognition and location, a lure for food, a warning signal, illumination, etc. Your students may have theories of their own that merit discussion.

10. The answers will vary. Reward creativity with praise rather than with criticism. One possible experiment might involve altering the lighted pattern on selected members of a species of fish and observing their interaction with non-altered fish of the same species and with similarly altered fish.

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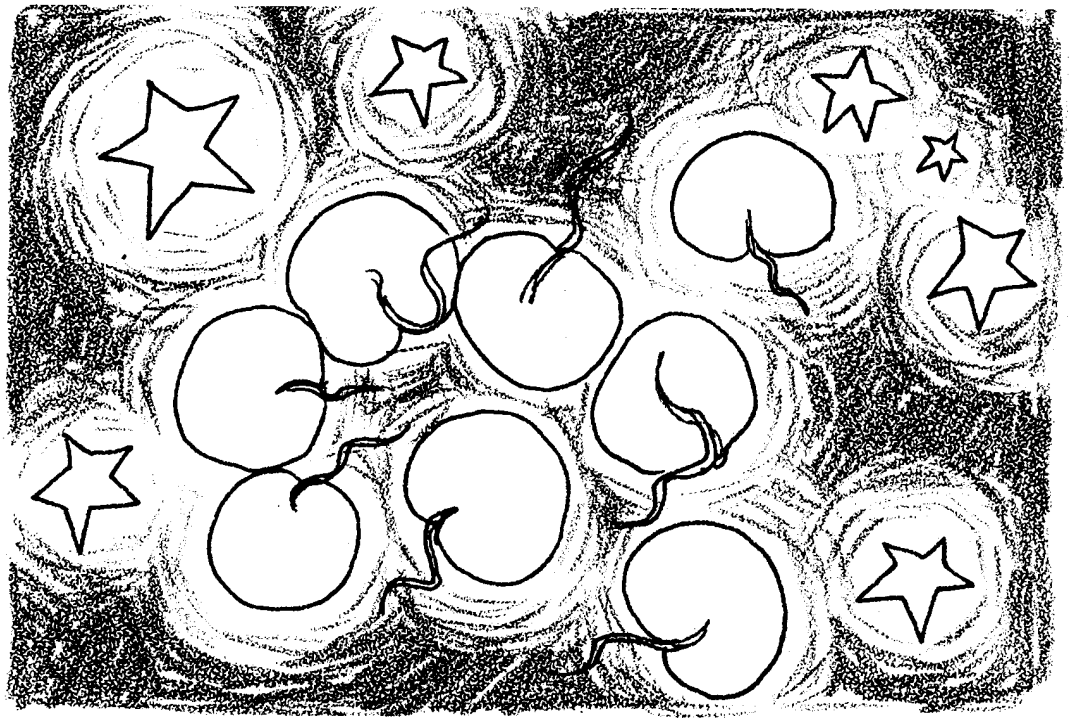

The dinoflagellate *Noctiluca* whose name translates as: "Night light"

The cold depths of the sea are without sunlight. Life goes on there in perpetual darkness. Or is it totally dark? Many of the plants and animals cope with this lack of sunlight by producing light of their own. This feeble light is a poor substitute for sunlight, but it does serve to light some of the inhabitants of the seas. Biological luminescence or **bioluminescence**, the production of light by living organisms, is common in marine plants and animals. The surface of the sea often puts on fiery displays as microorganisms at the surface produce light. Almost all of the invertebrate groups in the open ocean bioluminesce. The depths are lighted by microorganisms.

What causes this glow? Hundreds of years ago people hypothesized that the ocean absorbed the light of the sun by day and emitted it again at night. Today we know that the light production by living creatures is due to a series of complex chemical reactions. Within the light producing organisms a chemical called "luciferin" (from the Latin for "light bearer") combines with oxygen to yield oxyluciferin and water. In the process light energy is released. It is this light energy that we see. This reaction cannot occur without the presence of an

enzyme, luciferase. This enzyme acts as a catalyst; it speeds the reaction but is not consumed by the reaction. We might write the reaction in this manner:

$$\text{luciferin} + \text{oxygen} \xrightarrow[\text{luciferase}]{⑦} \text{oxyluciferin} + \text{water} + \text{light}$$

The arrows show that the reaction can occur in either direction. The reaction occurs in the presence of luciferase.

1. What is meant by the term bioluminescence?

2. How does luciferase affect the production of light by living organisms?

The number of organisms in the sea that exhibit bioluminescence is great. Bacteria, fungi, radiolarians, dinoflagellates, sponges, jellyfish, marine worms, squids, crustaceans, clams, snails and fish all have bioluminescent members. Interestingly enough, some of the bioluminescent fish actually have light producing bacteria colonies which live on their surface. It is these bacteria that produce the light. The light patterns always occur in the same place on the same kind of fish. How can this be? The bacteria cannot be inherited in the normal sense of the word since they are a separate organism. Perhaps the developing fish is "inoculated" by the bacteria. An explanation of the regular patterns of light producing colonies awaits further experimentation.

3. How might we write the light producing reaction?

Light production that we are familiar with produces heat. Anyone who has touched a glowing light bulb can witness the heat production. The heat produced is a waste product; we don't want the heat, we want the light. Bioluminescence is a cold light. The reactions are about 100% efficient in light production. All of the energy changed produces light; none produces heat. One molecule of luciferin consumed or burned gives one unit of light. Research into the chemistry of bioluminescence may give us some techniques for improving our own light producing capabilities. Our present lighting is about 50% efficient. Perhaps we can take a lesson from Mother Nature!

4. What do we mean by "cold light"?

In this activity you will have a chance to observe bioluminescence in marine bacteria. The bacteria can obtained from the outer slime covering of a marine fish. The fish has been kept refrigerated for two days. In this exercise, you will need to follow the same sterile technique used in the activity "Microscopic Forms: Marine Bacteriology".

Materials

- 1 sterile scalpel
- 3 sterile petri dishes
- 1 sterile test tube
- 1 sterile stopper for the test tube
- 1 inoculating loop or cotton swab
- Marine Luminescent Bacterial Medium

  20 grams peptone

  10 grams glycerine

  15 grams agar

  1 liter sterile sea water

To prepare the medium, bring the water to a simmer. Add the agar and other ingredients and stir until they dissolve. Pour the medium into test tubes and autoclave at 15 lbs of pressure or 120°C for 20 minutes.

Procedure:

1. Prepare 3 petri dishes of medium. Use one dish as a control plate.
2. Scrape a layer of surface slime from the side of the fish, using a sterile scalpel or similar instrument.
3. Place the scrapings in a sterile test tube containing 10 ml. of sterile sea water. Stopper the test tube.
4. Agitate the test tube to mix thoroughly.
5. Use the inoculating loop or cotton swab to streak two petri dishes with the solution. Label these dishes (l) and (2), Label the third dish (3).
6. Incubate all three petri dishes in a cool dark place (not the refrigerator) and observe daily in a dark room. (Allow several minutes for your eyes to become dark adapted).

   Observe and record daily:
   - number of colonies
   - size of colonies
   - form of colonies
   - time of appearance of each colony
   - amount of luminescence per colony (high, medium, low)
   - amount of luminescence in test tube before and after shaking (high, medium, low)

7. When luminescent colonies of bacteria are found, use the inoculating loop to scrape the colony from the surface of the agar.
8. Transfer the bacteria to a sterile test tube containing 5 ml. of sterile sea water and observe in the dark. (Allow several minutes for your eyes to become dark adapted.)
9. Shake the tube and repeat observations.

Analysis and Interpretation

1. Which petri dish had the most colonies?

2. Which dish had the most luminescent colonies?

3. What was the role of petri dish number 3?

4. How can you account for any colonies in dish number three?

5. At what point(s) in the procedure is contamination most likely?

6. Were the bacteria originally present in the sea water or in the fish scrapings? Explain your answer.

7. What conclusion does the refrigeration of the fish and the manner of incubation of the petri dishes lead you to make about the temperature preferences of these bacteria?

8. In the relationship between the fish and the bacteria, who benefits and how?

9. While it is difficult to see the benefit of bioluminescence to the bacteria, benefits of bioluminescence for other organisms may be more obvious. How may deep sea fish benefit from the ability to produce light?

10. Since different deep sea fish have different patterns of illumination it is thought that these patterns may help fish identify their own species. Design an experiment that might help prove or disprove the hypothesis that light patterns are used for species recognition. Be sure to include the materials you will need as well as the procedure you will follow.