



NOAA Ship *Okeanos Explorer*: America's Ship for Ocean Exploration.
Image credit: NOAA. For more information, see the following
Web site:
<http://oceanexplorer.noaa.gov/okeanos/welcome.html>

Section 5: Key Topic – Human Health

Watch the Screen!

(adapted from the 2003 Medicines from the Deep Sea: Exploration of the Gulf of Mexico Expedition)

Focus

Screening natural products for biological activity

Grade Level

9-12 (Life Science)

Focus Question

How can natural products be tested for biological activity?

Learning Objectives

- Students will explain and carry out a simple process for screening natural products for biological activity.
- Students will infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Materials

For each student group:

- 1 Copy of *Active Ingredient Screening Guide*
- Disposable plastic serological pipettes, 1 ml (Carolina Biological Supply No. 736122)
- 1 Mortar and pestle set (Carolina Biological Supply No. 742892)
- 3 Disposable plastic Petri dishes, 100 mm x 10 mm (Carolina Biological Supply No. 741248), three for each student group
- 1 1-liter Erlenmeyer flask, one for each student group
- Forceps, one for each student group

For preparation and for class:

- *Escherichia coli* B culture (Carolina Biological Supply No. 124300)
- Dehydrated nutrient agar, pre-measured packs (Carolina Biological Supply No. 789642)
- Luria Broth (Carolina Biological Supply No. 216620)
- Nichrome wire inoculating loops (Carolina Biological Supply No. 703060)
- Antibiotic sensitivity disks, blank, sterile (Carolina Biological Supply No. 805091)
- Incubator
- Autoclave or pressure cooker
- Alcohol or gas burner for sterilization of forceps
- Distilled water
- Strapping tape

Audiovisual Materials

- Marker board, blackboard (or digital equivalent), or overhead projector with transparencies for group discussions

Teaching Time

One or two 45-minute class periods, plus time for student research

Seating Arrangement

Groups of 2-4 students

Maximum Number of Students

32

Key Words and Concepts

Cardiovascular disease
Cancer
Arthritis
Natural products
Active ingredient screening

Background Information

NOTE: Explanations and procedures in this lesson are written at a level appropriate to professional educators. In presenting and discussing this material with students, educators may need to adapt the language and instructional approach to styles that are best suited to specific student groups.

Despite the many advances of modern medicine, disease is still the leading cause of death in the United States. Cardiovascular disease and cancer together account for more than 1.5 million deaths annually (40% and 25% of all deaths, respectively). In addition, one in six Americans have some form of arthritis, and hospitalized patients are increasingly threatened by infections that are resistant to conventional antibiotics. The cost of these diseases is staggering: \$285 billion per year for cardiovascular disease; \$107 billion per year for cancer; \$65 billion per year for arthritis. Death rates, costs of treatment and lost productivity, and emergence of drug-resistant diseases all point to the need for new and more effective treatments.

Most drugs in use today are produced from compounds that come from nature. Aspirin, for example, was first isolated from the willow tree. Morphine is extracted from the opium poppy. Penicillin was discovered from common bread mold. To date, almost all of the drugs derived from natural sources come from terrestrial organisms. But recently, systematic searches for new drugs have shown that marine invertebrates produce more antibiotic, anti-cancer, and anti-inflammatory substances than any group of terrestrial organisms. Particularly promising invertebrate groups include sponges, tunicates, ascidians, bryozoans, octocorals, and some molluscs, annelids, and echinoderms.

The list of drugs derived from marine invertebrates includes:

Ecteinascidin – Extracted from tunicates; being tested in humans for treatment of breast and ovarian cancers and other solid tumors; acts by blocking transcription of DNA

Toposentin – Extracted from the sponges *Topsentia genitrix*, *Hexadella* sp., and *Spongosorites* sp.; anti-inflammatory agent; mode of action not certain



The deepwater sponge *Discodermia* is now in clinical trials for the treatment of cancer. Image credit: NOAA.

<http://oceanexplorer.noaa.gov/explorations/03bio/background/plan/media/discodermia.html>



Though they may be visually unimpressive, *Forcepia* sponges (left) are the source of the lasonolides and tunicates (right) are the source of ecteinascidin, potential new drugs for treating cancer.

Image credit: NOAA.

http://oceanexplorer.noaa.gov/explorations/03bio/logs/hirez/lasonolide1_hirez.jpg

http://oceanexplorer.noaa.gov/explorations/03bio/logs/hirez/figure4_hirez.jpg



Harbor Branch Oceanographic Institution researcher Dr. Shirley Pomponi removes a bright yellow sponge from a rock collected by an underwater robot during the 2003 Medicines from the Deep Sea Expedition. Extracts from the sponge were tested for anti-cancer properties. Image credit: Laura Rear, NOAA.

http://oceanexplorer.noaa.gov/explorations/03bio/logs/summary/media/10249_bio_600.jpg



An agar plate with microorganisms isolated from a deepwater sponge. Image credit: NOAA.

http://oceanexplorer.noaa.gov/explorations/03bio/background/microbiology/media/figure_03.html

Lasonolide – Extracted from the sponge *Forcepia* sp.; anti-tumor agent; acts by binding with DNA

Discodermalide – Extracted from deep-sea sponges belonging to the genus *Discodermia*; anti-tumor agent; acts by interfering with microtubule networks

Bryostatins – Extracted from the bryozoan *Bugula neritina*; potential treatment for leukemia and melanoma; acts as a differentiating agent, forcing cancer cells to mature and thus halting uncontrolled cell division

Pseudopterostins – Extracted from the octocoral *Pseudopterogorgia elisabethae* (sea whip); anti-inflammatory and analgesic agents that reduce swelling and skin irritation and accelerate wound healing; acts as an inhibitor of phospholipase A, which is a key enzyme in inflammatory reactions

ω-conotoxin MVIIA – Extracted from the cone snail *Conus magnus*; potent pain-killer; acts by interfering with calcium ion flux, thereby reducing the release of neurotransmitters

This list reflects an interesting fact about invertebrates that produce pharmacologically-active substances: most species are sessile; they are immobile and live all or most of their lives attached to some sort of surface. Several reasons have been suggested to explain why these particular animals produce potent chemicals. One possibility is that they use these chemicals to repel predators, because they are sessile, and are basically “sitting ducks.” Since many of these species are filter feeders, and consequently are exposed to all sorts of parasites and pathogens in the water, they may use powerful chemicals to repel parasites or as antibiotics against disease-causing organisms. Competition for space may explain why some of these invertebrates produce anti-cancer agents: if two species are competing for the same piece of bottom space, it would be helpful to produce a substance that would attack rapidly dividing cells of the competing organism. Since cancer cells often divide more rapidly than normal cells, the same substance might have anti-cancer properties.

The goal of the 2003 Medicines from the Deep Sea Expedition was to discover new resources with pharmaceutical potential in the Gulf of Mexico. To achieve this goal, the expedition:

- Collected selected benthic invertebrates from deepwater bottom communities (sponges, octocorals, molluscs, annelids, echinoderms, tunicates), identified these organisms, and obtained samples of DNA and RNA from the collected organisms;
- Isolated and cultured microorganisms that live in association with deep-sea marine invertebrates;
- Prepared extracts of benthic invertebrates and associated microorganisms, and tested these extracts to identify those that might be useful in treatment of cancer, cardiovascular disease, infections, inflammation, and disorders of the central nervous system;
- Isolated chemicals from extracts that show pharmacological potential and determined the structure of these chemicals;
- Studied the pharmacological properties of active compounds; and
- Developed methods for the sustainable use of biomedically important marine resources.

The last activity is particularly important, since many potentially useful compounds found in animals are present in very small quantities. This makes it impossible to obtain useful amounts of the substances simply by harvesting large numbers of

animals from the sea. Some alternatives are chemical synthesis of specific products, aquaculture to produce large numbers of productive species, or culture of the cells that produce the desired compounds. Some techniques for producing specific drugs are based on the cells' own machinery for chemical synthesis: enzymes, guided by information contained in the cells' DNA and RNA.

This activity is designed to acquaint students with the process of screening for active ingredients in biological materials.


Learning Procedure

[NOTE: This lesson is based upon an activity designed by Jane Settle while participating in the 1993 Woodrow Wilson Biology Institute (<http://www.woodrow.org/teachers/bi/1993/active.html>). This activity is used with permission from the Woodrow Wilson National Fellowship Foundation. Visit <http://woodrow.org> for information on other activities and current programs.]

1. To prepare for this lesson:
 - Review established safety procedures for working with microbial materials, including school, district, and state policies; also see <http://www.nsta.org/portals/safety.aspx#elem> and http://www.isbe.net/ils/science/pdf/science_safety.pdf.
 - Review introductory information on the NOAA Ship *Okeanos Explorer* at <http://oceanexplorer.noaa.gov/okeanos/welcome.html>. You may also want to consider having students complete some or all of the lesson, *To Boldly Go...*
 - Review the *Active Ingredient Screening Guide*, and prepare one copy for each student group. You may also want to review "Active Ingredient Screening Test for Plants" from <http://www.woodrow.org/teachers/bi/1993/active.html>
 - One day before the lab, prepare Luria broth for culturing *E. coli* bacteria, and inoculate the broth medium with a loopful of culture using sterile technique. Incubate at 35 – 37°C for 24 hours.
 - Before the lab begins, prepare nutrient agar and sterilize by autoclaving or in a pressure cooker. If you use a pressure cooker, place the agar container in a basket just above the water level. Seal the lid onto the cooker and allow steam to flow freely for 10 minutes. Place the pressure control on the vent and maintain the pressure at 15 pounds for 30 minutes. At the end of this time, let the cooker cool, then keep the agar warm in a water bath on a hot plate to prevent gelling.
2. If you have not previously done so, briefly introduce the NOAA Ship *Okeanos Explorer*, emphasizing that this is the first Federal vessel specifically dedicated to exploring Earth's largely unknown ocean. Lead a discussion of reasons that ocean exploration is important, which should include human health.

Several days before beginning the lab investigation, review the importance of finding new drugs for the treatment of cardiovascular disease, cancer, inflammatory diseases, and infections. Describe the potential of marine communities as sources for these drugs, and briefly discuss some potentially useful drugs that have been discovered from these communities. Ask students to list some reasons that these kinds of drugs might be found primarily among sessile invertebrates. Briefly discuss the initial steps in the search for new drugs, and tell students that they will soon be testing various plant extracts for antibiotic activity using techniques similar to those used to screen for biologically active





ingredients in the field. Brainstorm plants that students think may have antibiotic properties, and develop a list of plants for the students to collect. Jane Settle suggests yew, golden meadow parsnips, parsley, pussy willow leaves and/or bark, wild garlic, wild onion, wild iris, bedstraw, larkspur, blue-eyed grass, penstemon, wild licorice, four o'clock, big bluestem grass, and basil. Have students bring at least 5 leaves from the plants they choose to test. If wild plants are not available, try commonly available herbs or other food that may have medicinal properties, such as garlic, mustard (leaves and seeds), banana, honey, goldenseal, and *Echinacea* root.

3. Provide each student group with a copy of the *Active Ingredient Screening Guide*. Have students prepare Petri dishes and inoculate them with *E. coli* culture as directed by the *Guide*. While the agar is cooling, have students prepare plant extracts as directed (Steps B1 through B3). Each group should prepare extracts from four plants, and test them in three replicate Petri dishes (Steps C1 through C3).
4. After 48 hours of incubation, have students examine their petri dishes and look for zones of inhibition (a clear area formed around the test disks due to the inhibition of *E. coli* growth by the plant extract). Have students measure the diameter of any zones of inhibition they observe. Each group should summarize their results on the *Active Ingredient Screening Data Sheet*, and prepare a brief written analysis of their conclusions based on these tests. You may also want to require that these reports include answers to the questions on the *Active Ingredient Screening Guide*.
5. Upon completion of this activity, collect the culture dishes, and immerse them in a 10% bleach solution for at least 15 minutes. Drain the excess solution and seal the dishes in a plastic bag for disposal. Alternatively, you may sterilize the dishes for 30 minutes in a pressure cooker at 15 lb pressure.
6. Have each group make a brief presentation of their results. Summarize these results on a marker board or overhead transparency. Lead a discussion of how this lab activity relates to the process of actually searching for new drugs. Students should recognize that scientists might want to screen for other types of biological activity in addition to antibiotic properties.

Discuss the process of developing a useful drug from a marine organism. The first step, of course, is to locate a promising candidate. This involves “prospecting” among many different species, though past experience suggests some groups (sessile invertebrates) that may be particularly promising. Extracts of each species are prepared, usually by grinding tissue from the organisms in organic solvents. Next, the extracts are tested for pharmacological activity through a series of bioassays (for example, finding out whether an extract can kill leukemia cells or reduce inflammation). When an extract is found to have positive biological activity, the active substance in the extract is isolated and identified. If the isolated chemical turns out to be new, the next step is to test the chemical in animal models (for example, mice with tumors). If animal testing is successful, the chemical may be approved for evaluation in humans. If the chemical is effective in humans without toxic side effects, it may be approved as a new drug. The entire process can take a lot of time and money: a new anti-cancer drug may require 10 – 20 years and an average of \$40,000,000 to develop to the point of commercialization.

The BRIDGE Connection

www.vims.edu/bridge/ – Scroll over “Ocean Science” in the navigation menu to the left, then “Human Activities” then “Technology” for resources on biotechnology and drugs from the sea.

The “Me” Connection

Have students write a short essay about natural products that are of personal importance, and why it is important to protect rare or unknown species.

Connections to Other Subjects

English/Language Arts

Assessment

Students’ responses to the *Guide* questions and class discussions provide opportunities for assessment.

Extensions

1. Visit <http://oceanexplorer.noaa.gov/explorations/03bio/welcome.html> to find out more about the Deep Sea Medicines 2003 Expedition.
2. Visit <http://www.woodrow.org/teachers/bi/1993/> for more activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute.
3. Visit the following web sites for other activities related to microorganisms:
www.glogerm.com
http://ceprap.ucdavis.edu/index.php?option=com_content&view=article&id=56&Itemid=138 - Click on “Microbe Laboratories” and it will automatically download
<http://spikesworld.spike-jamie.com/science/index.html>
<http://www.umsl.edu/~microbes/>

Multimedia Discovery Missions

<http://oceanexplorer.noaa.gov/edu/learning/welcome.html> Click on the links to Lessons 12 for interactive multimedia presentations and Learning Activities on Food, Water, and Medicines from the Sea.

Other Relevant Lesson Plans from NOAA’s Ocean Explorer Program

(The following Lesson Plans are targeted toward grades 9-12)

Cell Mates (from the 2003 Medicines from the Deep Sea Expedition)

http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_cellmates.pdf


Focus: Bacterial endosymbionts and organelles of eukaryotic cells (Life Science)
Students will compare and contrast prokaryotic and eukaryotic cells, explain the endosymbiont theory for the origin of eukaryotic cell organelles, and explain evidence that suggests an endosymbiotic origin for at least two common eukaryotic cell organelles.

The Benthic Drugstore (from the 2003 Medicines from the Deep Sea Expedition)

http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_drugstore.pdf

Focus: Pharmacologically-active chemicals derived from marine invertebrates (Life Science)





Students will identify at least three pharmacologically-active chemicals derived from marine invertebrates, describe the disease-fighting action of at least three pharmacologically-active chemicals derived from marine invertebrates, and infer why sessile marine invertebrates appear to be promising sources of new drugs.

The Electric Sieve (from the 2003 Medicines from the Deep Sea Expedition)

http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_elecsieve.pdf

Focus: Separation of complex mixtures (Chemistry)

Students will explain and carry out a simple process for separating complex mixtures, and will infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Other Resources

See page 217 for Other Resources.

Next Generation Science Standards

Lesson plans developed for Volume 1 are correlated with *Ocean Literacy Essential Principles and Fundamental Concepts* as indicated in the back of this book. Additionally, a separate online document illustrates individual lesson support for the Performance Expectations and three dimensions of the Next Generation Science Standards and associated Common Core State Standards for Mathematics and for English Language Arts & Literacy. This information is provided to educators as a context or point of departure for addressing particular standards and does not necessarily mean that any lesson fully develops a particular standard, principle or concept. Please see: http://oceanexplorer.noaa.gov/okeanos/edu/collection/wdwe_ngss.pdf.

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Active Ingredient Screening Guide

(adapted from *Active Ingredient Screening Test for Plants* by Jane Settle, 1993 Woodrow Wilson Biology Institute)

Purpose

To determine if various plant materials contain active ingredients that will inhibit the growth of bacteria.

Procedure

A. Prepare and Inoculate Petri Dishes

1. Prepare three empty, sterile Petri dishes by marking off the bottom of each Petri dish into quadrants, using a permanent felt-tip marker. Place a letter near the periphery of each quadrant that represents the plant material you will test. Label each dish with your name, class period and date.
2. Use a pipette to transfer 1 ml of the inoculum of *Escherichia coli* to each of the three sterile Petri dishes. Add 20 ml of the sterile liquid nutrient agar. Gently agitate the plate to diffuse the inoculum and allow it to gel and cool.

B. Prepare Plant Materials for Testing

1. Grind the plant material from one plant with a little distilled water in a mortar and pestle.
2. Use a sterile pipette to transfer the liquid from the mortar into an empty sterile Petri dish. Label the Petri dish with the name of the plant material.
3. Repeat Steps B1 and B2 for three other plant materials.

C. Test Plant Extracts for Bacterial Inhibition

1. Sterilize forceps by flaming or soaking in alcohol. Use the sterilized forceps to place 3 sterile disks into each of the liquid plant materials. Allow to soak for one minute.
2. After the agar has gelled in the Petri dishes prepared in Part A, use sterile forceps to carefully place one disk (blot any excess liquid before placing it on the Petri dish) in the correctly labeled quadrant, about 2 cm from the outer edge of the Petri dish. Place a control disk saturated with sterile distilled water in the center of each dish. Seal the dish with strapping tape.

Repeat this step twice more, so that you have three replicate Petri dishes in which you are testing four plant materials for their ability to inhibit bacterial growth.

3. Invert the Petri dishes and incubate at 35–37° C for 48 hours.
4. If desired, you may save the plant extracts in the refrigerator for future use.
5. After 48 hours, examine the plates with the plant disks and look for zones of inhibition. This is a clear area formed around the disc due to inhibitory action of the substances in the plant material. If a zone of inhibition is present, measure the diameter of this clear area and record your results on the *Data Sheet*.



Analysis

1. Which of the plant materials inhibited the bacteria growth?

2. Which of the plant materials had no effect on the bacteria growth?

3. What does the clear zone around the disk indicate in this investigation?

4. Why is it important to use sterile techniques in this investigation?

5. What variable factors could affect the zone of inhibition in this investigation?

6. Why do plants vary in their active ingredients? How might these active ingredients be advantageous to plants that produce them?

Active Ingredient Screening Data Sheet

Plant Material	Zone of Inhibition (Diameter)	Zone of Inhibition (Degree of Sensitivity)*
Petri Dish A.		
1.		
2.		
3.		
4.		
Control:		
 Petri Dish B.		
1.		
2.		
3.		
4.		
Control:		
 Petri Dish C.		
1.		
2.		
3.		
4.		
Control:		

*Identify inhibition zones as:

No Effect = 0, Slightly Sensitive = +, Sensitive = ++, Very Sensitive = +++