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Medicines from the Deep Sea: Exploration of the Gulf of Mexico

Watch the Screen!

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 be able to explain and carry out a simple process for screening natural products for biological activity.

Students will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Materials
- ❒ *Escherichia coli B* culture (Carolina Biological Supply No. WW-12-4300)
- ❒ Dehydrated nutrient agar, premeasured packs (Carolina Biological Supply No. WW-78-9662)
- ❒ Luria Broth (Carolina Biological Supply No. WW-21-6620)
- ❒ Nichrome wire innoculating loops (Carolina Biological Supply No. WW-70-3060)
- ❒ Disposal plastic serological pipettes, 1 ml (Carolina Biological Supply No. WW-73-6095)
- ❒ Mortar and pestle set (Carolina Biological Supply No. WW-74-2892)
- ❒ Disposable plastic petri dishes, 100 mm x 10 mm (Carolina Biological Supply No. WW-74-1248), one or more for each student group
- ❒ Antibiotic sensitivity disks, blank, sterile (Carolina Biological Supply No. WW-80-5091)
- ❒ Incubator
- ❒ Autoclave or pressure cooker
- ❒ 1 liter Ehrlenmeyer flask, one for each student group
- ❒ Forceps, one for each student group
- ❒ Distilled water
- ❒ Marker board, blackboard, or overhead projector with transparencies for group discussions
- ❒ Student instruction handout for each student (see "Learning Procedure")

Audio/Visual Materials
None

Teaching Time
Two or three 45-minute class periods

Seating Arrangement
Groups of 2-3 students

Maximum Number of Students
30

Key Words
Cardiovascular disease
Cancer
Arthritis
Natural products
Active ingredient screening

Background Information
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 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

Topsentin – Extracted from the sponges T*opsentia genitrix, Hexadella* sp., and *Spongosorites* sp.; anti-inflammatory agent

Lasonolide – Extracted from the sponge *Forcepia* sp.; anti-tumor agent

Discodermalide – Extracted from deep-sea sponges belonging to the genus *Discodermia;* anti-tumor agent

Bryostatin – Extracted from the bryozoan *Bugula neritina;* potential treatment for leukemia and melanoma

Pseudopterosins – Extracted from the octocoral (sea whip) *Pseudopterogorgia elisabethae;* anti-inflammatory and analgesic agents that reduce swelling and skin irritation and accelerate wound healing

ω-conotoxin MVIIA – Extracted from the cone snail *Conus magnus;* potent pain-killer

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, since they are sessile, and thus 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 is to discover new resources with pharmaceutical potential in the Gulf of Mexico. To achieve this goal, the expedition will:

- collect selected benthic invertebrates from deep-water bottom communities in the Gulf of Mexico (sponges, octocorals, molluscs, anne-

lids, echinoderms, tunicates), identify these organisms, and obtain samples of DNA and RNA from the collected organisms;
- isolate and culture microorganisms that live in association with deep-sea marine invertebrates;
- prepare extracts of benthic invertebrates and associated microorganisms, and test these extracts to identify those that may be useful in treatment of cancer, cardiovascular disease, infections, inflammation, and disorders of the central nervous system;
- isolate chemicals from extracts that show pharmacological potential and determine the structure of these chemicals;
- further study the pharmacological properties of active compounds; and
- develop methods for the sustainable use of biomedically-important marine resources.

The last objective is particularly important, since many potentially useful drugs are present in very small quantities in the animals that produce these drugs. This makes it impossible to obtain useful amounts of the drugs simply by harvesting large numbers of animals from the sea. Some alternatives are chemical synthesis of specific compounds, aquaculture to produce large numbers of productive species, or culture of the cells that produce the drugs. 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. 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. Download the following activity:
   "Active Ingredient Screening Test for Plants"
   from http://www.woodrow.org/teachers/bi/1993/

2. Several days before the lab, 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 introduce the objectives of the 2003 Medicines from the Deep Sea Expedition. Highlight 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 student 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.

   One day before the lab, prepare Luria broth for culturing *E. coli* bacteria, and innoculate the broth medium with a loopful of culture using sterile technique. Incubate at 35 – 37°C for 24 hours. Prepare student instruction sheets from the downloaded activity.

   Before the lab begins, prepare nutrient agar and sterilize by autoclaving or in a pressure cooker (see the "Microfriends" lesson plan for details on using a pressure cooker). Keep the agar warm in a water bath on a hot plate to prevent gelling.

3. Have students prepare petri dishes and innoculate them with *E. coli* culture as directed by the student worksheet. While the agar is cooling, have students prepare plant extracts as directed, and place disks saturated with the extracts in the appropriate petri dishes. Seal the dishes with strapping tape, turn upside down, and incubate at 35 – 37°C for 48 hours.

4. 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 student 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 student data sheet.

5. Following this activity, collect the culture dishes and sterilize them in an autoclave or pressure cooker for 30 minutes 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 (this is a good opportunity to interact with the scientists on the Medicines from the Deep Sea Expedition). 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/ – Click on "Ocean Science" in the navigation menu to the left, then "Chemistry" for resources on drugs from the sea. Click on "Ecology" then deep sea for resources on deep-sea communities. Click on "Human Activities" then "Technology" then "Biotechnology" for resources on biotechnology.

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

Evaluation

Written and oral reports in Steps 4 and 5 provide opportunities for assessment.

Extensions

Log on to http://oceanexplorer.noaa.gov to keep up to date with the latest discoveries of the 2003 Medicines from the Deep Sea Expedition.

Visit http://www.woodrow.org/teachers/bi/1993/ for more

activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute.

Resources

http://oceanica.cofc.edu/activities.htm – Project Oceanica website, with a variety of resources on ocean exploration topics

http://www.reefcheck.org/headlines/june/pdf/marine_pharmacology.pdf – Marine pharmacology

Faulkner, D. J. 2000. Marine pharmacology. Antonie van Leeuwenhoek 77: 135-145. Available online at http://www.reefcheck.org/headlines/june/pdf/marine_pharmacology.pdf.

www.nci.nih.gov – Website of the National Cancer Institute

http://www.woodrow.org/teachers/bi/1993/ – Background and activities from the 1993 Woodrow Wilson Biology Institute on biotechnology

http://www.umsl.edu/~microbes/pdf/steriletechnique.pdf - Worksheet on sterile technique

http://www.umsl.edu/~microbes/ – Website of the Science in the Real World: Microbes in Action project of the Department of Biology, University of Missouri - St. Louis

www.glogerm.com – Website of the Glo-Germ Company, with activity ideas related to microorganisms

http://ceprap.ucdavis.edu/acrobat/microkit_00.pdf – Activity manual developed during the 1996/97 teacher internship program of the Center for Engineering Plants for Resistance Against Pathogens at the University of California, Davis

http://spikesworld.spike-jamie.com/science/index.html — Website with lots of background and activities on multiple science topics, including microorganisms

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard C: Life Science

- The cell
- Interdependence of organisms
- Behavior of organisms

Content Standard E: Science and Technology

- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Personal and community health
- Natural resources
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

For More Information

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