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2007 Cayman Island Twilight Zone Expedition

Watch the Screen!

(adapted from the 2003 Deep Sea Medicines 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 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

- ❒ Marker board, blackboard, or overhead projector with transparencies for group discussions

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
Coral reef

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 *Topsentia 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.

Coral reefs provide habitats for some of the most diverse biological communities on Earth. Most people have seen photographs and video images of shallow-water coral reefs, and many have visited these reefs in person. Historically, scientists have believed that reef-building corals were

confined to relatively shallow depths, but ocean explorers have recently discovered extensive mounds of living coral in depths from 400 m to 700 m. Studies indicate that the diversity of species in deep-water coral ecosystems may be comparable to that of coral reefs in shallow waters, and that there are just as many species of deep-water corals (slightly more, in fact) as there are species of shallow-water corals. This high species diversity makes these ecosystems very promising sources of powerful new antibiotic, anti-cancer and anti-inflammatory drugs.

Around the world, shallow water coral reefs have been intensively studied by scientists using self-contained underwater breathing (SCUBA) equipment, while deep coral systems are being investigated with submersibles and remotely operated underwater vehicles (ROVs). Recent explorations have found a third type of coral ecosystem between depths of 50 m and 150 m: light-limited deep reefs living in what coral ecologists call the "twilight zone." These reefs have been studied much less than shallow and deep-water reefs because they are beyond the safe range of conventional SCUBA equipment, yet are too shallow and close to shore to justify the use of expensive submersibles and ROVs. The few studies of twilight zone reefs suggest that these ecosystems not only include species unique to this depth range, but may also provide important refuges and nursery habitats for corals and fishes that inhabit shallower reefs. This is particularly important in areas where shallow reefs are severely stressed, since twilight zone coral ecosystems may provide a natural option for recovery.

Scientific exploration of twilight zone coral reef ecosystems is urgently needed to provide information for their protection, as well as to identify potentially important sources of drugs and other biological products from organisms that are endemic to these systems. These are key objectives of the 2007 Ocean Explorer Cayman Island Twilight Zone Expedition.

This lesson 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/active.html.

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 2007 Cayman Island Twilight Zone 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 (oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_microfriends.pdf) 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. 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

Assessment

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

Extensions

Log on to http://oceanexplorer.noaa.gov to keep up with the latest 2007 Cayman Island Twilight Zone Expedition discoveries, and to find out what reserachers are learning about deep fore reef communities.

Visit http://www.woodrow.org/teachers/bi/1993/ for more activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute.

Multimedia Learning Objects

http://www.learningdemo.com/noaa/ – Click on the links to Lessons 3 and 12 for interactive multimedia presentations and Learning Activities on deep-sea corals and biotechnology.

Other Relevant Lesson Plans from the Ocean Exploration Program

History's Thermometers [http://oceanexplorer.noaa.gov/explorations/02alaska/background/edu/media/thermo9_12.pdf] (5 pages, 80k) (from the 2002 Alaska Seamount Expedition)

Focus: Use of deep-water corals be used to determine long-term patterns of climate change (Physics)

In this activity, students will be able to explain the concept of paleoclimatological proxies, learn how oxygen isotope ratios are related to water temperature, and interpret data on oxygen isotope ratios to make inferences about climate and climate change in the geologic past.

Cut-off Genes [http://oceanexplorer.noaa.gov/explorations/04mountains/background/edu/media/MTS04.genes.pdf] (12 pages, 648k) (from the Mountains in the Sea 2004 Expedition)

Focus: Gene sequencing and phylogenetic expressions (Life Science)

In this activity, students will be able to explain the concept of gene-sequence analysis; and, given gene sequence data, will be able to draw inferences about phylogenetic similarities of different organisms.

Feeding in the Flow [http://oceanexplorer.noaa.gov/explorations/03bump/background/edu/media/03cbfeedflow.pdf] (6 pages, 268k) (from the 2003 Charleston Bump Expedition)

Focus: Effect of water currents on feeding efficiency in corals (Life Science)

In this activity, students will be able to describe at least two ways in which current flow may affect the feeding efficiency of particle-feeding organisms and explain how interactions between current flow and the morphology of a particle-feeding organism may affect the organism's feeding efficiency. Students will also be able to identify at least two environmental factors in addition to current flow that may affect the morphology of reef-building corals.

Cool Corals [http://oceanexplorer.noaa.gov/explorations/03edge/background/edu/media/cool.pdf] (7 pages, 476k) (from the 2003 Life on the Edge Expedition)

Focus: Biology and ecology of *Lophelia* corals (Life Science)

In this activity, students will describe the basic morphology of *Lophelia* corals and explain the significance of these organisms, interpret preliminary observations on the behavior of *Lophelia* polyps, and infer possible explanations for these observations. Students will also discuss why biological communities associated with *Lophelia* corals are the focus of major worldwide conservation efforts.

Keep It Complex! [http://oceanexplorer.noaa.gov/explorations/03bump/background/edu/media/03cb_complex.pdf] (5 pages, 272k) (from The Charleston Bump 2003 Expedition)

Focus: Effects of habitat complexity on biological diversity (Life Science)

In this activity, students will be able to describe the significance of complexity in benthic habitats to organisms that live in these habitats and will describe at least three attributes of benthic habitats that can increase the physical complexity of these habitats. Students will also be able to give examples of organisms that increase the structural complexity of their communities and infer and explain relationships between species diversity and habitat complexity in benthic communities.

Are You Related? [http://oceanexplorer.noaa.gov/explorations/05deepcorals/background/edu/media/05deepcorals_related.pdf] (11 pages, 465k) (from the Florida Coast Deep Corals 2005 Expedition)

Focus: Molecular genetics of deepwater corals (Life Science)

In this activity, students will define "microsatellite markers" and explain how they may be used to identify different populations and species, explain two definitions of "species," and describe processes that result in speciation. Students will also use microsatellite data to make inferences about populations of deep-sea corals.

How Does Your (Coral) Garden Grow? [http://oceanexplorer.noaa.gov/explorations/03mex/background/edu/media/mexdh_growth.pdf] (6 pages, 456k) (from the Gulf of Mexico Deep Sea Habitats 2003 Expedition)

Focus: Growth rate estimates based on isotope ratios (Life Science/Chemistry)

In this activity, students will identify and briefly explain two methods for estimating the age of hard corals, learn how oxygen isotope ratios are related to water temperature, and interpret data on oxygen isotope ratios to make inferences about the growth rate of deep-sea corals.

How Diverse is That? [http://oceanexplorer.noaa.gov/explorations/03windows/background/education/media/03win_hdiverse.pdf] (6 pages, 552k) (from the 2003 Windows to the Deep Expedition)

Focus: Quantifying biological diversity (Life Science)

In this activity, students will be able to discuss the meaning of biological diversity and will be able to compare and contrast the concepts of variety and relative abundance as they relate to biological diversity. Given abundance and distribution data of species in two communities, students will be able to calculate an appropriate numeric indicator that describes the biological diversity of these communities.

Other Links and Resources

The Web links below are provided for informational purposes only. Links outside of Ocean Explorer have been checked at the time of this page's publication, but the linking sites may become outdated or non-operational over time.

http://oceanexplorer.noaa.gov – Web site for NOAA's Ocean Exploration program

http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean_coral.html – Ocean Explorer image gallery

http://www-biol.paisley.ac.uk/courses/Tatner/biomedia/units/cnid1.htm – Phylum Cnidaria on Biomedia of the Glasgow University Zoological Museum on the Biological Sciences, University of Paisley, Scotland Web site; includes explanations of the major classes, a glossary of terms and diagrams and photos

http://www.calacademy.org/research/izg/calwildfall2000.pdf – Article from California Wild: "Stinging Seas - Tread Softly In Tropical Waters" by Gary C. Williams; an introduction to the venomous nature of tropical cnidarians, why and how they do it

http://www.cancerquest.org – CancerQuest Web site; includes an introduction to cell biology; structure and action of anti-cancer drugs

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

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

http://www.mcbi.org/publications/pub_pdfs/Deep-Sea%20Coral%20issue%20of%20Current.pdf – A special issue of Current: the Journal of Marine Education on deep-sea corals.

http://www.mesa.edu.au/friends/seashores/index.html – "Life on Australian Seashores" by Keith Davey on the Marine Education Society of Australasia Web site, with an easy introduction to Cnidaria, including their method of reproduction

http://www.cancer.gov/ – Web site of the National Cancer Institute

http://www.oceanicresearch.org/ – The Oceanic Research Group Web site; lots of photos, but note that they are very explicit about their copyrights; check out "Cnidarians: Simple but Deadly Animals!" by Jonathan Bird, which provides an easy introduction designed for classroom use

http://www.science.fau.edu/drugs.htm – An overview article on drugs from the sea

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

http://www.ucmp.berkeley.edu/cnidaria/cnidaria.html – Introduction to Cnidaria from the University of California Museum of Paleontology

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

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

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

Maxwell, S. 2005. An Aquatic Pharmacy: The Biomedical Potential of the Deep Sea. Current 21(4):31-32; available online at http://www.mcbi.org/what/what_pdfs/Current_Magazine/Pharmacy.pdf

Morgan, L. E. 2005. What are deep-sea corals? Current 21(4):2-4; available online at http://www.mcbi.org/what/what_pdfs/Current_Magazine/What_are_DSC.pdf

Reed, J. K. and S. W. Ross. 2005. Deep-water reefs off the southeastern U.S.: Recent discoveries and research. Current 21(4):33-37; available online at http://www.mcbi.org/what/what_pdfs/Current_Magazine/Southeastern_US.pdf

Roberts, S. and M. Hirshfield. Deep Sea Corals: Out of sight but no longer out of mind. http://www.oceana.org/fileadmin/oceana/uploads/reports/oceana_coral_report_final.pdf — Background on deep-water coral reefs

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

Ocean Literacy Essential Principles and Fundamental Concepts

Essential Principle 1.
The Earth has one big ocean with many features.

Fundamental Concept h. Although the ocean is large, it is finite and resources are limited.

Essential Principle 5.
The ocean supports a great diversity of life and ecosystems.

Fundamental Concept c. Some major groups are found exclusively in the ocean. The diversity of major groups of organisms is much greater in the ocean than on land.

Fundamental Concept d. Ocean biology provides many unique examples of life cycles, adaptations and important relationships among organisms (such as symbiosis, predator-prey dynamics and energy transfer) that do not occur on land.

Fundamental Concept e. The ocean is three-dimensional, offering vast living space and diverse habitats from the surface through the water column to the seafloor. Most of the living space on Earth is in the ocean.

Fundamental Concept f. Ocean habitats are defined by environmental factors. Due to interactions of abiotic factors such as salinity, temperature, oxygen, pH, light, nutrients, pressure, substrate and circulation, ocean life is not evenly distributed temporally or spatially, i.e., it is "patchy." Some regions of the ocean support more diverse and abundant life than anywhere on Earth, while much of the ocean is considered a desert.

Essential Principle 6.
The ocean and humans are inextricably interconnected.

Fundamental Concept a. The ocean affects every human life. It supplies freshwater (most rain comes from the ocean) and nearly all Earth's oxygen. It moderates the Earth's climate, influences our weather, and affects human health.

Fundamental Concept b. From the ocean we get foods, medicines, and mineral and energy resources. In addition, it provides jobs, supports our nation's economy, serves as a highway for transportation of goods and people, and plays a role in national security.

Fundamental Concept e. Humans affect the ocean in a variety of ways. Laws, regulations and resource management affect what is taken out and put into the ocean. Human development and activity leads to pollution (such as point source, non-point source, and noise pollution) and physical modifications (such as changes to beaches, shores and rivers). In addition, humans have removed most of the large vertebrates from the ocean.

Fundamental Concept g. Everyone is responsible for caring for the ocean. The ocean sustains life on Earth and humans must live in ways that sustain the ocean. Individual and collective actions are needed to effectively manage ocean resources for all.

Essential Principle 7.
The ocean is largely unexplored.

Fundamental Concept a. The ocean is the last and largest unexplored place on Earth—less than 5% of it has been explored. This is the great frontier for the next generation's explorers and

researchers, where they will find great opportunities for inquiry and investigation.
Fundamental Concept b. Understanding the ocean is more than a matter of curiosity. Exploration, inquiry and study are required to better understand ocean systems and processes.
Fundamental Concept c. Over the last 40 years, use of ocean resources has increased significantly, therefore the future sustainability of ocean resources depends on our understanding of those resources and their potential and limitations.
Fundamental Concept d. New technologies, sensors and tools are expanding our ability to explore the ocean. Ocean scientists are relying more and more on satellites, drifters, buoys, subsea observatories and unmanned submersibles.
Fundamental Concept f. Ocean exploration is truly interdisciplinary. It requires close collaboration among biologists, chemists, climatologists, computer programmers, engineers, geologists, meteorologists, and physicists, and new ways of thinking.

For More Information

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