

Antiseptic Nature of Plants

by Regina Sievert

Grade level 9-12

Time required

Two 45 minute periods,
separated by 24 to 48 hours

**Materials/Technology
required**

(per pair of students)

2 sterile agar-filled petri
dishes
4 short pieces of scotch tape
Wax pencil
Forceps
Bunsen burner
4 sterile filter paper disks
1 ml of yarrow extract
1 ml of mint extract
1 ml of antibacterial soap
Incubator (one for the class)

Summary

Students compare the antiseptic properties of native plants by growing bacteria on agar plates containing plant extracts. Plants used are traditional medicinal plants of the indigenous people of the Flathead Reservation. The concepts of homeostasis and the development of resistance in microbes are also examined.

Objectives

The student will:

- 1) understand the concepts of homeostasis, competition, pathogens and antiseptics.
- 2) examine how antiseptics affect microbial growth and the development of new strains of microbes
- 3) be able to employ sterile technique.
- 4) be able to graph and interpret experimental results in a meaningful manner.
- 5) become aware of contributions of traditional indigenous knowledge to modern medicine.
- 6) learn about some traditional uses of indigenous plants by native people of the Flathead Reservation.

Montana Science standards addressed

- 1) Students demonstrate knowledge of characteristics, structures, and function of living things, the process and diversity of life, and how living organisms interact with each other and their environment.
- 2) Students understand historical developments in science and technology.

Assessment

Evaluate students on their proficiency in completing the lab procedure, graphing and interpreting their results, answering the questions provided and working with their lab partners. Also possible would be an assessment of their participation in class discussion.

Procedural notes

- 1) *Plant extracts* - The two plants chosen, Yarrow (*Achillea millefolium*) and Horsemint (*Monarda fistulosa*) were traditionally used by the native people of the Flathead Reservation, and are still in use today. They are relatively easy to find and collect, although

be aware that collecting plants on tribal land is generally illegal if you are not a tribal member. Collect the yarrow when it is in flower, between early summer and early fall. Collect the mint before the flowers open. For both plants, collect the aerial parts, i.e., the stems, leaves and flowers.

Tinctures are generally the best form of plant extract to use and are easily made. Chop or grind the plants into fine pieces and add them to alcohol or glycerin (glycerin is recommended) in a container that can be closed tightly. Use about 120 grams or 4 ounces of dried herb to 500 ml or 1 pint of liquid. If the herbs are fresh, use about twice as much, or 8 ounces, of the plant. Place the container in a warm location and shake it twice daily. After two weeks, strain the contents in a piece of cloth, removing all the solid material and retaining the liquid.

In case these particular plants are not available, some other traditional plants used as antiseptics include stinging nettles, rattlesnake plantain, sagebrush buttercup, kinnickinnick and snowberry.

- 2) *The agar plates and filter paper disks* - For best results, the agar filled petri dishes and filter paper disks should be sterilized before using. Filter paper disks can be made using a large paper punch and any standard filter paper. The dishes should be autoclaved after use and prior to disposal to destroy any potentially dangerous microbes.
- 3) *Visit with a native expert on plants* - Arrange a visit for your students with an elder or tribal botanist knowledgeable about traditional plants. To find a suitable guest, call the culture committees, the Peoples' Center or the Natural Resources Department at the tribal complex. Ask the person to talk about plants with your students. Be aware that some topics, such as medicinal uses of plants, may be sensitive information, not suitable for transmission in all settings.

Further information

For further information about the experiment, contact Regina Sievert via electronic mail at wenonah@centurytel.net. For further information about the traditional use of plants, contact either the Salish or Kootenai Culture Committees.

Answer key

Introduction questions:

- 1) the best or most favorable
- 2) For example, water content, acidity of blood, respiratory rate, etc.
- 3) Temperature, moisture, nutrition, pH
- 4) Generally, an increase in moisture and temperature
- 5) Depression of the immune system
- 6) Native, not introduced, living naturally in an area
- 7) Destroy microorganisms
- 8) A) Sterilize utensils like forceps, etc. B) Autoclave the agar plate C) Do not touch the agar plate to any surface, except to inoculate it and keep the plate closed at all times.

Post lab questions:

- 1) Answers will vary
- 2) Answers may vary, but probably not exactly the same
- 3) Because each substance may be effective against different microbes
- 4) A) Internally, for bladder infections, etc. B) Externally, on wounds
- 5) A) Other microbes, like fungi, viruses, protozoa, bacteria, etc. B) Food, space
- 6) A) By killing off competing microbes, drug resistant microbes will have less competition, and be able to survive more easily B) There will be no means of controlling them if necessary
- 7) The reproductive rate of bacteria is so much greater than that of humans, allowing for the development of new gene combinations and mutations at a faster pace.
- 8) An agent that causes disease
- 9) To avoid potential contamination by the microbes that are growing inside the dish
- 10) To see what types of microbes would grow with no antiseptic present and be able to compare the other results to it
- 11) Garlic, rattlesnake plantain, nettles, snowberry, etc.

Homeostasis, the Antiseptic Nature of Plants and the Development of Resistance in Microbes

Introduction

Most everything on Mother Earth tends toward a state of equilibrium or balance. One way that healthy living things attempt to achieve this state is by maintaining *optimal* physiological conditions for life, also known as *homeostasis*. For example, human beings have a mechanism to control their internal temperature at an optimal 98.6 degrees Fahrenheit (or near that). A series of feedback mechanisms within our bodies help to maintain homeostasis by monitoring the body's internal environment and signaling the brain when adjustments are required to stay within the optimal ranges for comfort and health.

- 1) What does the word optimal mean?

- 2) Name some other examples of physiological conditions which the human body regulates in order to maintain homeostasis:
 - A)

 - B)

Many forms of disease are the result of disturbances of homeostasis. One example involves the microbes normally present on or in the human body. Like other living things, microbes have certain requirements for survival and reproduction. Although some of these organisms are beneficial to humans and required for normal functioning, others are potential *pathogens*. When the body strays from its state of homeostasis, shifting physiological conditions can provide an opportunity for the pathogens to reproduce and cause disease. A specific example is dental decay, which is caused by the over accumulation of certain streptococcus bacteria normally present in the mouth. If a person does not practice good dental hygiene, the unhygienic conditions along with the presence of sugar as a food source promote the bacteria's rapid reproduction which often results in tooth decay.

- 3) What might be some requirements or conditions important for a microbe's survival?
 - A)

 - B)

 - C)

 - D)

- 4) Athlete's foot is a disease that is caused by a fungal pathogen often present in healthy individuals. What changes in homeostasis promote the development of the fungus into a disease?

- 5) Often when a person acquires AIDS, they die of complications such as pneumonia, rather than directly from the virus itself. What change in homeostasis allows a pneumonia infection to occur in these cases?

Today's lab involves *ethnobotany*, the use of indigenous plants by native people. The Salish, Kootenai and Pend O'Reille people of the Flathead Reservation have long used plants in many ways, including medicinally, long before contact with Europeans. More than half of the medicines used in modern Western medicine trace their origin to botanicals used by native peoples, in fact.

- 6) What does the word indigenous mean?

In this lab we will be investigating the *antiseptic* property of certain plants found on the Flathead Reservation, and how they can be used to treat and prevent disease. We will be growing bacteria on agar dishes in the presence of plant extracts known to have antiseptic properties. In order for the experiment to be valid, the use of *sterile technique* is required.

- 7) What does an antiseptic do?

- 8) What steps must be taken to maintain sterile technique?

A)

B)

C)

Materials (per pair of partners)

2 sterile agar-filled petri dishes

Wax pencil

Forceps

Bunsen burner

4 short pieces of scotch tape

4 sterile paper disks

1 ml of yarrow extract

1 ml of mint extract

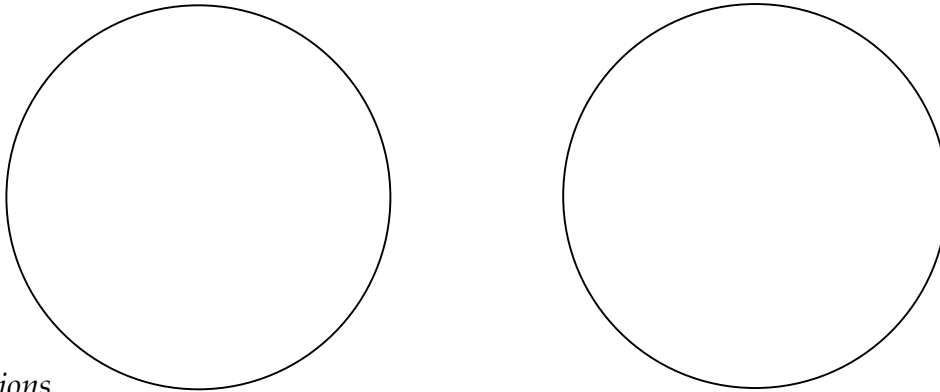
1 ml of antibacterial soap

**** NOTE:** Once the agar plates in this experiment have been inoculated and placed in the incubator, the plates must not be opened.

Procedure

- 1) Obtain two agar-filled petri dishes. Remember to keep them closed. Make a line down the middle of the bottom of the plates using a wax pencil. On one half of plate #1, you will be testing extract from a yarrow plant, so write the word *yarrow*. On the other half write *soap*. On one half of plate #2 write the word *mint*. What do you think you should write on the other half of plate #2? (In other words, what substance will be tested on this half of plate #2?)
- 2) Rub your finger lightly and evenly back and forth across the top of the agar in each dish. You shouldn't make a dent in the agar. You are *inoculating* the dish with the bacteria from your hands. The same person should inoculate both dishes for your group.
- 3) Sterilize your forceps in the flame of the Bunsen burner for ten seconds. Pick up a sterile disk and dip it in the yarrow extract. Place the disk in the middle of the yarrow half of dish #1.
- 4) Repeat step 3 with a disk for the mint extract and one for the soap, remembering to sterilize your forceps each time. Place each disk in the appropriately labeled position on the plates.
- 5) Place the fourth disk on the control side of plate #2.
- 6) Tape the plates shut and place them in an incubator at 38 degrees Celsius. Without opening the plates, observe them after 24 hours. If you do not see significant bacterial growth on the control side of plate #2 at that time, leave the dishes in the incubator for another 24 hours.
- 7) At the end of the experiment, examine the plates without opening them. In the space on the next page, accurately draw all of the microbial growth that you observe on each side of the two dishes, then give the unopened dishes to the teacher for safe disposal.

Draw the observed colonies here. Label each side of the plates either yarrow, mint, soap or control.



Questions

- 1) Which of the three substances tested appeared to be the most effective antiseptic?
- 2) Did you see the same types of colonies on each of the four halves of the plates?
- 3) Why might the type of microbial colonies differ in some cases?
- 4) List some ways that native people may have used yarrow and mint as antiseptics.
 - A)
 - B)
- 5) *Competition* is one factor that affects the survival of living organisms.
 - A) What organisms might a microbe be competing with for survival?
 - B) What types of things are microbes competing for?
- 6) Antibiotics are another type of substance that can destroy pathogens. The overuse of antibiotics, antibacterial soaps and antiseptics can hasten the development of new strains of microbes.
 - A) How might the overuse of antimicrobial substances lead to development of new strains of microbes?

B) These new strains of microbes are often resistant to antimicrobial substances. Why is this a dangerous situation?

7) Theoretically, the evolution of bacteria should occur much more quickly than that of humans. Why is this so?

8) Define a pathogen:

9) Why was it important to not open the petri dishes after incubation?

10) What was the purpose of the control side of plate #2?

11) List any other indigenous plants that you know of that have antiseptic properties: