Spice up your life: Spices as Antibiotics in Foods

SUMMARY
In this activity, students will collect spices and place them on a plate containing bacteria commonly found in foods. If the spices contain antibiotic-producing qualities, the growth of the bacteria on the plate will be visibly inhibited. Students can compare different types of spices and their effect on 3 different bacteria, which cause food spoilage, but are safely used in lab with proper laboratory procedures. Information is included about the medicinal use of plants. Students are also encouraged to think about how they would design experiments of this type themselves.

LEARNING OBJECTIVES
- Observe that some spices contain antibiotic properties.
- Explore and learn how different spices affect 3 different food-spoiling bacteria and how they may be used to naturally preserve foods as well as add flavor.

INTENDED GRADE LEVEL(S)
High School

CONTENT THAT STUDENTS SHOULD UNDERSTAND PRIOR TO LAB
During this activity students will conduct an independent investigation in the antibiotic effects of spices on some food spoiling bacteria. They will use the scientific method to carry out this investigation.
Scientific Method
1. Choose a question
2. Design a hypothesis (possible answer) to the question
3. Make testable predictions based on the hypothesis
4. Design experiments to answer the question and see whether the predictions are met.
5. Perform experiments and collect data.
6. Analyze data including graphs and tables if necessary.
7. Determine whether your results support or falsify your hypothesis.
8. Additional conclusions.
9. Present your investigation to the class

Although the scientific method is written as a series of distinct steps above, it is actually a process where all steps influence each other. For example, the question one chooses may be influenced by what materials are available and what experiments can be performed. Thus, it is often impossible to do steps 1 and 4 separately from one another. Similarly, once an experiment is performed, it may become obvious immediately that the experimental design is flawed, which will necessitate redesign of steps 1-4 without completion of steps 6-8.
Vocabulary:
- Antibiotic
- Zone of Inhibition (mm)
- Spice
- Colony
- Bacteria
- Nutrient Agar

BACKGROUND INFORMATION
In contemporary western culture, we have come to rely on chemical preservatives to prevent food spoilage and antibiotics to treat bacterial diseases. While the use of these has helped to diminish the impact of many common diseases, many pathogens have evolved resistance to some or all of the known antibiotics. The source for our arsenal of antibacterial drugs has traditionally been the natural world of fungi, plants, and bacteria. As we see more resistant strains of bacteria evolve, we must turn to that source again for potential new drugs. Food poisoning affects millions of people each year. Spices have been used for centuries to

flavor food while killing selective bacteria. For example, spices used in making sausages will inhibit bacteria that cause spoilage while not harming the bacteria that add flavor to the sausage.

- IMPORTANCE OF PLANT-DERIVED MEDICINES:
  Many of the medicines used to treat a variety of ailments are produced naturally by a diverse assortment of organisms including many plants, fungi, bacteria and animals. The use of naturally occurring medicines is not new. Traditional cultures throughout human history have used naturally occurring medicines. There is even some evidence that non-human animals use plants medicinally. It is estimated that in many developing countries, 85% of health problems are treated with plant-derived medicines. In the U.S>, roughly 25% of all prescription drugs contain compounds extracted directly from plants. Currently, we have the technology and knowledge necessary to synthesize many chemical compounds in our search for new medicines. However, there are many chemical compounds found in nature that we cannot synthesize. Moreover, a vast number of plant species (250,000+) have never been screened for potential medicinal value. Many have never even been described. The task of surveying the earth's immense diversity of plants, animals, fungi and bacteria for chemical compounds of practical use has been coined "chemical prospecting" by one of the field's pioneers, Dr. Thomas Eisner at Cornell.

  - BIODIVERSITY AND BIG BUSINESS:
    Much of the planet's untapped reserve of biological diversity is in the tropics. However, most of the world's money for collecting and testing species for useful chemical compounds is in the hands of corporations in North America, Europe, and Australia. Hence, many pharmaceutical companies and tropical countries have forged agreements to put these two resources together. Needless to say, testing the many biological samples collected in the wild for bioactive compounds is a time and resource-consuming process. In order to narrow the search, chemical prospectors use various criteria to select the species, which are most likely to have useful compounds.

MATERIALS LIST

- **Plant material:** You may provide this for students or have them bring in their own spices, they may wish to compare the effectiveness of fresh vs. packaged. The material may be from any part of the plant. In fact different parts of the same plant may have different concentrations of chemicals (e.g. there may be more of an antiherbivore compound concentrated in the seeds or antifungal compound in the roots). Material should be labeled and stored in an airtight container such as a resealable plastic bag.

  If students collect plant material on their own, you may wish to have them identify criteria for selecting which plants are most likely to have antibacterial or antifungal properties. There are web sites (www.fiery-foods.com/dave/spices.html) and books (*Spices* by Paul Sherman) that can be used as resources. Encourage students to brainstorm and decide upon their own criteria for collection.

  The materials listed can be purchased through your favorite Biological supply catalog.
  For a classroom of 30 you will need the following:
- Nutrient Agar
- Sterile petri dishes
- Bacillus cereus
- E. coli
- Pseudomonas fluorescens
- Spices
- Incubator
- Sterile Q-tips
- Mortar and pestle

OUTLINE

1. Background: Provide students with information on the process of science and the Scientific Method. Discuss the role of plants as antibiotics and the use of spices in limiting bacterial growth.

2. Choose spices to be tested. Determine the experimental and control variables to be used.
3. Choose a question: Students work in groups, brainstorming questions about the effectiveness of certain spices on bacterial growth. Each group selects one question to investigate. Following the Scientific Method, students write a hypothesis, prediction and design their own experiment to test their prediction. For example, students may choose to investigate the effects of 3 different spices on one type of bacteria or they may choose to study the effects on one spice on the 3 different bacteria.
4. Students write a hypothesis (or hypotheses) and make prediction(s) based on their hypothesis. Students then design an experiment to test their prediction(s) and perform their experiment. Students then write a paragraph summarizing how their results support or falsify their hypothesis.
5. Teacher background: Prepare Nutrient agar and pour sterile agar plates the night before the students perform their experiment. B. cereus, E. coli and Pseudomonas fluorescens bacteria are common organisms that spoil food, but are safe for High School Biology students. These can be purchased through Carolina Biological Supply Co. You need to make bacterial broths for covering plates. Provide a mortar and pestle for students to grind any fresh / large spices.
6. This is a guided inquiry lab; students will work in groups of 2. A student worksheet with a suggested way to explore the antibiotic effects of spices on bacteria is provided. You may want to let students devise their own set-up based on their question and hypothesis.
7. Incubate the student experimental agar plates for 24 – 48 hours (depending on the school schedule) and let students record results and draw conclusions. Include data comparisons between student groups and encourage the construction of charts and graphs where appropriate.
8. Furthering Investigations based on data should be encouraged. Exploration of the effects of spices on food molds would be another option for student experiments. What questions does the data generate? A formal lab write up can be optional. Students answer discussion questions.

TEACHER PREP INSTRUCTIONS AND ORDERING INFORMATION

Safety tips
The food spoiling bacteria in this lab are considered "safe" for student use in High Schools when standard microbial handing techniques are observed. However, when performing this experiment in the classroom, have the students **tape their plates shut** after they have inoculated the plates with the bacteria (or fungi if appropriate). The next class period - **do not allow your students to open the plates in order to examine them!**

Suggestions for safely adapting this exercise to different maturity levels.
- The bacteria you will use, E. coli, Bacillus cereus, and Pseudomonas fluorescens, should not pose any danger to healthy individuals when proper lab techniques are applied. However, if you have doubts as to how carefully you students will work with a broth culture of bacteria, or if you feel uncomfortable having your students do this, you can swab the plates with bacteria prior to the start of class (immediately before) and then have them place the spice samples on pre-swabbed plates. This will eliminate the chance of students spilling bacterial cultures on themselves or their desks.
- If you have doubts that you students will follow your instructions not to open the plates, you can set this activity up as a demonstration with several plates and show them the results while you supervise.
- Students may choose to wear gloves or goggles while performing labwork.
- No papers or books should be left on student workspace during the experiment.
- Students should wash the workspace and their hands with disinfectant soap after completing the exercise.
- No food or drink should be allowed in the room while performing this experiment.

Modifications, Observations, and Suggestions
- This exercise is written for use with 3 food spoiling bacteria considered "safe" for high school students. You could consider other bacteria or fungi as well. Consulting the Food Safety Specialist in your state may provide additional "safe" species, or you may try the Internet.
- I found web sites dedicated to principle food spoilage and food poisoning microorganisms. Other sites listed plant material commonly used as microbe inhibitors. The following spices are listed in order of their strength of inhibition from strongest to weakest: garlic, onion, allspice, oregano, thyme,

cinnamon, tarragon, cumin, cloves, lemon grass, bay leaf, chili peppers, rosemary, marjoram, mustard, caraway, mint sage, fennel, coriander, dill, nutmeg, basil, parsley, cardamom, pepper, ginger, anise seed, celery seed, lemon/lime [www.fiery-foods.com].

- Very little spice is needed. You might want students to determine the quantity of spice used as well as the consistency. They can use a mortar and pestle to grind spices making them more consistent (control variable). Spices can be sprinkled in the designated areas on the petri plate.
- This experiment works well when plates are incubated at 37 C. You may let students vary this in order to investigate the effects of temperature when other control variables are in place.
- This investigation is designed to be a guided inquiry. Therefore, students are provided with a possible procedure, but may choose to deviate from that using the scientific method and their own design.

Supplies and Materials Needed

- Cultures of E. coli, Bacillus cereus, Pseudomonas fluorescens should be stored in a non-frost free freezer because repeated cycles of freezing and thawing will kill the bacteria.
- Nutrient agar plates – one per group of 2 students.
- Tubes of Nutrient broth (to make bacterial broth for covering plates)
- Sterile Q-tips (if you don't have an autoclave, an unopened box of Q-tips is good enough)
- Spices
- Incubator or warm place to incubate plates
- Tape
- Markers to write on plates
- Mortar and pestle for grinding spices
- Balance to mass spices (optional)

Preparation other than materials and supplies

Media preparation

- Nutrient agar plates:
  you can buy premixed, dehydrated Nutrient agar.
  Prepare agar according to instructions and autoclave at 121 C, 20 psi, for 30 minutes. Or you can boil the media or cook it in a pressure cooker for 1 hour.
  Allow media to cool to about 55 C, then pour into plates.
  Let plates cool on a table/workspace 2-3 days (This is longer than usually required, but for this activity the plates must be allowed to dry more. This minimizes condensation on the lids since the plates will need to be stored right side up rather than upside down). To check for contamination, you can incubate the plates upside down in an incubator overnight. If no growth has occurred overnight, the plates are not contaminated. Store plates inverted in the refrigerator.

- Nutrient agar broth:
  Use premixed nutrient broth. Aliquot the broth into tubes, 5 mls per tube.
  Cap tubes with non-sealing caps and autoclave for 30 min.

- Bacterial Culture Prep:
  Streak out several fresh plates of each bacterial stock sample several days prior to when you plan to do this activity in class and incubate overnight at 37 C.

  The morning of the lab activity, use a sterile swab to remove bacteria from the plate and mix the swab in a tube of nutrient broth. The broth should be very cloudy when you are done. If there are not enough bacteria in the broth, the students will not get a solid enough lawn of bacteria. The same tube may be used for several classes. You can leave the swab in the culture tube for student use. Repeat the procedure for each of the 3 bacteria.

  Ordering information

  Fisher Scientific
  1-800-766-7000 (phone)
  1-800-926-1166 (FAX)

  Carolina Biological

Agar, Fisher, #DF0140-15-4, 100g
Premixed, dehydrated nutrient agar
Premixed, nutrient broth
Bacteria stock cultures:
E. coli,
Bacillus cereus
Pseudomonos fluorenscens

STUDENT HANDOUTS

1. Student Pre-Activity Exploration

Introduction – Anti-microbial agents kill or inhibit the growth of microorganisms. They're several different sources of these agents.

Naturally occurring – many antibiotics are produced by plants
Semi-synthetic – some antibiotics are modifications of those produced by plants
Chemically synthesized – other anti-microbial agents are synthesized by a chemical process.

This exercise deals with the antibiotic properties of plants, specifically spices. Spices have been used for centuries to flavor foods, but they may also have the ability to kill bacteria that spoil food. Some may be more useful as antibiotics than others.

Questions for thought

1. Consider several spices. List them and the part of the plant they represent

2. Where would a plant concentrate its anti-microbial chemicals?

3. If spices inhibit microbial growth on foods, what other uses might be possible for spices?

4. Does the antibiotic effectiveness of a spice vary with different bacteria?

5. Are some bacteria more resistant to spices than other bacteria? What factors might play a role in antibiotic resistance of some bacteria?

2. Antibiotic affects of Spices on Food Spoiling Bacteria
Student Worksheet

LEARNING OBJECTIVES

- Observe that plants, especially spices, can have antibiotic qualities
- Learn how the effectiveness of spices as antibiotics can be tested
- Compare which spices are most effective as antibiotic agents on certain food spoiling bacteria

MATERIALS

For this activity each group will need the following

- 1 agar plate
- 1 broth culture of bacteria
- 1 sterile swab (Q-tip)
- marker
- tape
- bacterial sample (s)

- spice (s)

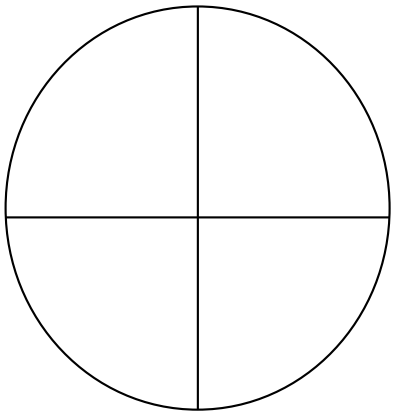
INSTRUCTIONS

General Laboratory Technique
- No papers or books (other than these instructions and a data sheet) should be left on your desk or workstation during the experiment.
- Wash your desk/workstation and hands with disinfectant soap after completing the exercise.
- No food or drink should be in the room while performing this experiment.
- Do not open your plate after allowing the organisms to grow overnight.
- Use latex gloves if needed

Below is one possible way to look for antibiotic effectiveness of spices on food spoiling bacteria. You may follow the basic procedure with modifications designed to address the specific question, hypothesis and prediction your group came up with.

1. Select 3 different spices and one bacterial culture. The amount (mass) of the spices used must be the same for all samples (control variable, a "pinch" of the spice is recommended so you will need to determine the mass of a "pinch", less is more. You may want grind each spice individually.

2. Obtain a sterile plate containing nutrient agar. Label the plate as shown below.



[each quadrant should be labeled as 1,2,3,4 I was not able to do this on my computer]

(refer to BSI lab Antibiotic Producing Bacteria and Fungi Student Worksheet for similar format)

3. Dip a sterile Q-tip into the bacterial broth you selected and swab the surface of your plate thoroughly, in several different directions as shown below so that the entire surface of the plate is completely covered with the bacterial solution.

Place 2 drawings in this space to demonstrate how the swabing is to be done. Refer to BSI lab Antibiotic Producing Bacteria and Fungi Stud. Wkst.

4. Place Spice #1 onto the quarter of your plate marked 1. You should use just a "pinch" of spice (mass to be determined). Repeat this process for spices #2 and #3. Leave the quadrant #4 of your plate empty as a control.

5. Tape your plate shut. The plate must remain taped throughout the experiment for safety.

6. Place your plate in a 37 C incubator for 24 to 48 hours. Incubate your plate right side up to prevent loss of the spices from the surface of the agar. (Bacterial plates are usually stored upside down to prevent moisture from condensing on the lid).

OPTION*
Inquiry Lab Approach:
Given the background information, students can generate their own question (s) regarding the antibiotic / antibacterial properties of spices on food spoiling bacteria. Applying the scientific method, they can choose a question which is testable and design a hypothesis or hypotheses related to their questions, make testable predictions, design an experiment, etc. The format for such an investigation may look like BSI lab *Adherence Part II – Identification of Host Cell Receptors* OR BSI Lab *Independent Investigations in Infectious Disease.* The basic format can be designed to fit this lab activity. Students may choose to test 3 bacteria and one spice, or apply other experimental variables in their design.

PRE-LAB INQUIRY QUESTIONS (some of these may depend on the format used by the students, as some of these may be built into the inquiry lab procedure)

1. What is your question?
2. What is your hypothesis?
3. What are your experimental variables? (Independent and dependant variables)
4. What are your control variables? (so that you can be sure any change in the dependent variable is due to changes in the independent variable only?)
5. What is your prediction?
6. How will you measure the effects of the independent variable on the dependent one?
7. What will the units of the measurements be?
8. How will you design your experiment so that all variables except the independent variable are kept constant?
9. How will you design your data table to record the data as the experiment is being done?

POST LAB QUESTIONS

1. DO NOT OPEN YOUR PLATE FOLLOWING THE INCUBATION. Observe your plate. Sketch you results below.

*Draw a circle here with quadrants to represent the bacterial plate, like in the lab

2. Which spice sample (s) appeared to be the most effective antibiotic? Explain.

3. Which bacteria appear to be the least resistant to spices? You may need to compare your results with those of other classmates in order to determine this.

4. Did your control quadrant give the expected results? Explain.

5. * Using the chart from BSI activity *Antibiotic Producing Bacteria and Fungi Student Worksheet*, construct a similar chart for students to compare class data from this lab.

6. Looking at the results of the entire class, are there any spices which seem to be more effective than others as an antibiotic against food spoiling bacteria? If so, what, if anything, do these spices have in common?

7. Do you see any patterns from this experiment? Explain

8. What experiments could you perform to help clear up or explain any unexpected results?

9. What new questions did the results bring up?

10. What would you do next if you were to continue this work?

11. Explain how the Board of Health in your state could use this experimental design to support the use of spices in food preparation/processing.

FURTHER INVESTIGATIONS

As part of the post lab, or even as a pre-lab activity, students can use the internet and research or visit web sites which deal with the effects of spices as antibiotics or anti-bacterial agents for food spoilage bacteria. There are many search engines that provide web sites which contain information on spices as antibiotic/antibacterial agents and others which give info on food spoiling bacteria. Historical, current and future medicinal uses of spices may be interesting to students.

RESOURCES

1. www.fiery-foods.com/dave/spices.html
2. www.firey-foods.com/dave/bbqhealth.html
3. www.woodrow.org/teachers/bi/1999/projects/group5/presentation/sid003.html
4. www3.airproducts.com/freshline/principle_foods_organisms.html#Acintobacter
5. Dr. Pat Kimball, Food Safety Specialist, Colorado State Univ. 970-491-7334
6. Biological Sciences Initiative, University of Colorado

- Julie, this is still a bit jumbled and the exact format needs work. My computer skills aren't the best and I was unable to construct some of the drawings/charts and tables which should be a part of the format. I indicated the labs from BSI, which I used as models for some of this work. Please call or email me if you have any questions or want revision. Thanks for your patience.
- Marilyn Schmidt