Science in the Real World

Microbes In Action

"Spice Up Your Life!" is a curriculum unit developed as part of the *Science In The Real World: Microbes In Action Program*. The curriculum units were developed with support from the National Science Foundation, The Coordinating Board for Higher Education, Sigma Chemical Company, Pfizer Foundation and the Foundation for Microbiology.

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At a Glance

Description

In this lab students will evaluate various spice aromas for their ability to kill bacteria.

Time Requirements

This lab will require one class period for setup and one class period for analysis and conclusions.

Curriculum Placement

This lab would fit well into a microbiology unit (natural inhibitors of bacteria), a botany unit (plants as producers of antimicrobial agents) or relating to our human body in a study of our senses (aromas and sense of smell.)

Equipment

Autoclave or pressure cooker

Materials

Nutrient agar plates
Various spices to be supplied by the students
Bacillus subtilis
Marking Pens
Sterile water
Sterile cotton tipped applicators
10% bleach solution
Scissors
3″ x 5″ note cards

Spice Up Your Life

Using spice aromas as bacterial inhibitors

Background

How many of us would like the taste of food without the addition of spices? People have used spices and herbs for centuries to improve the flavor of foods. Also some spices, salt in particular, has been used to dry foods and aid in its preservation. There are many, many herbal products on the market that claim to improve health, and many spices have documented health benefits. Just what makes spices so great? Some contain anti-oxidants that have been shown to reduce free-radicals in the blood. Free-radicals run amuck in our bloodstream causing changes to our body's chemistry, and sometimes these changes lead to disease. Other spices contain aromatic essential oils. Essential oils are natural aromatic compounds produced by some plants. Essential oils have been used for centuries in medicine, foods, perfume, and cosmetics. Even the well-known Hippocrates used essential oil aromatherapy for healing in 2000 B.C. Nowadays they are used as "lemon" disinfectants, in healing ointments, in toothpaste, as flavorings, perfume, deodorants, soaps, salves and many new body and hair care items. In the last few years the market demand for essential oils has increased dramatically.

The usefulness of spices continues to be explored by scientists and manufacturers alike. Consumers usually judge spices on their aroma and flavor. Could the aroma of a spice have something to do with its health benefits? Could the aroma alone inhibit bacterial growth? Could spices be used as a natural preservative in food for both enhancing flavor and extending shelf life?

Purpose

1. To evaluate various spice aromas to determine their affect on bacterial growth.

Procedure- Day 1

1. Obtain materials from your teacher. Each group will need:
    a. One nutrient agar plate for each spice you will be testing, plus one for a control plate (no spice).
    b. One 5 ml tube of sterile water.
    c. One sterile cotton swab.
    d. Marking pens for labeling plates.
    e. Various spices (to be provided by students)
    f. One plate of *B. subtilis* bacteria.
    g. One 3 x 5 inch note card.
    h. Scissors

2. On the bottom and along the edge label your plates with the following information:
    a. Group name
    b. Date
    c. Spice
    d. Name of bacteria

3. Smell your spice and record your description of its aroma on the analysis page (7):

-	**No smell**
+	**Weak**
++	**Strong**
+++	**Very Strong**

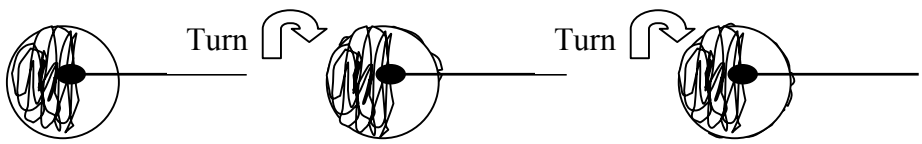
Note***Steps 5-7 need to be completed quickly once started, make sure you read all of the steps before beginning.**

4. Locate your plate of *Bacillus subtilis* bacteria and your tube of 5 ml sterile water. Insert your sterile swab into the tube of sterile water. Rub the moistened swab on the surface of the agar containing bacteria. Turn the swab as you rub to pick up more bacteria. Return the swab to the tube of water.

5. Move the swab up and down rapidly to mix the bacteria in the water.

6. Use the following technique to apply bacteria to **all of your plates**:

    A. Rapidly mix the bacterial solution by moving the swab up and down 8 times.
        i. Apply the moistened swab to the **entire surface** of a nutrient agar plate in a series of 3 steps. Lift the lid just enough so you can apply the bacteria, do not set it down on your tabletop. See diagram below.
        ii. Start in the middle and rub up and down toward the outside edge.
        iii. Turn the plate 90 degrees clockwise and repeat.
        iv. Turn the plate 135 degrees clockwise and repeat.



    B. Return the swab to the bacterial tube. Repeat step A. for all plates.

7. Dispose of the used swab in the 10% bleach solution provided.

8. On the lid of the closed petri dish, draw a line down the center. 

9. Invert plates so the bottoms are face up.

Note: **********The plates will remain upside down for the remainder of the lab!!!**********

10. Open the petri dish by lifting the bottom (which is now on top) straight up and place it open side-down onto your lab table.

11. Gently shake or pour out enough spice to cover the bottom of <u>only one half</u> of your petri dish lid. (You can use a note card held vertically against the lid to help place the spice in only one half of the plate. Replace the bottom half of your Petri dish.

12. Draw lines on the side of the Petri dish and across the bottom; extending the line you drew on the lid. Put and X on the half that contains spice. This will allow us to know where the spice was, in case your dish gets turned or dropped accidentally.

13. Repeat steps 10-12 for each spice plate.

14. Carefully, without disturbing the spice, place your dish in an area as designated by your teacher. They will grow for 24-48 hours at room temperature.

Procedure- Day 2

1. After 24-48 hours, retrieve your plates again being careful not to turn them over or disturb the spices.

2. Observe the plates. The bacteria will look cream colored and will grow in spots (colonies). Sometimes the dots all grow together and it looks more like a film (lawn). Draw a picture of the plates that were tested by your group on the analysis page.

Name _____

Date _____

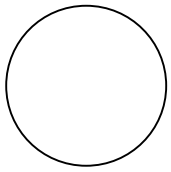
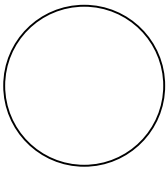
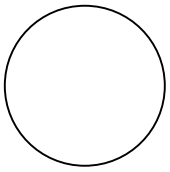
Spice Up Your Life
Data and Analysis

1. What spice did you use?

2. How strong of a smell does your spice have?

3. Is there a correlation between your spice's smell and its ability to kill bacteria? Explain your answer.

4. Draw a line dividing the plate and shade in the areas where bacteria grew. Be sure to label the spice tested and which half of the plate contained the spice. Draw and label your control plate also.

5. Based on your results classify your the spices according to their effect. Label your above drawings using the key below.

-	**No effect**	looks the same as the control plate
+	**Weak**	slightly less bacteria, as compared to no spice
++	**Strong**	area above spice is cleared of bacteria
+++	**Very Strong**	area above spice clear, as well as part of the no spice area

6. On a separate sheet of paper, create a class data table to hold all of the classifications (weak, strong, etc.) of all of the spices tested by the class. Include both the observation of the spice's aroma on day 1 as well as the observation of its antimicrobial strength on day 2. Be sure to label your data table and include a key to the information included.

7. Also on a separate sheet of paper, create a chart that groups the spices according to their antimicrobial effects.

8. What conclusion(s) can you draw from the class data (if any)?

9. How can a spice that does not touch the bacteria cause its death?

10. Which spice has the most surprising result? Explain your answer.

11. What further experiments would you like to try using spices?

12. How might you use the information you learned from this lab as a consumer?

Teacher's Guide

Overview

This lab is a smelly, colorful, fun experiment, which determines the ability of various spice aromas to inhibit bacterial growth. The students will be amazed by the power of these odors, and it lends itself to the discussion of natural bacterial inhibitors, commercial uses and research issues.

Instructional Objectives

At the end of this activity, the students should be able to:

1. Demonstrate the following laboratory skills:
   a. Comparing and contrasting
   b. Following the directions to complete a variety of tests
   c. Constructing and compiling a data table.

2. Demonstrate the methods of scientific inquiry by:
   a. Perform an experiment according to given directions
   b. Gathering and organizing data
   c. Analyzing data
   d. Applying understanding of a specific principle to a more general purpose

3. Demonstrate the understanding of the following scientific concepts:
   a. Some spices contain aromatic compounds that may inhibit bacterial growth.
   b. Spices and herb have been used for centuries and have many uses.

Background

Students are familiar with spices, and use them everyday. Many of them probably have a few that they "hate" to smell. This lab incorporates the students' previous knowledge about "stinky" spices, and allows them to test the effect of these odors on the growth of bacteria. Herbal supplements and remedies are available everywhere, even with little testing to support their supposed claims. Essential oils are used in massage and aromatherapy, and constitute another high volume industry. Most students are aware of the disinfectant properties of many cleaners, soaps, etc., but they will be surprised by the effect of just the aroma of certain spices will have on *B. subtilis*.

Spice Information

The active ingredients in spices are presumed to be the essential oils. See the table below for a partial list of spices and their antimicrobial compounds.

Spice	**% Essential oil**	**Antimicrobial Compounds**
Cloves	16 –18	Eugenol
Cinnamon	0.5 – 2.0	Cinnamaldehyde, Eugenol
Sage	0.7 – 2.0	Thymol, Eugenol
Mustard	0.5 – 1.0	Allyl isothiocyanate
Oregano	0.8 – 0.9	Thymol, Carvacrol
Garlic	0.3 – 0.5	Allicin

Allicin and allyl isothocyanate are sulfur-containing compounds. Eugenol, carvacrol, and thymol are phenol compounds. These have been shown to inhibit fungal growth as well. Oleoresin, which is an extract of rosemary, has anti-oxidant capabilities.

Could spices replace other preservatives? Probably not…but their effects are easy to see and their presence could add to the flavor while also prolonging shelf life. Listed below are some common spices and their antimicrobial abilities (this is based on general properties, but it can vary based on the type of bacteria.)

Spices and Herbs	**Inhibitory Effects**
Cinnamon, Cloves, Mustard	Strong
Allspice, bay leaf, caraway, coriander, cumin, oregano, sage, thyme, onion, garlic	Medium
Black pepper, red pepper, ginger, salt	Weak—may be too weak to observe in this lab

Sources of Supplies

Description	Stock Number	Quantity	Cost
Petri dishes	CE-74-1251	500	$ 95.50
Bacillus subtilis plate	CE-15-4922	1	$ 9.00
Nutrient Agar (dry)	CE-78-5320	100 g	$ 28.80
Sharpie Markers (Set of 4)	CE-64-4298	4 (16 total)	$18.60
Sterile Cotton-tipped Applicators	CE-70-3033	200	$19.95
Test Tubes (13 x 100mm)	CE-73-1437	250	$14.10

Preparation

- At least one week before the lab (but no more than 3 weeks before), obtain a plate of *Bacillus subtilis*. Store culture in refrigerator.
- One or two days before prepare several streak plates of *B. subtilis*. Incubate the plates at room temperature or 30 degrees for 24-48 hours. You will need one plate per group for each class, but you should be able to use the same plates for all of your classes that day. For a typical class you would need 5-7 plates of bacteria.
- Prepare tubes of 5 ml sterile water. You can autoclave the water when you make the agar, or use bottled water.
- Prepare nutrient agar plates. One liter of medium will provide about 50 plates. Dissolve 23 grams of nutrient agar powder into 1 liter of distilled water (tap water will work). Prepare enough to use approximately 20 mL per plate. Prepare agar batches that only fill the flasks half way to prevent boil over. Once you have prepared the nutrient agar, cover the flasks with aluminum foil and place them in an autoclave or pressure cooker.

  Follow the instructions on your sterilizer to complete the sterilization process. Generally, you need 15 lbs. of pressure and a temperature of 121^{O} C for 20-25 minutes to achieve sterility.

  Once the materials have been sterilized, the nutrient agar must be poured into the plates before it solidifies. It is best to pour the agar when it has cooled enough to be held comfortably in your hand (45-50 ^{O} C), but still feels hot. Spread the plates out on the lab tables; lift the lid on one side like a clamshell. Fill the plate with enough agar to cover about 2/3 of the surface. Cover and slide the dish back and forth to distribute the agar. **Thin plates work best with this lab.** You may want to pour 15mL of water into a plate to give you an idea of how far to fill the plate. Once all of the plates have been poured, let them sit until the agar solidifies. Store the plates upside down until you are going to use them. If it is going to be several days, put the plates back into the sleeve that they came in and store upside down in the refrigerator.

- Prepare one 10% bleach waste container for each group.

Teacher's Hints and Troubleshooting

- This is a fun, colorful and smelly lab that is relatively easy and the students will enjoy it. The results are easy to see, and students get to test a spice they bring from home. Other than the cost of nutrient agar plates, this lab is inexpensive.

- If an autoclave or pressure cooker is not available for sterilization procedures you could purchase the pre-made bottles of nutrient agar although this is more costly.

- To reduce the amount of plates you can alter the number of spices tested. Also, you can make one control for the whole class to look at, instead of each group making one.

- Instruct the students to bring in a spice from home a day or two before the lab begins. One tablespoon should be enough for one test, but it is easier to apply the spice if they bring the bottle it came in. You may want to have some spices on hand. Make sure they are fresh, old spices will not work!

- Students should be instructed to bring single ingredient spices only to cut down on variables. Testing mixtures such as chili powder, onion salt, etc., could be used as an extension of the lab if desired.

- Water is used as the medium to transfer the bacteria from one petri dish to another. Water will kill bacteria over time, so it is important that the students do not add their bacteria to their tube of water until they are ready to go on. The limits are unknown, but it was tested for up to 5 minutes successfully.

- You may want to make the paper or cardboard dividers ahead of time. If note cards aren't available you can use a thin piece of cardboard like the cover of a spiral notebook. Box cardboard may be too big and paper works, but the separation isn't as precise as with cardboard.

- As mentioned above, thin agar plates work better than thick in this lab because this aids in keeping the spice form touching the surface of the agar accidentally.

- You could consider measuring the width of clearing if you wanted students to graph their data.
- Whole spices are difficult to use. Powders worked the best. When whole cloves and bay leaves were used, there was a problem in keeping them from touching the bacteria.

Dear Parents,

Did you know that some spices could kill bacteria? It's true. Most foods are preserved with chemical additives, but the addition of spices to food can prolong its shelf-life. We are going to do an experiment testing the ability of different spices to kill bacteria. Please help us by helping your child pick out one spice from home and allowing them to bring it to school. It needs to be a relatively new spice or else the experiment will not work. For the most proper testing procedures we would like to only use single ingredient spices. Please look at the ingredients to make sure that the spice does not contain additives. If there is no ingredient list then it only contains one spice, whatever is named on the front.

Thanks for your help and participation in your child's education. Your spice will be returned as soon as we finish the lab. You could use this opportunity to ask your child about this lab, and talk to him or her about what is going on in school. We appreciate your help!

Thank you,

Biology Teacher