Cornell Institute for Biology Teachers

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Title: **Some Like it Hot- Testing Spices for Antibiotic Properties**

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Appropriate Level: Grades 3-8, Life Science

High School

Abstract:

Time Required: 1 class period for designing and setting up experiments, about 30 minutes for looking at results.

Special Needs:

NYS Learning Standards:

NYS Learning Standard 1. Analysis, Inquiry and Design
Students will use mathematical analysis, scientific inquiry and engineering design, as appropriate, to pose questions, seek answers and develop solutions.

NYS Living Environment Standards
Standard 1. Key Idea 1:
The central purpose of scientific inquiry is to develop explanations of natural phenomena in a continuing and
creative process.

Standard 1. Key Idea 2:
Beyond the use of reasoning and consensus, scientific inquiry involves the testing of proposed explanations
involving the use of conventional techniques and procedures and usually requiring considerable ingenuity.

Background

Humans have added spices to their foods for centuries. Hieroglyphs in the Great Pyramid at Giza, built around 2500 BC, show workers eating garlic and onions for strength. Around 400 BC Hippocrates, the Greek physician, listed more than 400 medicines made with spices and herbs, about half of which we still use today. In the American southwest, chile pepper seeds have been found in Anasazi ruins, which date to around 1200 AD. Most likely they obtained them from traders that came to the New World through South America and Mexico (they also find parrot feathers!). In 1563, Garcia de Orta writes "Colloquies on Drugs and Simples of India" the first scientific book on oriental spices published in the western world.

There are two schools of thought to explain why our history seems very connected with the ingestion of spices and herbs. The first line of reasoning is that spices were simply used to cover up the bad flavor of what was then unrefrigerated foods, especially meats. The other school of thought is that spices are used to inhibit spoilage or as therapeutics. It is thought that the Anasazi smoked most of their meat into jerky to preserve it over the winter and "remains" of ancient jerky contain chile seeds, juniper berries and salt.

In many cultures, spices and herbs are used therapeutically. There has been substantial research done in India on the anti-microbial, anti-parasitic, and anti-cancer qualities of commonly used spices. This activity allows students to test the antibiotic properties of spices and herbs.

Supplies and Equipment needed:

100x15 plastic Petri dishes	(Wards 18V1702 – about 20 for 9.95) (Divided plates are nice, if available)
Nutrient agar- premade in plastic bottles	(Ward's #88V1500 – about 600 mls for 21.95).Need about 15-20 mls agar/Petri dish
Control bacteria if desired	Bacillus, Vibrio, E.coli and Serratia. All of these are not dangerous to humans. All are available at Ward's
Spices	Best are cloves, cinnamon, allspice, and turmeric. Other spices inhibit some, but not all bacteria
Plastic spoons	
Forceps and Rubbing Ethanol	Optional

Water Bath or Microwave to warm media

Tape or Parafilm to seal plates shut

Cotton tipped swabs or Streaking needles

Anti-bacterial soap or hand sanitizer

Preparation
Almost any general agar will do. Most of the bacteria that grow in air will grow on nutrient agar. The easiest form to use is pre-made agar in 100 ml bottles that are available from life science supply companies. If you have a pressure cooker or an autoclave, you can prepare the media from dry powder.

Melt the nutrient agar either in the microwave or in a water bath. If you use a microwave, be careful that the agar does not "bump" or overheat. It will be extremely hot. Place in hot water to finish melting.

Experimental Design
Students can work alone or in groups. Students should be allowed to develop their own hypothesis, after either hearing an introduction to spices or looking at the resources on the Internet.

To create their experimental dishes, they should place a small amount of the spice they are using into the Petri dish and pour a thin layer of agar over the spice. If they are using whole spice, (cloves, allspice, cinnamon, bay leaves) they should put one or two in the center of the plate. They will need something to hold the spice in place while the agar cools. The plates should be allowed to cool (10-15 minutes) and then another layer of agar should be poured into the plate until it covers the spices. When the agar cools again and solidifies, it is ready to be used.

The chemicals in the spice will leach out and in most cases the zone of bacterial inhibition will correlate with the colored pigments.

Bacteria inoculation
If doing this lab with younger students, use a Sharpie marker to divide the plate into a number of sections. Be sure to leave a control section that will not get bacteria. Have each student rub their finger over the agar in one of the sections. Close the plate and incubate on the counter upside down (with the agar up and the cover down). Most bacteria will grow up overnight. Have the students wash their hands after inoculating the plate.

For older students, make a bacterial culture by inoculating a liquid media culture. Allow the culture to grow up overnight. The students can then use a cotton-tipped swab to inoculate their plate. Remind students to think about controls!

Safety Concerns
Once the students have finished their work, the plates should be sealed shut with tape or Parafilm and not re-opened.

If the lab is used to investigate what spices kill bacteria in food, do not use meat or chicken, which can easily harbor pathogenic bacteria. Most fruits and vegetables will have safer bacteria on their skins.

Some students will have problems with the smell of the spices, the agar or the smell of the bacteria when the plates are grown up. Do not allow the students to open the plates and smell them- if the plates are left closed, the odor is reduced.

Ways to Use this Lab

This lab is an excellent way to teach students about the scientific method.

1. Intergenerational information- Have students talk to parents and grandparents or older people or do some research about plants and or herbs that their families may have used in earlier times to help inhibit infections.

2. Since plants often sequester chemicals in specific parts of the plants (for example, most of the oxalic acid in rhubarb is found in the leaves- the small amount that stays in the stem imparts the characteristic tart taste) students can test different parts of a plant to see if there is more activity in one part than another. Some spices to try different parts of the plant are garlic (different parts of the bulb, green scapes if available, Coriander or cilantro- leaves versus the seeds, and Hot Peppers – veins, meat, or seeds

3. Does preparation affect spice potency? Tried fresh versus dried. Be careful with spices that have salt added- like garlic salt or onion salt. The salt alone will kill the bacteria.

4. Spices that are mixtures are interesting to study. Pickling spices works very well and with some sorting students can determine that the cloves and cinnamon are the most potent, not the black or chili peppers that seem more obvious.

5. This lab is an excellent interdisciplinary lab that can be used to teach history, cultural studies, and plant biology.

Additional Teacher Information

Examples of spices that work well in this lab

Turmeric

Turmeric comes from the root of Curcuma longa, a leafy plant in the ginger family. The root, or rhizome, has a tough brown skin and bright orange flesh. Ground Turmeric comes from fingers, which extend from the root. It is boiled or steamed and then dried, and ground. India is the world's primary producer of Turmeric. It is also grown in China and Indonesia.

Turmeric, with its brilliant yellow color, has been used as a dye, medicine, and flavoring since 600 BC. In 1280, Marco Polo described Turmeric as "a vegetable with the properties of saffron, yet it is not really saffron." Indonesians used Turmeric to dye their bodies as part of their wedding ritual. Turmeric has been used medicinally throughout Asia to treat stomach and liver ailments. It also was used externally, to heal sores, and as a cosmetic.

Cilantro (Coriander)

Coriander has been used as a folk medicine for the relief of anxiety and insomnia in Iranian folk medicine. Experiments in mice support its use as an anxiolytic. [2]

Coriander essential oil showed a delay in E. Coli growth, suggesting possible agricultural anti-bacterial applications.

Coriander seeds have also been used to prepare a traditional diuretic in India. The diuretic is prepared by boiling equal amounts of coriander seeds and cumin seeds. The extract is then cooled and consumed as a diuretic

Cinnamon

Cinnamon bark is widely used as a spice. It is principally employed in cookery as a condiment and flavoring material, being largely used in the preparation of some kinds of desserts, chocolate and spicy candies and liqueurs. In the Middle East, it is often used in savory dishes of chicken and lamb. Cinnamon can also be used in pickling. In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhea and other problems of the digestive system.

Cinnamon is high in antioxidant activity. The essential oil of cinnamon also has antimicrobial properties This property may allow cinnamon to

extend the shelf life of foods

Cinnamon is also used as an insect repellent. It is widely used when a manufactured insecticide is not wanted or cannot be used because of possible health side effects or allergies.

Cloves

Syzygium aromaticum (L.) Merr. & L. M. Perry

Cloves are the aromatic dried flower buds of a tree in the family Myrtaceae. It is native to Indonesia and used as a spice in cuisine all over the world. The name derives from French clou, a nail, as the buds vaguely resemble small irregular nails in shape.

Clove essential oil is used in aromatherapy and oil of cloves is widely used to treat toothache in dental emergencies. Cloves have historically been used in Indian cuisine (both North Indian and South Indian). In the north Indian cuisine, it is used in almost every sauce or side dish made, mostly ground up along with other spices.

Along with the recreational uses of cloves, they are also said to be a natural antihelmintic. The compound responsible for the cloves' aroma is eugenol. It is the main component in the essential oil extracted from cloves, comprising 72–90%. Eugenol has pronounced antiseptic and anesthetic properties.

Supplemental Reading

Spice references

Sherman, P.W. and J. Billing 1999.
Darwinian gastronomy: Why we use spices. BioScience 49:453-463.

http://www.sciencedaily.com/releases/1998/03/980305053307.htm

Billing, J. and P.W. Sherman 1998.
Antimicrobial functions of spice use: Why some like it hot.
Quarterly Review of Biology 73:3-49.

Why We Get Sick:
The new science of Darwinian medicine
By Randolph M. Nesse and George C. Williams. 291 pp. New York, Times Books, 1995.
ISBN 0-8129-2224-7.

Web references

UCLA Spice Exhibit
http://unitproj.library.ucla.edu/biomed/spice/index.cfm

Epicentre Spices
http://www.theepicentre.com/

McCormick Spices
http://www.mccormick.com/content.cfm?id=8291

History of Spices
http://www.mccormick.com/content.cfm?ID=10109

Herbs and Spices and Medicines

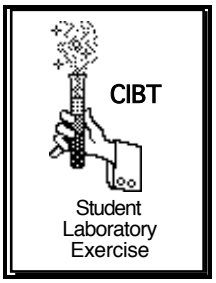
http://www.chaddsfordhistory.org/history/herbs2.htm

Wikipedia
http://en.wikipedia.org/wiki/Main_Page

Search for spices, ethnobotany

Some Like it Hot!

An investigation of the antibiotic properties of spices, herbs and teas



Questions to Consider:

When you have a bacteria infection, such as strep throat, your doctor gives you antibiotics to kill the infection. In 1928, Alexander Fleming discovered that molds make chemicals that stop bacteria from growing. These chemicals became our first antibiotics. (He won a Nobel Prize for this discovery!)

Think about early human beings. They could not drive to the pharmacy for medicine. They used what they had- in many cases, plants. Spices are parts of plants and some evolutionary biologists think that we eat spices because they protect us from infections. In this lab you are going to investigate whether common spices are capable of killing bacteria.

How to Set up Your Experiment:

1. Decide what spice or spices you are going to test. Think about what controls you will need for your experiment. Pick up Petri dishes, spices, spoons and a marker. Label your plate with the name of your group and your spice.

2. Place a small amount of spice in the plate. If you are using a whole spice, like clove or cinnamon or allspice, put 1 or 2 in the middle of the Petri dish. If you are using a ground spice, put about 1/2 tsp in the bottom of the plate. Get a bottle of melted agar and pour a small amount into the dish. If the whole spice moves around, use the spoon to center it in the plate. Allow the agar in your dishes to cool. Once the agar is solid, pour another thin layer of agar over the spices to completely cover them. Let this layer of agar cool.

3. Once the agar is solid, it is time to add bacteria to your plate. If you are using your fingers, dip your finger into water and rub it around the space in your plate. If you are using a liquid culture of bacteria, use a cotton-tipped swab to completely cover the area. Let the dishes sit for a few minutes, then turn them upside down and give them to your teacher. The plates will be incubated to allow the bacteria to grow.

4.

	# Some Like it Hot! An investigation of the antibiotic properties of spices, herbs and teas
Group Members:	
Hypothesis:	
Spices Being Tested:	

EXPERIMENTAL PLATES

Results:

After your plates have incubated, look to see what sections of your plate have bacterial growth on them.

Then, answer these questions.

What results did you obtain?

What controls did you include?

Did your results surprise you? Why?

Did your spice kill more than one kind of bacteria?

Vocabulary:

Agar
Agar is a carbohydrate obtained from the cell walls of red algae or seaweed. The word agar comes from the Malay word agar-agar (meaning jelly). Chemically, agar is a polymer made up of subunits of the sugar galactose. Agar polysaccharides serve as the primary structural support for the algae's cell walls. Dissolved in hot water and cooled, agar becomes gelatinous. Its chief use is as a culture medium for microbiological work. Other uses are as a laxative, a vegetarian gelatin substitute, a thickener for soups, in jellies, ice cream and Japanese desserts such as anmitsu, as a clarifying agent in brewing, and for paper sizing fabrics.