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NHGRI Short Course in Genomics

You Are What You Eat — Exploring the Microbiome Through Inquiry-Based Labs

Karla Fuller, Ph.D.

Associate Professor of Biology

Stella Charles Guttman Community College

NIH National Human Genome Research Institute

The Forefront of Genomics®

Forward

"We can compare the gut of a person with inflammatory bowel disease to a dying coral reef or a fallow field: a battered ecosystem where the balance of organisms has gone awry."
*(***Ed Yong,** *I Contain Multitudes: The Microbes Within Us and a Grander View of Life)*

This quote from Ed Yong illustrates how the study of the human microbiome is comparable to the way in which ecologists study complex communities. While people are probably most familiar with the human microbiome, every ecological niche on Earth has its own collection of distinctive microorganisms. This includes coral reefs and barnyard soils, as well as hostile environments like hot springs or acidic outflows from mining operations. These microbial communities are critical to important planet-wide processes like carbon sequestration, nitrogen fixation and the breakdown of toxic chemicals. These wildly differing environments and functions reflect another important feature of the microbiome: diversity. Despite a bias for focusing on the bacterial component of the microbiome, microbial communities are complex and draw organisms from across the tree of life, including fungi, viruses, protists and metazoans.

Bacteria are often the focus of microbiome studies because microbiologists have developed robust tools for growing and identifying bacteria. Part of this stems from our frequently adversarial relationship with bacteria. Pathogens like *Yersinia pestis*, the causative agent of the Black Plague, have fundamentally shaped human history. The careers of famous microbiologists like Louis Pasteur (1822-1895), Robert Koch (1843-1910) and Alexander Fleming (1881-1955) were built on understanding and eradicating bacterial infections. While modern life wouldn't be possible without medical advances to treat bacterial infections, it's important to understand that bacteria far more often play a beneficial role in the world. Unlike pathogenic bacteria, which usually act alone, beneficial bacteria often function as a community made up of tens or hundreds of different microbes. Consortia of bacteria (and also fungi) are indispensable for making bread, cheese, yogurt, kimchi and many other foods. The microbiome is important to human health and the development of a strong immune system. Microbial communities are critical for nitrogen fixation and the health of our crops and livestock.

This collection of lesson plans is designed to introduce students to the microbiome through discussions of current science and hands-on experiments.

- **A Glimpse into the Microbiome provides an overview of the microbiome** starting with the ecological paradigms that defined early microbiome science and finishing with the impact of the microbiome on human health.

- **Exploring the Microbiome and Its Connection to Metabolic Syndrome** offers a detailed look at the current science linking the microbiome to a constellation of health problems called metabolic syndrome. Studies connecting obesity, diabetes and hypertension to the microbiome are some of the most frequently cited examples of microbiome-mediated health conditions.

- **You Are What You Eat — Exploring the Microbiome Through Inquiry-Based Labs** teaches students important concepts like the scientific method, sterile technique and reproducibility.

- **Microbiome Virtual Lab Exploration!** gives student the opportunity to analyze real microbiome data using web-based analysis tools.

Taken together, these lesson plans can be adapted to a variety of settings, student populations and educational goals. We hope students will be inspired to learn more about the hidden communities of microbes that shape human health and the environment.

Sean P. Conlan, Ph.D.
Associate Investigator
Microbial Genomics Section
Translational and Functional Genomics Branch
National Human Genome Research Institute

You Are What You Eat — Exploring the Microbiome Through Inquiry-Based Labs

Timeframe

1. Introduction to Microbiome lecture (powerpoint slides) (45 minutes)
2. Science in the Classroom module: https://www.scienceintheclassroom.org/research-papers/1958/guide
    a. Read open access article (45 minutes or homework)
    b. Discussion questions (45 minutes or assigned as homework when students read the article)
    c. Active engagement activity (minimum 45-minute class period)
    d. Optional: Use another article (or several and do a jigsaw reading).
    The discussion questions are broad enough to be used for other readings.
3. Guided lab (You Are What You Eat)
    a. Lab overview and experiment planning (45 minutes)
    b. Pre-lab (45 minutes or homework)
    c. Lab experiment (45 minutes for initial experiment, follow up to collect results as needed)
    d. Post-lab (45 minutes or homework)
4. Microbiome Inquiry Activity
    a. Lab overview and experiment planning (45 minutes)
    b. Pre-lab (45 minutes or homework)
    c. Lab experiment (45 minutes for initial experiment, follow up to collect results as needed)
    d. Post-lab (45 minutes or homework)
    e. Optional: oral or poster presentations

You Are What You Eat: Do curry and cinnamon inhibit bacterial growth?

Background

Photo: Gettyimages/artlinegraphics

There is significant interest in natural products, including herbs, spices and folk remedies, as potential treatments for disease. In particular, with the rise of antibiotic-resistant bacteria, scientists are looking to the natural world as inspiration for new antibacterial and antiviral agents. Our diets have been shown to significantly alter the composition and functioning of the microbial population residing inside of our bodies.

These diet-induced changes in our gut microbe population may play a role in our overall health and wellness. For example, turmeric and curry have been shown to have broad spectrum antimicrobial/antiviral activity.[1,2] Similarly, cinnamon stick extract has been shown to have anti-microbial activity against *Helibactor pylori*[3] and some strains of *Candida.*[1,4]

If these commonly used spices have the ability to inhibit pathogenic bacterial growth, could they also potentially inhibit the growth of normal, harmless bacteria that live in your body? In this lab, we will test common bacteria for resistance to food additives.

It is important to note that demonstrating activity in the lab is only the first of many steps in developing a new therapy, and the vast majority of natural products aren't found to be suitable or effective for therapeutic use. Furthermore, it is important to emphasize that, while food-as-medicine is an attractive idea, that sort of thinking without the science to back it up can have tragic results. For instance, Samoa recently experienced a deadly measles outbreak, partially fueled by anti-vaccination activists promoting vitamin C to cure measles (https://www.npr.org/2019/12/06/785487606/samoa-arrests-anti-vaccination-activist-as-measles-death-toll-rises). Natural product research requires careful laboratory testing like that seen in the paper by Wong et al. on natural compounds and Mycobacterium avium (Wong et al., AEM 2008).

Objectives

- Observe the effects of food additives (in this case cinnamon and curry) on the growth of bacteria in your body.
- Learn how to culture bacteria in a safe and sterile manner.

Pre-lab questions

1. What are some of the effects that diet is known to have on the human microbiome?
2. What methods can you use to ensure that you do not contaminate your plate with bacteria that are not from your sample? Research and describe two of these methods.
3. What steps can you take to keep you and your classmates safe when working with bacteria?
4. Look up and describe five safety techniques.
5. Would you expect to find the same type of bacteria in a person's stomach as you would find in their mouth? Why/why not? Explain your answer.

Materials

- Curry oil
- Cinnamon oil
- Prepared petri dishes containing agar medium and nutrients (9 for each person or group)
- Sterile cotton swabs

Safety precautions

1. Protect hands with gloves and do not touch face or mouth with gloves.
2. Do not smell the plates.
3. Keep petri dishes with bacteria taped shut.
4. Kill bacteria on plates with bleach when you are finished, and properly dispose of plates in a biohazard container.

Procedure

1. Label three petri dishes “control,” three “curry,” and three “cinnamon.”
2. Dip a sterile swab into the curry oil and spread the curry oil all over the plates labeled “curry.” Repeat the same procedure with a new sterile swab using cinnamon for the plates labeled “cinnamon.”
3. Use a new sterile swab to take a sample from your mouth by swabbing along your inner cheek. Apply the sample to a plate with the swab. Repeat this step for all of your plates using a new sterile swab each time. *Note: encourage students to try to follow the same “swab path” created when the spice was swabbed on.*
4. Store the petri dishes at room temperature for several days until you begin to see bacterial growth. Each day make qualitative observations about the bacteria on the petri dishes. Have bacteria started growing? How large are the colonies? What are the colors of the bacteria? Take pictures or sketch what you see in your lab notebook for future reference.
5. After several days make more detailed observations about the bacteria growing on the petri dishes. Make qualitative observations about the dishes first. Are certain color bacteria found on only one type of dish? Are bacteria on certain dishes smaller than the others? What color are the bacteria on the plates?
6. Count and record the number of bacteria found on each dish. Take the average of the number of bacteria found on the control plates, curry plates and cinnamon dishes.

Post-lab questions

1. What are the chemicals found in cinnamon and curry that scientists believe might have antibiotic properties?
2. Based on your data, do you think that the antibiotic properties of cinnamon and curry should be advertised to customers? Explain your reasoning.
3. What are other naturally occurring food additives that are believed to have antibiotic properties?
4. What could be done to improve this experiment to give us more information about the antibiotic properties of curry and cinnamon?
5. What happens when antibiotic compounds inhibit the growth of your naturally occurring, non-pathogenic bacteria? Give two examples.

Standards (based on NGSS)

- HS-LS2-2 Ecosystems: Interactions, Energy, and Dynamics
  Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.provide specific functions within multicellular organisms.
- HS-LS2-6 Ecosystems: Interactions, Energy, and Dynamics
  Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.
- HS-LS3-3
  Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.
- HS-LS4-5 Biological Evolution: Unity and Diversity
  Evaluate the evidence supporting claims that changes in environmental conditions may result in (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.

Microbiome Inquiry Activity

Can be completed after, or in place of, the guided activity (depending on student level and time).

In this experiment, students can choose from a provided list or select their own dietary items to test. Some examples are: probiotic supplements, antibacterial tinctures, antimicrobial folk treatments and/or other homeopathic remedies

Objective

Use the scientific process to design and implement an experiment that tests the effects of dietary items on bacteria.

Pre-lab questions

1. What dietary items will you use for your test?
2. What is the advertised effect of these dietary items on the microbiome/bacteria?
3. How do you predict these dietary item will affect the growth of your bacteria?
4. What are your positive and negative controls?
5. What is your hypothesis?

Materials

- Approved dietary items
- Prepared petri dishes (enough for each student have triplicate test and control plates)
- Sterile cotton swabs

Lab safety reminders

- Protect hands with gloves and do not touch face or mouth with gloves.
- Do not smell the plates.
- Keep Petri dishes closed.
- Dispose of plates properly, as directed by your instructor, at the conclusion of the experiment.
- Use proper sterile procedure at all times.

Procedure

1. Label petri dishes in triplicate with the name of each test and control.
2. Prepare your dietary items as directed by your instructor.
3. Cover your triplicate set(s) of test plates with the test solution.
4. Use a clean, sterile swab to take a sample from your mouth by swabbing along your inner cheek.
5. Mix the swab in 300-500ml of nutrient broth and aliquot the entire mixture to each petri dish using a cell spreader to inoculate the plates. Repeat this step for all of your plates using a new sterile swab mixture each time.
6. Store the petri dishes at room temperature for several days until you begin to see bacterial growth.

Adapted from http://www.education.com/science-fair/article/curry-inhibit-bacterial-growth/

[1]Praditya, D., et al. (2019) “Anti-infective Properties of the Golden Spice Curcumin.” *Front Microbiol*.

[2]Katoch, et al. (2013) “Screening of murraya koenigii (curry) and camellia sinensis (tea) leaves for antimicrobial activity against strains of staphylococcus aureus, pseudomonas aeruginosa and candida species and their phytochemical analysis.” IJPSR.0975-8232.4(2) pp. 862-68.

[3]Tabak, M., et al. (1999) “Cinnamon extracts’ inhibitory effect on Helicobacter pylori.” *J Ethnopharmacol*, 67, pp. 269-277.

[4]Quale, J.M., et al. (1996) “In vitro activity of Cinnamomum zeylanicum against azole resistant and sensitive Candida species and a pilot study of cinnamon for oral candidiasis.” *Am J Chin Med*, 24, pp. 103-109.


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