Using An In Vivo Model System to Investigate Innate Immunity & the Microbiome

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Funded through The American Association of Immunologists

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TEACHER GUIDE

I. Science Background

Scientists have recently revealed the microbiome's decisive role on our health. This curriculum helps students explore the role of this microbial community on our immune system by investigating a model system used in biomedical science, *C. elegans* worms. In addition, plants collected in the field, such as common milkweed, will be studied for its phytochemical influence on the nematodes, directly or indirectly, via their microbiome. Procedures include collecting wild plants, preparing plant extracts, culturing bacteria using aseptic microbiology techniques, and animal husbandry involving the care of *C. elegans* worms.

II. Student Outcomes

• Science concepts covered in the unit:

a) The role of the colon's mucosal membranes and microbial community in influencing gut homeostasis and immunity.
b) Innate immunity and signal transduction pathways, including p38 MAPK, IIS cascade, and HSF-1 pathways (also highly conserved between *C. elegans* and mammals).
c) The biomedical use of model organisms pertains to shared ancestry via evolution by common descent.

• Next Generation Science Standards (NGSS) and/or AP Biology alignment:

This inquiry-based curriculum will help students build NGSS science practices, including: asking questions, developing and using models, planning and carrying out investigations, analyzing and interpreting data, employing mathematical and computational thinking, constructing explanations, engaging in arguments from evidence and communicating information.

• Recommended placement for the materials within a middle or high school course:

This curriculum is intended for motivated students in grades 9-12 in a science research elective to help them prepare for possible school-wide and/or local science fairs. It may also work for AP Biology students in districts which meet for 4-6 weeks after the exam in early-to-mid-May.

• What students will do and what technical skills they will learn:

This lab series will prepare students for pursuing a laboratory position during high school and provide fundamental microbiological techniques for life science majors in college.

• Relevance to other science concepts and students' lives:

The microbiome plays a critical role in the maturation of the immune system. In particular, our colon has sensors for detecting microbes in our gut, which is triggered when microbial diversity is abnormal. Chronic diseases linked with obesity, diabetes, and a variety of autoimmune diseases are associated with dysbiosis, a condition of microbiome imbalance. In vivo model systems using organisms such as *C. elegans,* allow investigators to test how plant compounds interact with the worm's innate immune system and the microbiome.

III. Learning Objectives

• Observable and measurable skills include:

Gather plants (ex. milkweed) to (a) measure the effect on *C. elegans'* survival, (b) influence on the microbiome of *C. elegans,* (c) infection by a pathogen, and (d) expression of green fluorescent reporters.

• Students will demonstrate specific knowledge and skills in the following manner:

1. Explore, plan, and conduct investigations using Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans.*
2. Explore and interpret data using Homologous Disease Genes in Flies & Worms.
3. Explore the microbiome, deconstruct a research paper, display research, and present findings to an audience using the Human Microbiome Mini Project.
4. Explain and evaluate, communicate information on how the innate immune system responds to phytochemicals using Modeling Signal-Transduction Pathways with Playdough.

IV. Time Requirements

Requires eleven 60 minute periods.

V. Advance Preparation

• Equipment and materials:

The following equipment is required: Petri dishes, electronic balances, weighing dishes, 1 liter and 500 ml bottles, foam buckets, bunsen burners and/or alcohol lamps, 75% ethyl alcohol spray bottles, sterile inoculation loops, an autoclave or pressure cooker, autoclave tape, oven mits or gloves, stereomicroscopes, a stereo microscope fluorescence adapter, refrigerator, micropippetors with tips, serological pipettes, glass pipettes, 2 liter erlenmeyer flasks, magnetic stir bar, a stirring hotplate, 500 ml graduated cylinder, and platinum wire.

Reagents include: 100% methanol, NaCl, Bacto Agar, peptone, Milli-Q Water (or some type of ultra purified water), CaCl2, 1 M cholesterol, MgSO4, KPO4, LB Medium agar, streptomycin, ampicillin, and tryptic soy agar.

Worms and the relevant bacteria can be obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota. The following worms will be used: N2 (aka wild isolate), Clec-60, hlh-30, and sysm-1. Bacterial strains that are part of *C. elegans'* microbiome, also ordered from the CGC, which include: *Lelliottia amnigena*, *Acinetobacter guillouiae*, *Sphingomonas molluscorum*, *Pseudomonas lurida*, *Comamonas piscis*, *Pantoea nemavictus*, *Sphingobacterium multivorum*, and *Chryseobacterium scophthalmum*.

• Directions for preparing solutions and other reagents

- Nematode Culture Media (NGM) - used to grow colonies of OP50 *E. coli* bacteria and *C. elegans* worms
    - Make 1 liter
    - 3 g NaCl
    - 17 g Bacto Agar - labeld “Difco Agar”
    - 2.5 g peptone
    - 975 ml mini Q water
    - Stir and autoclave (see autoclave protocol below):
        - Add a stir bar
        - Leave cap slightly ajar
        - Add autoclave tap to the top of the cap
        - Place the flask on a metal cart
        - Use heat-resistant gloves
        - Follow the autoclave directions so the liquid is at 121°C for 20 minutes.
        - Once autoclaved, cool to 40 °C
    - To cooled, autoclaved liquid add the following:
        - 1 ml M CaCl2 (see photo)
        - 1 ml 1 M 5mg/ml cholesterol on ethanol
        - 1 ml 1 MgSO4
        - 25 ml 1 KPO4

- Add the 1 liter of aqueous NGM to an autoclaved beaker while in a hood or sterile area.
- Mix and pour into Petri dishes using a serological pipette.
- Store 1 liter NGM stock in a refrigerator at 4 °C.

- LB Liquid Agar - used to grow a liquid culture OP50, a strain of *E. coli,* used as food for *C. elegans)*
    - Make 500 ml
    - LB Medium agar powder
    - Mass 12.5 g of agar powder
    - Add to a 1,000 ml sterile bottle
    - Use a sterile graduated cylinder to measure the volume of 500 ml of Milli-Q water
    - Add a stir bar
    - Leave cap slightly ajar
    - Add autoclave tape to the top of the cap
    - Place the flask on a metal cart
    - Stir and autoclave (see autoclave protocol below):
        - Add a stir bar
        - Leave cap slightly ajar
        - Add autoclave tap to the top of the cap
        - Place the flask on a metal cart
        - Use heat-resistant gloves
        - Follow the autoclave directions so the liquid is at 121°C for 20 minutes.
        - Once autoclaved, cool to 40 °C
    - When in need of a sample of the liquid agar, pipette 25 ul of streptomycin into the liquid agar.
    - Obtain a petri dish of OP50 and use an inoculating loop to transfer one colony into the 50 ml tube. Seal cap and place on a shaker table to mix for 24 hours.

- Tryptic Soy Agar (TSA) - used to culture bacterial strains that are part of C. elegans' microbiome
    - 4 g of tryptic soy agar powder
    - 100 ml of Milli-Q water
    - Add agar powder + water to a sterile 250 ml erlenmeyer flask
    - Include a sterile stir bar
    - Add new autoclave tape to foil cap
    - Stir and autoclave (see autoclave protocol below):
        - Add a stir bar
        - Leave cap slightly ajar
        - Add autoclave tap to the top of the cap
        - Place the flask on a metal cart

- Use heat-resistant gloves
- Follow the autoclave directions so the liquid is at 121°C for 20 minutes.
- Once autoclaved, cool to 40 °C

• Approximate preparation time

3 hours

VI. Materials and Equipment

• For the number of students

15 - 25 students

• Procedure, as described in Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of C. elegans:

1) PLANT HARVESTING - Obtain leaves, stems, branches, and/or fruit/seeds of a desired plant growing in its natural habitat. Common milkweed *(Asclepias syriaca)* leaves will be used in this lab series.
2) PREPARATION OF EXTRACT - Use 100% methanol to make a 20% solution of the collected plant.
3) MILKWEED CONTAMINATION SCREENING - Determine the presence of bacteria or fungi that may not have been killed by the 24 hours of methanol steeping. The milkweed (MW) solution must be microbe-free for the survival assay in the next step. Therefore, it is important to do this test before any other experimentation.
4) SURVIVAL ASSAY - Establish the effect of methanol and milkweed extract on *C. elegans* survival and reproduction.
5) CeMbio ASSAY - Observe how methanol and milkweed extract influence the 12 essential species of the *C. elegans'* microbiome (aka the "CeMbio"). These twelve bacteria include:
    - *Enterobacter hormaechei*
    - *Lelliottia amnigena*
    - *Acinetobacter guillouiae*
    - *Sphingomonas molluscorum*
    - *Stenotrophomonas indicatrix*
    - *Pseudomonas lurida*
    - *Pseudomonas berkeleyensis*
    - *Comamonas piscis*
    - *Pantoea nemavictus*
    - *Ochrobactrum vermis*
    - *Sphingobacterium multivorum*

    - *Chryseobacterium scophthalmum*
6) INFECTION ASSAY - Measure potential anal swelling after infection to *Microbacterium nematophilum* when exposed to milkweed extract (and as a control, no milkweed extract).
7) FLUORESCENT REPORTER FOR IMMUNE ACTIVATION OR SUPPRESSION IN VIVO ASSAY - Identify potential gene expression targets activated or suppressed by milkweed exposure.

• Estimated cost for materials

Approximately $500 - $1,000 for ordering laboratory-grade reagents, consumables, and live materials. Bacto Agar, peptone, mini Q water, CaCl2, cholesterol, ethanol, methanol, and tryptic soy agar powder can be ordered from Fisher Scientific. Consumables such as 50 mm Petri dishes, inoculating loops, glass pipettes, platinum wires, weighing dishes, serological pipettes, and pipette tips can be purchased from Fisher Scientific. *C. elegans* worms, OP50 bacteria, and most or all of the 12 essential species of the *C. elegans'* microbiome can be obtained from the Caenorhabditis Genetics Center (CGC).

• Possible substitutes

Carolina Biological Supply Company sells *C. elegans* live cultures and premade nematode growth agar. Instead of using the OP50 *E. coli* strain, C. elegans can consume Carolina Biology Supply Company's K-12 strain, a safe bacterial strain appropriate for high school students. Carolina also sells 50 mm Petri dishes and pipette tips. The total cost for these items (for a class of 25 students) is about $200.

• Explanation of equipment use:

Per Class:

Stereomicroscopes - for observing worms
Bunsen burner - to melt the tip of a glass pipette and attach to a short (1 cm long) platinum wire.
75% ethyl alcohol spray bottle - to sterilize a work area of a lab bench.
Autoclave or pressure cooker - to sterilize glassware such as Erlenmeyer flasks.
Stereo microscope fluorescence adapter - to observe GFP worms.
Refrigerator - to store reagents such as nematode growth medium.
Micropippetors (2-20 uL, 20-200 uL, and 100 uL) - to measure and prepare stock solutions of nematode growth medium and tryptic soy agar.
Serological pipettes - to measure and prepare stock solutions of nematode growth medium and tryptic soy agar.

Stirring magnetic hotplate w/ magnetic stir bar - to warm and mix reagents (ex. nematode growth medium and tryptic soy agar).
Glass pipettes - for creating a “worm picker,” a device made by melting the tip of a glass pipette and fusing it to a 1 cm long platinum wire.
Platinum wire - for creating a “worm picker,” a device made by melting the tip of a glass pipette and fusing it to a 1 cm long platinum wire.
2 liter Erlenmeyer flasks - for storing stock cultures of nematode growth medium and trypic soy agar.

Per Group:

6 Petri dishes, 1 weighing dish, 1 bunsen burner or alcohol lamp, 1 75% spay bottle, 5 sterile inoculation loops, 1 stereomicroscope, platinum wire.

- If students make growth media themselves, they need the following items below. (Alternatively, media can be ordered premade from Carolina Biological Supply Company, see “possible substitutes” above):
    - 1 liter and 500 ml bottles - for storing reagents.
    - Foam buckets - to keep antibiotics such as streptomycin and chemical reagents like cholesterol chilled.
    - 500 ml graduated cylinders - for measuring the volume of liquid reagents.
    - Electronic balances - for weighing reagents

• Possible sources for access to expensive equipment

- Stereomicroscopes - Leica Microsystems
- Stereo microscope fluorescence adapter - New York Microscope Company
- Micropippetors (2-20 uL, 20-200 uL, and 100 uL) - VWR or Ward’s
- Serological pipettors (aka a controller of “gun”) - USA Scientific
- Glass pipettes - Fisher Scientific
- Platinum wire - Fisher Scientific
- 2 liter Erlenmeyer flasks - Fisher Scientific

• Precautions and safety (chemical storage, handling, waste removal, etc.)

Precautions should be taken when handling bacteria. K-12 or OP50 bacteria are safe in a high school classroom, but instructors and students should use aseptic techniques such as washing hands before and after handling. Nitrile gloves can also be worn to increase safety. A 75% ethyl alcohol spray bottle should be applied to any working area before and after use. When agar plates are no longer used, a 10% bleach solution should be poured over the exposed agar before disposal.

VII. Student Prior Knowledge and Skills

• Expected prior content knowledge

Although this curriculum is written for advanced and motivated students, no prerequisite knowledge or skills are required.

• Expected prior technical skills

None

• Possible preconceptions

1. Misunderstanding of evolutionary connections between humans and *C. elegans* worms.
2. Preconceptions that all bacteria are germs and cause disease.

VIII. Daily Unit Plans

• Comprehensive plans for each day of the unit (see below):

Each day requires 60 minutes to complete. The listed pedagogy uses the 5 E model (Engage, Explore, Explain, Elaborate and Evaluate) and the Next Generation Science Standards (NGSS) (ask questions, develop and use models, plan and carry out investigations, analyze and interpret data, use mathematics and computational thinking, construct explanations, engage in argument from evidence, obtain, evaluate, and communicate information). NGSS Cross-Cutting Concepts include: patterns, cause and effect, scale, proportion and quantity, systems and system models, energy and matter, structure and function, and stability and change.

Timeline	Activity	Pedagogy	Materials
Day 1	- What are *C. elegans* & why study them? - Observe N2 worms	Engage, ask questions	Computers, stereomicroscopes, Petri dishes with N2 worms
Day 2	- Homologous Disease Genes in Flies & Worms	Explore, analyze, and interpret data	Computers
Day 3	- Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans*	Explore, plan, and carry out investigations	Per Class: Petri dishes, electronic balances, weighing dishes, 1 liter and 500 ml bottles, foam buckets, bunsen burners and/or alcohol lamps, 75% ethyl alcohol spray bottles, sterile

			inoculation loops, an autoclave or pressure cooker, autoclave tape, oven mits or gloves, stereomicroscopes, a stereo microscope fluorescence adapter, refrigerator, micropippetors with tips, serological pipettes, glass pipettes, 2 liter erlenmeyer flasks, magnetic stir bar, a stirring hotplate, 500 ml graduated cylinder, and platinum wire. Per Group: 6 Petri dishes, 1 weighing dish, 1 bunsen burner or alcohol lamp, 1 75% spay bottle, 5 sterile inoculation loops, 1 stereomicroscope, platinum wire.
Day 4	- Human Microbiome Mini Project - Identify & collect milkweed (and/or other plants) - Prepare plant extract(s)	Explore the microbiome, deconstruct a research paper, display research findings, and present to an audience.	Computers, mini white boards, or trifold posters
Day 5	- Milkweed Contamination Screening - Human Microbiome presentations	Explain, construct explanations, engage in argument from evidence	Computers, mini white boards, or trifold posters
Day 6	- Observe milkweed contamination screening data - Survival Assay (day 1)	Explore, plan and carry out investigations	Same as day 3
Day 7	- Survival Assay (day 2) - CeMbio Assay (day 1)	Explore, plan and carry out investigations	Same as day 3
Day 8	- CeMbio Assay (day 2) - Analyze data using DataClassroom - Infection Assay (day 1)	Elaborate and Evaluate, use mathematics and computational thinking, construct explanations	Same as day 3
Day 9	- Infection Assay (day 2) - Fluorescent Reporter Assay (day 1)	Explore, plan and carry out investigations	Same as day 3
Day 10	- Fluorescent Reporter Assay (day 2)	Explore, plan	Same as day 3

	- Observe fluorescence to infer innate pathway expression or suppression	and carry out investigations	
Day 11	- Modeling Signal-Transduction Pathways with Playdough	Explain and evaluate, communicate information	Computers, playdough, printed handouts

• Cited activities (also listed below in the appendix):

- What are *C. elegans* & why study them?
- Homologous Disease Genes in Flies & Worms
- Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans*
- Human Microbiome Mini Project
- Modeling Signal-Transduction Pathways with Playdough

• Formative assessments:

1. Feedback from paired conversations from What are *C. elegans* & why study them?
2. Feedback from paired conversations from Homologous Disease Genes in Flies & Worms
3. Feedback from paired conversations from Modeling Signal-Transduction Pathways with Playdough

Classroom Discussion (also included in the hyperlinked in the schedule {above}):

1. Discussion questions from What are *C. elegans*, why study them?
   Examples: *(a) Identify 3 features humans share with C. elegans. (b) Explain why people and nematode worms have these traits in common. (c) Describe why scientists study C. elegans.*
2. Discussion questions from Homologous Disease Genes in Flies & Worms
3. Examples: *(a) What percent similarity is there between the proteins coded by these orthogonal genes? (b) Compare the letters representing each amino acid between the fly and human proteins. Can you find where they differ?*
4. Discussion question from Modeling Signal-Transduction Pathways with Playdough
   Example: *Explain how inflammation might be suppressed with a particular phytochemical.*

IX. Summative Assessment

• The formative assessments described above highlight assessments where students may revise their work. In contrast, the summative assessment below includes laboratory

results, which, for practical purposes of time, may not permit students to re-do laboratory experiments:

> Student's laboratory results include (a) mortality of worms or the microbiome of C. elegans bacteria exposed to phytochemicals, (b) presence or absence of fluorescent signals in transgenic reporter worms indicating innate immunity activation or suppression after exposure to phytochemicals. Descriptions included in Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans*

• Additional Notes for Curriculum Developers

> The activities described in this curriculum can be modified for lessons about evolution and common ancestry (examples: What are C. elegans, why study them? + Homologous Disease Genes in Flies & Worms). Alternatively, the activity Modeling Signal-Transduction Pathways with Playdough, could be adapted for a lesson on the immune system. Finally, the project on the Human Microbiome could be integrated into a unit on health and preventive disease.

STUDENT SECTION

I. Rationale

Scientists recognize that trillions of bacteria reside in our body and play a vital role in health. This "microbiome" influences many aspects of our wellbeing, including our immune, digestive, and nervous systems. In this series of labs, you will investigate how plant-derived chemicals or "phytochemicals" influence the innate immune system using a model organism that can illuminate human biology.

During this three-week series of activities, labs, and projects, you will investigate the innate immune system using a nematode worm called *Caenorhabditis elegans* or simply *C. elegans.* You will explore this fascinating relationship as part of five lessons:

1. What are C. elegans & why study them?
2. Homologous Disease Genes in Flies & Worms
3. Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of C. elegans
4. Human Microbiome Mini Project
5. Modeling Signal-Transduction Pathways with Playdough

II. Materials (per group)

1. What are C. elegans & why study them?
    a. Materials:

i. Teacher-guided presentation - What are C. elegans & why study them?
ii. Observe wildtype (N2) worms using a stereo microscope.

2. Homologous Disease Genes in Flies & Worms
    a. Materials:
        i. Digital or Paper Handout - Homologous Disease Genes in Flies & Worms
        ii. A computer

3. Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of C. elegans
    a. Materials:
        i. 6 Petri dishes, 1 weighing dish, 1 bunsen burner or alcohol lamp, 1 75% spay bottle, 5 sterile inoculation loops, 1 stereomicroscope, platinum wire.
        ii. Digital or Paper Handout - Using Phytochemicals to Study the Microbiome & Innate Immunity of C. elegans

4. Human Microbiome Mini Project
    a. Materials:
        i. Computer, mini white boards, or trifold posters
        ii. Digital or Paper Handout - Human Microbiome Mini Project

5. Modeling Signal-Transduction Pathways with Playdough
    a. Materials:
        i. Computer and playdough
        ii. Digital or Paper Handouts:
            1. Modeling Signal-Transduction Pathways with Playdough
            2. iMotion or Stop Motion Studio
            3. List of Phytochemicals and Their Effect on Signal Transduction Pathways
            4. Description of how pathogens induce the p-38 pathway
            5. Signal-transduction pathways labels
            6. A biology textbook as reference (optional)

IV. Data Collection

The culmination of this series of investigations is to interpret results which include (a) mortality of worms or the microbiome of *C. elegans* bacteria exposed to phytochemicals, and (b) presence or absence of fluorescent signals in transgenic reporter worms indicating innate immunity activation or suppression after exposure to phytochemicals.

Maintaining a laboratory journal, which includes tables, photographs, and graphs, will allow you to document and analyze your findings.

Appendix 1 - What are C. elegans & why study them?

Slide #1:

What is a Nematode?

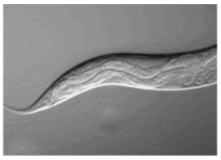
Video - The Most Important Animal You've Never Seen, Meet the Nematode (12:24)

1) How abundant are nematodes?
2) Which description most accurately describes nematodes: segmented worms, roundworms, or flatworms?
3) Why are nematodes like *C. elegans* used in medical research?

Slide #2:

Caenorhabditis elegans
An elegant model system

Greek caeno (recent), rhabditis (rod-like) and Latin elegans (elegant)



Images sources: Left: Wikipedia, Crawling C. elegans - https://en.wikipedia.org/wiki/File:CrawlingCelegans.gif

- Used as a model organism since the mid 1960's.
- 20,500 genes, similar to humans with about 19,900.
- Versatile organism used by over a thousand labs worldwide.

Images sources: Right: Facebook,NemaMetrix Inc.- https://www.facebook.com/photo.php?fbid=667372263688592&id=252078568551299&set=a.273743246384831&locale=id_ID

Slide #3:

Mutants!

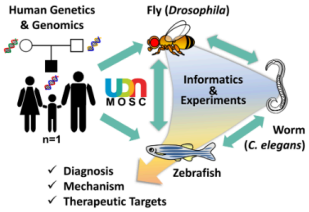
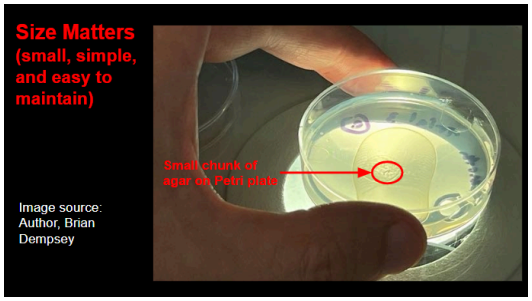


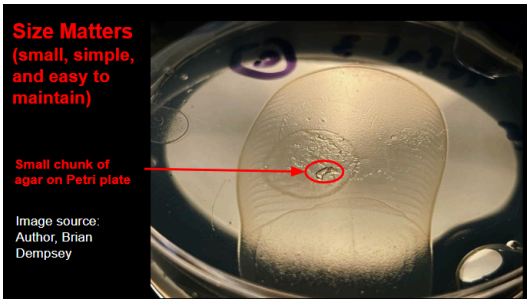
Image source: Undiagnosed Diseases Network - https://undiagnosed.hms.harvard.edu/research/model-organisms-phase-ii/

- About half of human disease genes are identical in *C. elegans* and two thirds are similar.
- Examples of human diseases studied using *C. elegans:*
  - Alzheimer's
  - Parkinson's
  - Polycystic kidney disease (PKD)
  - Amyotrophic lateral sclerosis (ALS)
  - Diabetes

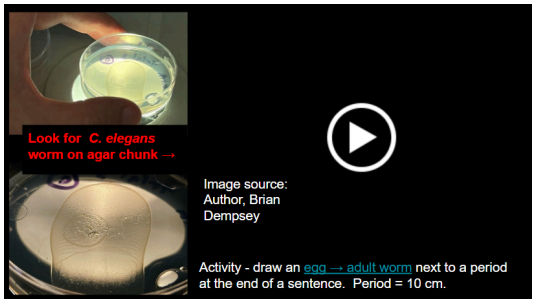
Slide #4:



Slide #5:

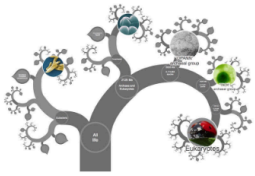


Slide #6:



Slide #7:

A Family Tree



Explore:
1) Identify 3 features humans share with *C. elegans.*
2) Explain why people and nematode worms have these traits in common.

Images sources: Left: Evolution Lab: Deep Tree - https://www.pbs.org/wgbh/nova/labs/lab/evolution/research#/chooser. Right: OneZoom tree of life explorer - https://www.onezoom.org/

Slide #8:

Why Study *C. elegans?*

Worms are cleverer than you think!

Read this short article, linked above, by the Medical Research Council (MRC) Laboratory of Molecular Biology.

- What are two facts that you find interesting?
- Describe why scientists study *C. elegans.*
- Identify something about *C. elegans* you want to learn more about.

Image source: Source: MRC Laboratory of Microbiology. - https://www2.mrc-lmb.cam.ac.uk/groups/worms/history-of-c-elegans-in-scientific-research/

Appendix 2 - Homologous Disease Genes in Flies & Worms

Homologous Disease Genes in Flies & Worms

Background: Bioinformatics, the alignment of biology and computer science, allows scientists to acquire, store, analyze, and disseminate biological data, most often DNA and amino acid sequences. In addition, computational biology allows scientists to use this data to create mathematical models to study evolutionary relationships and disease likelihood. In this lesson, we will explore how some of these methods can be used to identify disease genes in humans using fruit flies and worms as models.

Vocabulary:
Homologous or **"homologs"** - a gene inherited in two species by a common ancestor. This includes both orthologs and paralogs.
Orthologs - homologous genes where a gene diverges after a speciation event, but the gene and its main function are conserved.
Paralogs - are genes that arose from a duplication. As lineages diverge, the original and duplicate genes may evolve different sequences and functions.
→ See a diagram for an illustration of this concept. See the video - Homolog vs paralog vs ortholog vs analog in 4 minutes for more information.

[Ortholog] genes conserved between humans and flies:

1) Go to FlyBase
2) Scroll down to "QuickSearch." Click "Homologs"
3) Uncheck all. Now check H. sapiens (human),
4) Copy and paste the fly gene (in the table below) into the input search. Next, under output, uncheck all and then only check H. sapiens (Human).
5) Click "Search."
6) Next, under Gene Reports click "NCBI" to learn more about this gene.
7) Click the back arrow to return to the previous page.
8) Notice that the fly gene is matched with the corresponding orthogonal gene. For this gene, click "+" to align the amino acids transcribed by this gene.
9) What percent similarity is there between the proteins coded by these orthogonal genes?
10) Compare the letters representing each amino acid between the fly and human proteins (see table at the bottom of this document for a key). Can you find where they differ?

Fly Gene	Human Ortholog	Function of Gene in Humans
cindr	CD2AP	Related to synapse formation among neurons and mutations with it are associated with susceptibility to glomerular disease (NCBI) and Alzheimer's disease. (SA Ojelade, 2019)
Sox102F	SOX11	The protein may function in the developing nervous

		system and play a role in tumorigenesis (NCBI)
14-3-3epsilon	YWHAH	Mediates signal transduction by binding to phosphoserine-containing proteins. *Mutations in this gene are associated with forms of early-onset schizophrenia and psychotic bipolar disorder.* (YWHAH at NCBI)

→ For more examples of orthologous disease genes in humans and Drosophila, vist the Genes conserved between human and fly at the Bloomington Drosophila Stock Center and open the tab “”Human orthologs of Drosophila genes based on FlyBase -compiled data.”

Right: List of amino acids which form proteins. Use the one letter code to compare the homology between humans and fruit fly proteins.

Amino Acid	Three Letter Code	One Letter Code
Alanine	Ala	A
Arginine	Arg	R
Aspartic Acid	Asp	D
Asparagine	Asn	N
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[Ortholog] genes conserved between humans and worms:

1) Go to the Alliance of Genome Resources
2) You should see C. elegans and Disease already selected. However, if not, in the search menu select “Disease” and type C. elegans.
3) Once C. elegans and Disease appears near the top of your screen, you will see a menu on your left. Look for the Disease Group menu and click “show more.”
4) Choose the disease area you wish to investigate (ex. “central nervous system disease.”)
5) Once you click this disease region, you will see a list of disorders associated with it. Click on a disease and you will see a description of a mutation associated with the

disease. Scroll down to the Associated Genes and you will find *C. elegans* has an ortholog gene associated with the human disease gene.

Worm Gene	Human Ortholog	Function of Gene in Humans
abt-2	ABCA7	Involved in several processes, including amyloid-beta formation and is implicated in Alzheimer's disease 9. (NCBI)
zmp-3	MMP12	May play a role in aneurysm formation and mutations in this gene are associated with lung function and chronic obstructive pulmonary disease (COPD). (NCBI)
gpx-3	GPX1	Associated with certain cancers, including breast cancer risk in premenopausal women. (NCBI)

Appendix 3 - Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans*

Nature's Pharmacy: Using Phytochemicals to Study the Microbiome & Innate Immunity of *C. elegans*

Background:
Scientists recognize that trillions of bacteria reside in our body and play a vital role in health. This "microbiome" influences many aspects of our wellbeing, including our immune, digestive, and nervous systems. In this series of labs, you will investigate how plant-derived chemicals or "phytochemicals" influence the innate immune system using a model organism that can illuminate human biology.

Introduction:
You are not alone. You are a walking, talking ecosystem comprised of trillions of bacteria and other microbes that outnumber your human cells. The majority of these inhabitants live in your large intestine where they play a role in metabolizing what you eat. Chemicals in our food influence the biodiversity of our microbiome and may lead to inflammation when we are poorly nourished. This occurs when immune cells respond to a bacterial population that is unbalanced. As a result, you may have acute symptoms like an upset stomach or headache. Worse, diabetes or autoimmune diseases may slowly arise from chronic inflammation caused by a low quality diet that leads to an impoverished microbiome.

Taking medicine to remedy our ailments is one solution. Interestingly, about 25% of pharmaceutical drugs are derived from plants. In this series of labs, you will collect plants from around your school or home to prepare a chemical extract. You will use a type of nematode worm called *Caenorhabditis elegans,* abbreviated as *C. elegans,* to examine how its simple microbiome changes when exposed to common milkweed *(Asclepias syriaca),* a plant used by Native Americans to treat dysentery and inflammation. In addition, you will discover how the innate immune system of the worms is activated or suppressed from the milkweed extract.

Goals:
- Extract plant compounds using methanol as a solvent. Plants with potential for CeMbio disruption: Black-eyed Susan (Rudbeckia hirta), Coneflower (Echinacea sp.), Joe Pye Weed (Eupatorium maculatum), Goldenrod (Solidago sp.), Common milkweed *(Asclepias syriaca).*
- Survival Assay: establish the effect of methanol and milkweed extract on *C. elegans* survival and reproduction.
- CeMbio Assay: Observe how methanol and milkweed extract influence the CeMbio.
- Infection assay: Measure potential anal swelling after exposure to milkweed extract and later *Microbacterium nematophilum* infection.
- Fluorescent reporter for immune activation in vivo assay: identify potential gene expression targets activated by milkweed stress.

Vocabulary:

- **Phytochemicals** - chemical compounds produced by plants used to resist fungi, bacteria viruses and insects.
- ***Caenorhabditis elegans*** - a 1 mm transparent nematode worm that lives in temperate soils.
- **Microbiome** - the collection of microbes, such as bacteria, fungi, and viruses, that live on the body of a multicellular host.
- **Innate Immunity** - the non-specific defense system that is present from birth and protects against a wide range of pathogens and foreign substances.
- **Dysbiosis** - an imbalance in the gut microbiome that can lead to inflammation and disease.
- **CeMbio** - Caenorhabditis elegans microbiome. 12 essential species of bacteria that comprise the minimal microbiome of C. elegans. These bacteria are biosafety level 1 (BSL1) and can be ordered from the Caenorhabditis Genetics Center (CGC).
- **NGM** - Normal (aka Nematode) Growth Medium. Standard culture media for raising worms.
- **Lysogeny broth (LB)** - nutrient liquid for culturing bacteria in a solution.
- **LB agar** - the solidified version of LB broth used for growing bacteria on Petri dishes.
- **TSA** - tryptic soy agar used for culturing a range of bacteria, including the CeMbio, *E.coli,* and other microorganisms.
- **OP50** - used as a bacterial food in the laboratory maintenance of *Caenorhabditis elegans* on agar plates. It is resistant to streptomycin, allowing it to grow as a uniform culture in an LB liquid medium once this antibiotic is added.
- **Streptomycin** - an antibiotic that kills or stops the growth of certain bacteria.

Key Concepts:

1. The role of the colon's mucosal membranes and microbial community influences homeostasis of the gut and helps regulate immunity.
2. Innate immunity and signal transduction pathways such as p38 MAPK are conserved between *C. elegans* and mammals.
3. The biomedical use of model organisms pertains to shared ancestry via evolution by common descent.

Research Questions:

1) How does common milkweed influence the survival of *C. elegans?*
2) How does common milkweed influence the microbiome of the CeMbio?
3) How does exposure to common milkweed influence pathogen infection leading to anal swelling?
4) What impact will common milkweed have on fluorescent reporters for immune activation in *C. elegans?*

Equipment (per class): Petri dishes, electronic balances, weighing dishes, 1 liter and 500 ml bottles, foam buckets, bunsen burners and/or alcohol lamps, 75% ethyl alcohol spray bottles,

sterile inoculation loops, an autoclave or pressure cooker, autoclave tape, oven mits or gloves, stereomicroscopes, a stereo microscope fluorescence adapter, refrigerator, micropippetors with tips, serological pipettes, glass pipettes, 2 liter erlenmeyer flasks, magnetic stir bar, a stirring hotplate, 500 ml graduated cylinder, and platinum wire.

Equipment (per group): 6 Petri dishes, 1 weighing dish, 1 bunsen burner or alcohol lamp, 1 75% spay bottle, 5 sterile inoculation loops, 1 stereomicroscope, platinum wire.

Routine Lab Bench Skills:

- Aseptic technique
- Pipetting with serological and micropippetes
- Prepation of percent solutions
- Preparation of serial diluations
- Use of a stereomicroscope to observe nematodes

Experimental Protocols:

1) PLANT HARVESTING - Obtain leaves, stems, branches, and/or fruit/seeds of a desired plant growing in its natural habitat. Common milkweed *(Asclepias syriaca)* leaves will be used in this lab series.
2) PREPARATION OF EXTRACT - Use 100% methanol to make a 20% solution of the collected plant.
3) MILKWEED CONTAMINATION SCREENING - Determine the presence of bacteria or fungi that may not have been killed by the 24 hours of methanol steeping. The milkweed (MW) solution must be microbe-free for the survival assay in the next step. Therefore, it is important to do this test before any other experimentation.
4) SURVIVAL ASSAY - Establish the effect of methanol and milkweed extract on *C. elegans* survival and reproduction.
5) CeMbio ASSAY - Observe how methanol and milkweed extract influence the 12 essential species of the *C. elegans'* microbiome (aka the “CeMbio”). These twelve bacteria include:
    - *Enterobacter hormaechei*
    - *Lelliottia amnigena*
    - *Acinetobacter guillouiae*
    - *Sphingomonas molluscorum*
    - *Stenotrophomonas indicatrix*
    - *Pseudomonas lurida*
    - *Pseudomonas berkeleyensis*
    - *Comamonas piscis*
    - *Pantoea nemavictus*
    - *Ochrobactrum vermis*
    - *Sphingobacterium multivorum*
    - *Chryseobacterium scophthalmum*

6) INFECTION ASSAY - Measure potential anal swelling after infection to *Microbacterium nematophilum* when exposed to milkweed extract (and as a control, no milkweed extract).
7) FLUORESCENT REPORTER FOR IMMUNE ACTIVATION OR SUPPRESSION IN VIVO ASSAY - Identify potential gene expression targets activated or suppressed by milkweed exposure.

Data:
Use a physical or digital notebook to record the mortality of worms or the microbiome of C. elegans bacteria exposed to phytochemicals. Later observe the presence or absence of fluorescent signals in transgenic reporter worms indicating innate immunity activation or suppression after exposure to phytochemicals.

Results:
Count 30 or more worms per treatment (example: exposure or no exposure to phytochemicals) to create a scatterplot. Ideally, add standard deviation with error bars to conclude 95% confidence.

Conclusion:
Did the phytochemicals from the milkweed extract have an influence on the survival of worms and/or their microbiome? Did you detect fluorescent signals in transgenic reporter worms? If so what does this indicate about innate immunity activation or suppression?

Further Investigations:
Research phytochemicals from other plants in your region. Prepare extracts and repeat a similar experiment to the one used with milkweed.

Appendix 4 - [Human Microbiome Mini Project]

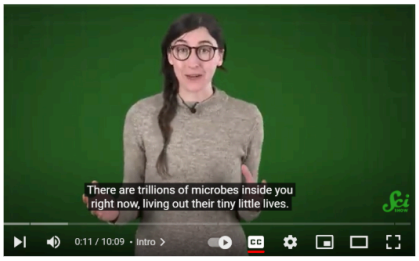
Slide #1:

Human Microbiome Mini Project

Investigating the influence of the microbial diversity on our brain & immune health

Slide #2:

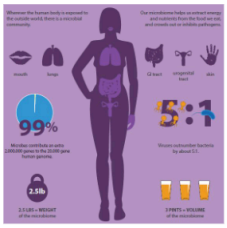
What is the Microbiome?



Video - The Truth About the Gut to Brain Connection (10:09)

Slide #3:

Meet Your Microbiome



Images sources:
Left: American Society for Microbiology (ASM) - https://x.com/ASMicrobiology/status/1011957534226173955. Right: Bob Englehart - https://www.cartoonstock.com/directory/m/microbe-human_interaction.asp

Slide #4:

Your Microbiome & Health

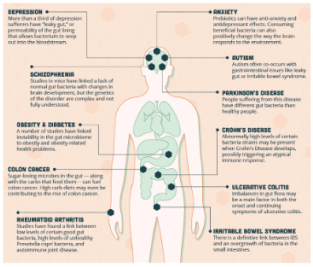
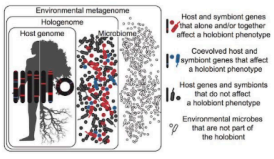


Image source Huffington Post - https://big.assets.huffingtonpost.com/2015_GutBacteriaA.png

Slide #5:

Our Extended Genome - the Hologenome



Images sources Learn Genetics: Left: Our Microbes Are Under Threat, and the Enemy is Us, USAToday http://usatoday30.usatoday.com/news/health/story/2012-07-13/body-bugs-microbes/56255904/1?csp=usattumblr . Right: Seth Bordenstein@Symbionticism - https://twitter.com/Symbionticism/status/694898586438467585

Slide #6:

AP Biology Microbiome Project

STEP 1 - Explore the Microbiome & Learn About an Assigned Area of the Body (Mouth, Respiratory Tract, Stomach, Intestines, Urogenital Tract, Skin)

Images source Learn Genetics: Learn.Genetics. https://learn.genetics.utah.edu/content/microbiome/friends/

Slide #7:

AP Biology Microbiome Project

STEP 2 - Find and Deconstruct a Research Paper about a Microbe found in Your Assigned Region of the Microbiome

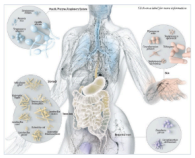


Image source: Scientific American, 2012. https://www.scientificamerican.com/article/microbiome-graphic-explore-human-microbiome/

Slide #8:

AP Biology Microbiome Project

STEP 3 - Display Research on a Trifold Poster and Prepare an 8-10 Minute Presentation on Your Topic

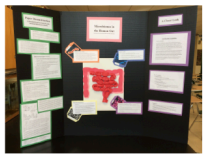


Image source: Author, Brian Dempsey, 2018.

Appendix 5 - Modeling Signal-Transduction Pathways with Playdough

Modeling Signal-Transduction Pathways with Playdough

Background: Understanding mechanisms for how cells respond to chemical signals is critical for treating disease. Historically, humans used medicinal plants to treat ailments, but their chemical action was a mystery. In-vivo systems have elucidated some cause-and-effect actions. Experiments using live organisms, including *C. elegans* worms, provide clues to this chemical signaling. From such research, scientists build models to understand biochemical and cellular relationships. The hope is that models will help us understand signal transduction pathways and allow researchers to develop drugs to treat inflammation caused by diseases.

Materials:

- Playdough (ex. Play-Doh® Mini Classics Set)
- Mini whiteboards, dry-erase markers, erasers, OR chalk markers, and a wipe cloth
- iMotion or Stop Motion Studio
- List of Phytochemicals and Their Effect on Signal Transduction Pathways
- Description of how pathogens induce the p-38 pathway
- Signal-transduction pathways labels
- A biology textbook as reference (optional)

Questions:

1) How do pathogens activate innate immunity such as the p-38 pathway?
2) How might phytochemicals modulate signaling pathways and reduce inflammation from pathogens?

Challenge: Build a stop-motion animation using playdough to model how signal-transduction pathways are activated or suppressed.

Procedure:

1) Use several colors of playdough at your station.
2) Look at the description of how pathogens induce the p-38 pathway (a conserved signaling pathway found in both *C. elegans* worms and humans).
3) Use the signal-transduction pathway labels and playdough to model how a pathogen causes inflammation, possibly leading to cell death.
4) Use the dry-erase markers or chalk markers to describe events that may occur as a pathogen infects a cell. See the list of potential vocabulary terms below.

   Vocabulary (include 3-4 terms in your animation):
   - Ligand (signal molecule)
   - Receptor protein
   - Conformational change
   - Second messenger

- Phosphorylation (includes phosphorylation cascades)
- Transcription factor
- Signal amplification
- Nuclear response
- Apoptosis

5) Finally, reference the list of phytochemicals and their effect on signal transduction pathways. At the end of your video animation, write a sentence that explains how inflammation might be suppressed with a particular phytochemical.
6) Save your video and share it with your teacher.