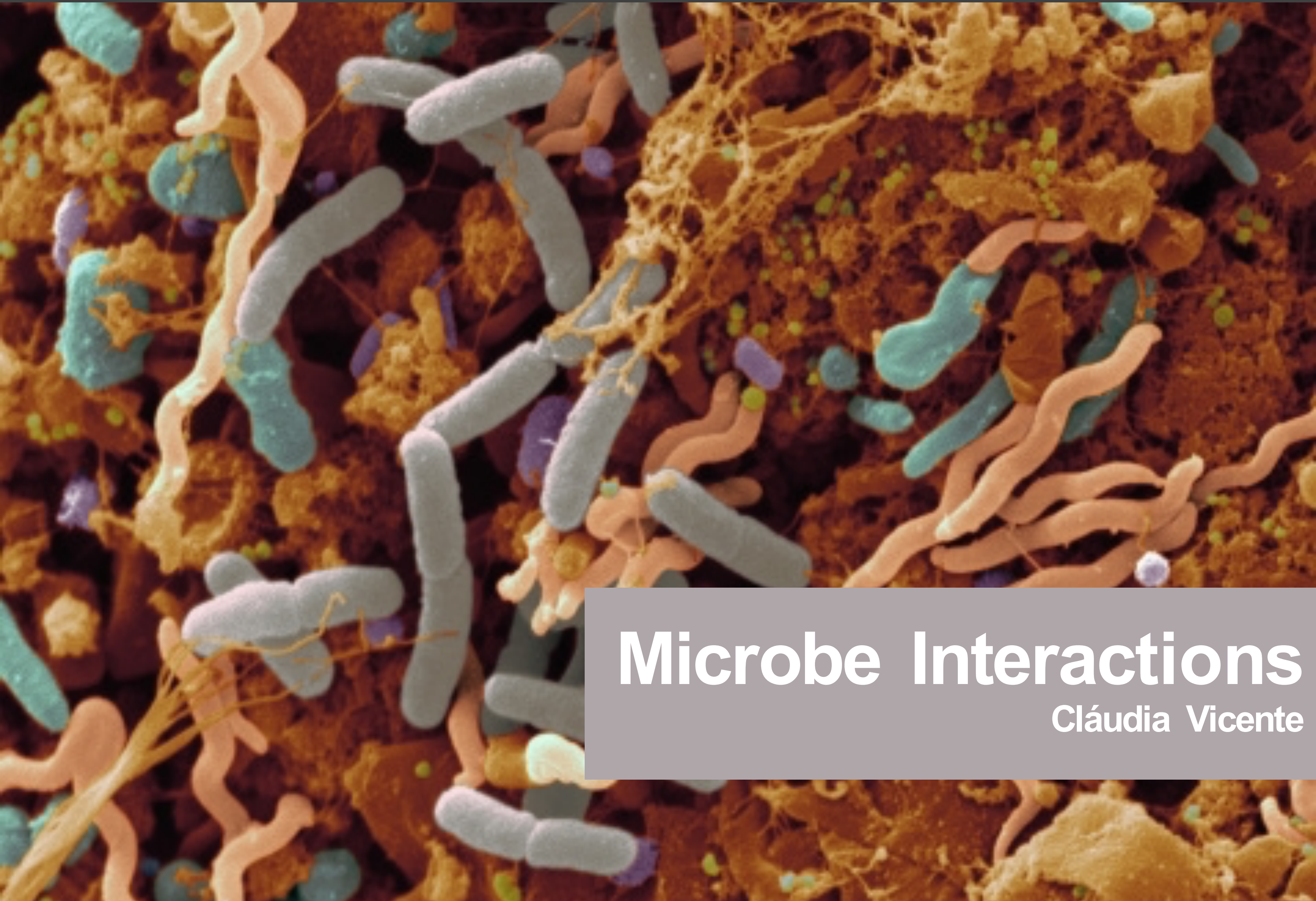


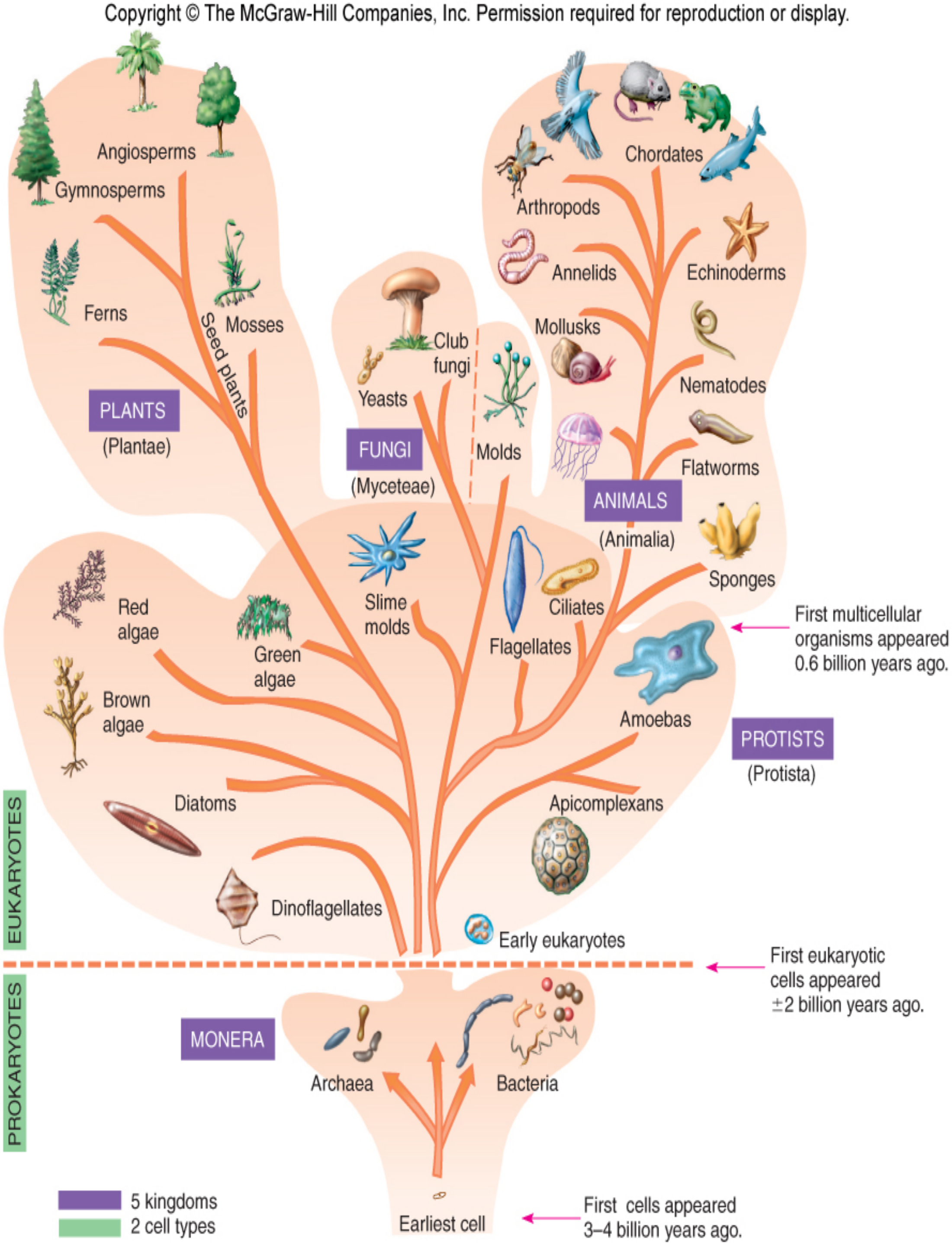
Microbe Interactions

Cláudia Vicente

JSPS Science Dialogue 2016, Kariya High School, July 6th 2016

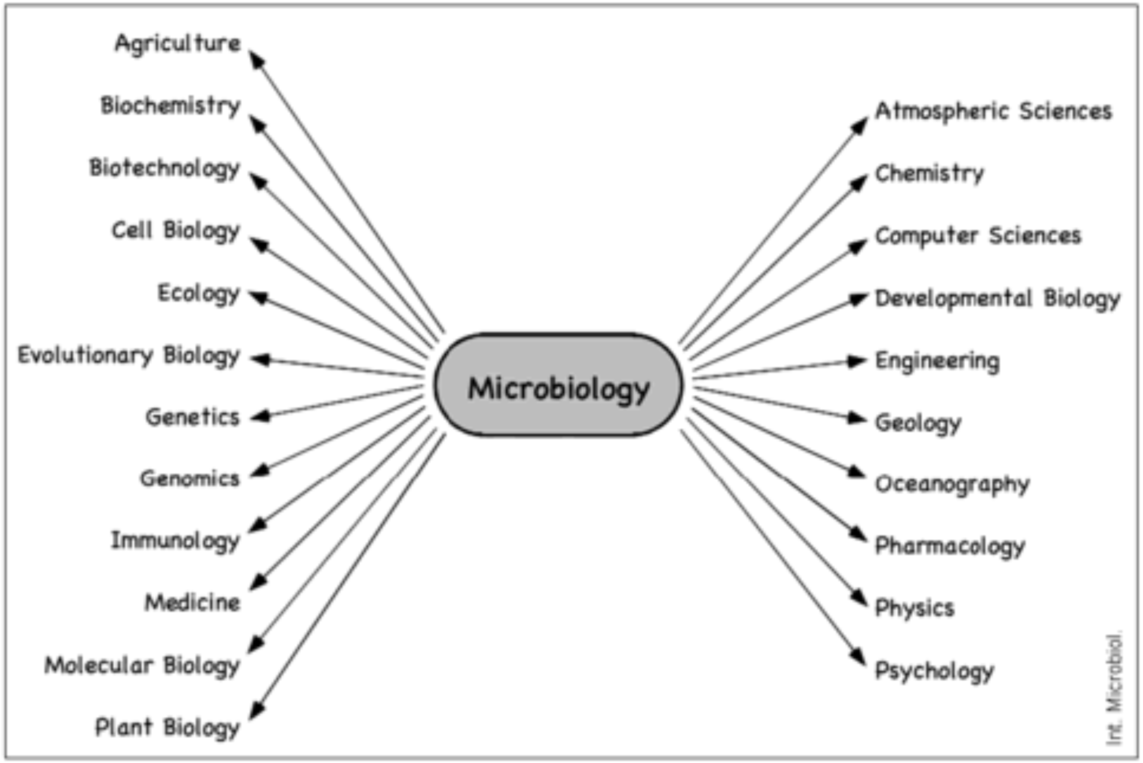
Topics of this lecture

1. Self-presentation
   - 1.1 My country
   - 1.2 Why I became a scientist?
   - 1.3 Portugal and Japan relations
2. Microbes (general introduction)
3. Microbe interactions
   - 3.1 Pine wilt disease (background)
     - Experiment 1 – Can bacteria help the nematode to survive?
     - Experiment 2 – Observation of *Bursaphelenchus xylophilus*
   - 3.2 Cockroach microbiome



Microbes

- Microscopic organisms, single-cell or multicellular, which inhabit all kinds of environments;
- Bacteria, Archaea, Fungi, Protists, Viruses and microscopic animals;
- The oldest form of life on earth (3.5 billion years ago);
- Different shapes and ecological interactions;
- Play important rules on earth and in our lives;



- The science that studies microbes or microorganisms is called **Microbiology**;

- **Microbiology** can interact with many different subjects – today you will learn about microbe interactions, mainly nematode-bacteria and insect-bacteria associations.

Microbiology mentors

Antonie Van Leeuwenhoek
(1632-1723)

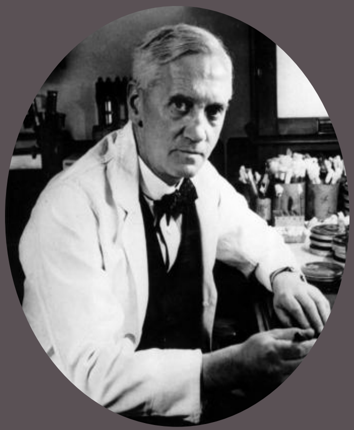
- Observed for the first time bacteria
- Developed the first microscope (single-lens)

Louis Pasteur
(1822-1895)

- Pasteurization
- Disprove the theory of spontaneous generation
- Invented several vaccines
- Considered the father of Microbiology

Robert Koch
(1843-1910)

- Developed the germ theory
- First to obtain a culture pure
- Considered the father of Medical Microbiology



Alexander Fleming
(1881-1955)

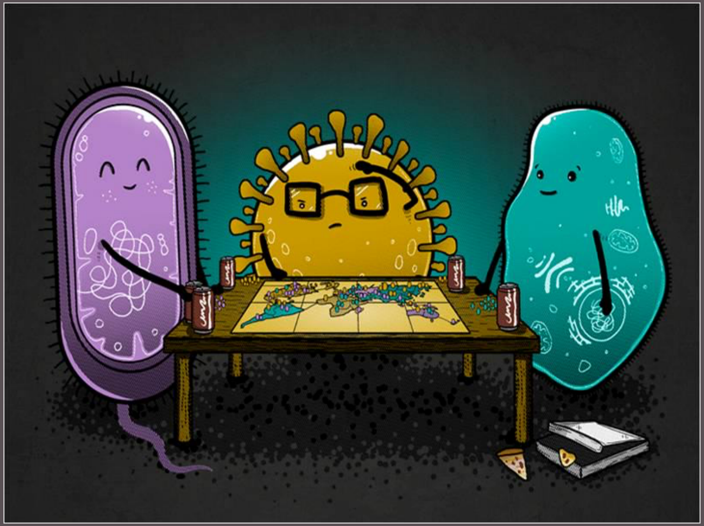
- Discovered of penicilin and the enzyme lyzozyme (present in tears, salive for example).
- Received Nobel Prize in Physiology and or Medicine in 1945.

Microbiology in our daily life

Some of the bacteria that grew when an 8-year-old boy who had been playing outside pressed his hand onto a large Petri dish.

Microbes interactions

1. Nematode – Bacteria interactions in Pine Wilt Disease
   a. Terms that you will learn:
      1. Disease – Host – Pathogen
      2. Nematode
      3. Associated Bacteria



2. Insect – Bacteria interactions
   a. Terms that you will learn:
      1. Microbiome
      2. Parasitism versus Mutualism
      3. Endosymbiont

Pine Wilt Disease

INTERACTION

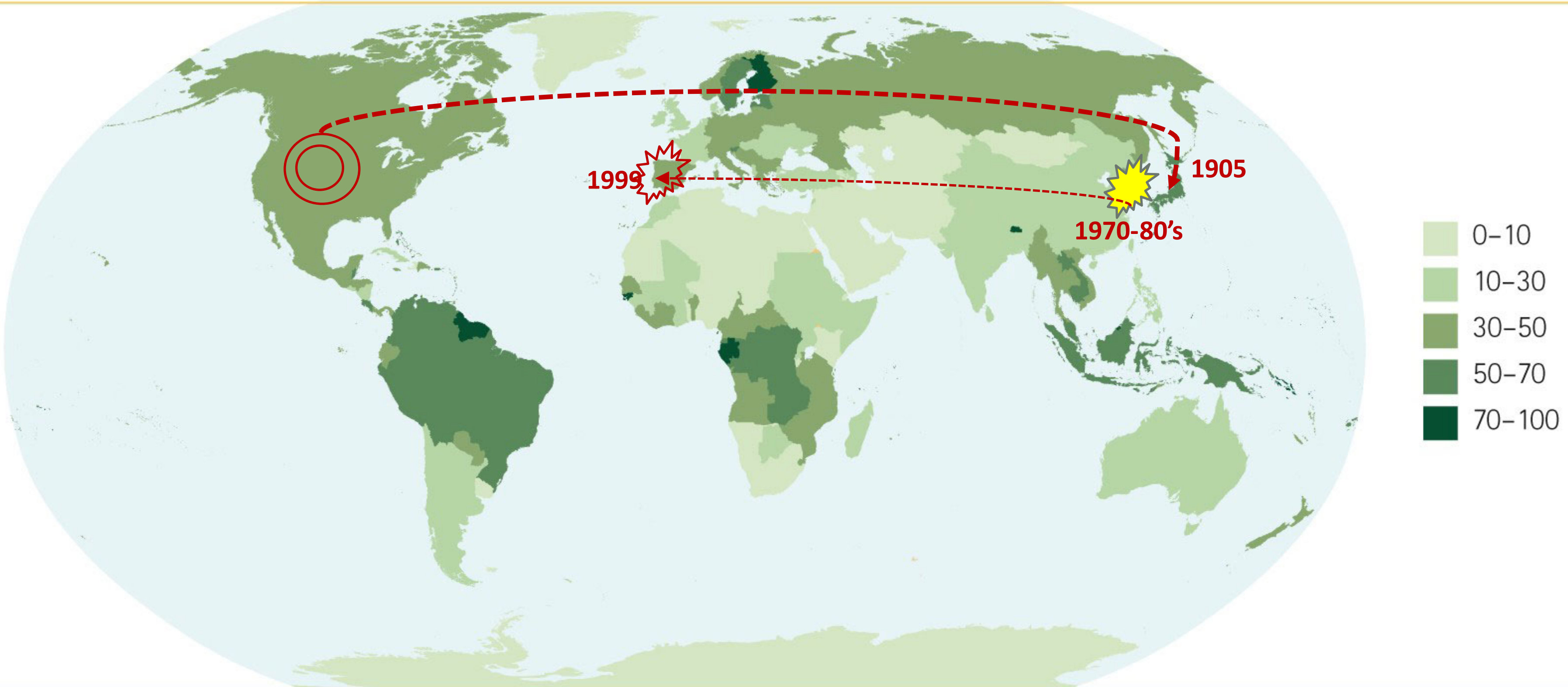
…and because bacteria are ubiquitous to all environments.

Pine Wilt Disease is considered the most devastating disease for pine forests worldwide.

PWD is a complex disease which results from the interaction between the plant parasitic nematode *Bursaphelenchus xylophilus* (PWN, pine wood nematode), the insect-vector *Monochamus* sp. and the host tree, *Pinus* sp.

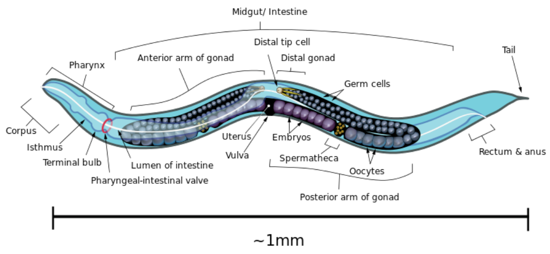
Bacterial communities associated with PWN are suggested to play a role in PWD development.

Forest area as percent of total land area by country, 2010



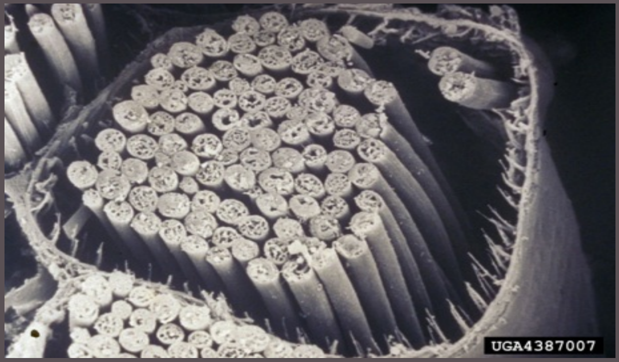
What is a nematode?

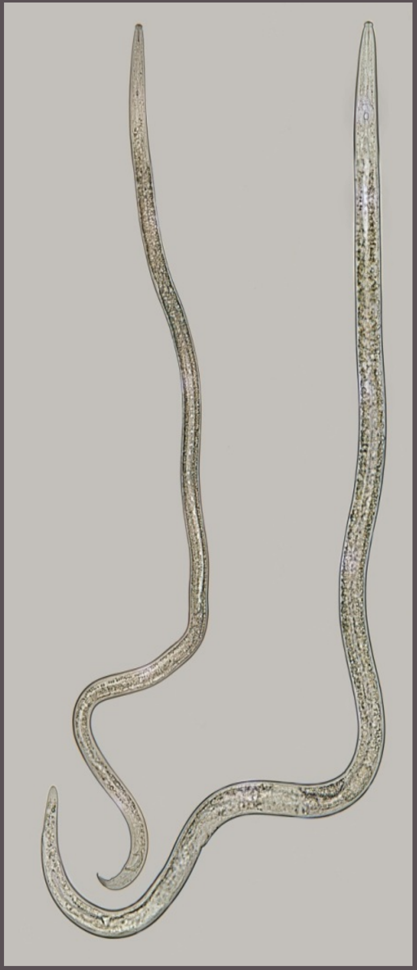
Any unsegmented worm of the phylum Nematoda, having an elongated, cylindrical body.



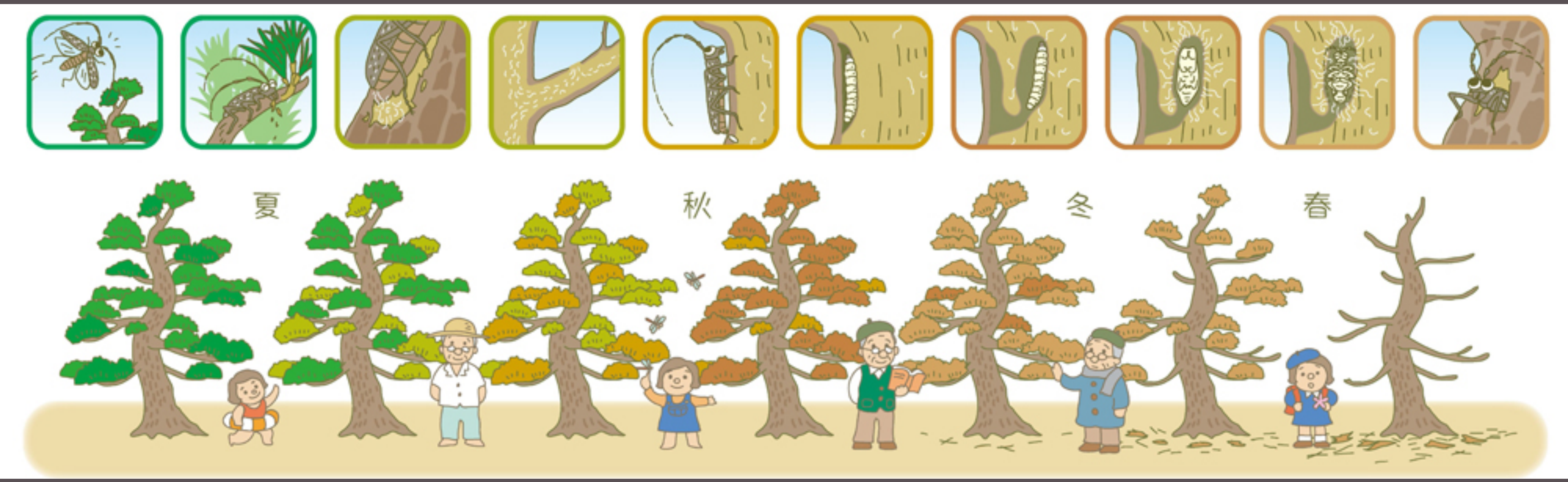
Bursaphelenchus xylophilus

- Also known by pinewood nematode (PWN);
- Plant parasitic nematode that feeds on plant cells (tree) and fungi;
- Pathogenic agent of Pine Wilt Disease;
- Phoretic relation with insect ***Monochamus*** **sp**.;





Pine Wilt Disease cycle



Protection strategy from host

Secondary metabolites
Cavitation
Xylem discoloration

↓ is not effective to pathogens

Dispersal of pathogen

↓ population of nematodes increase

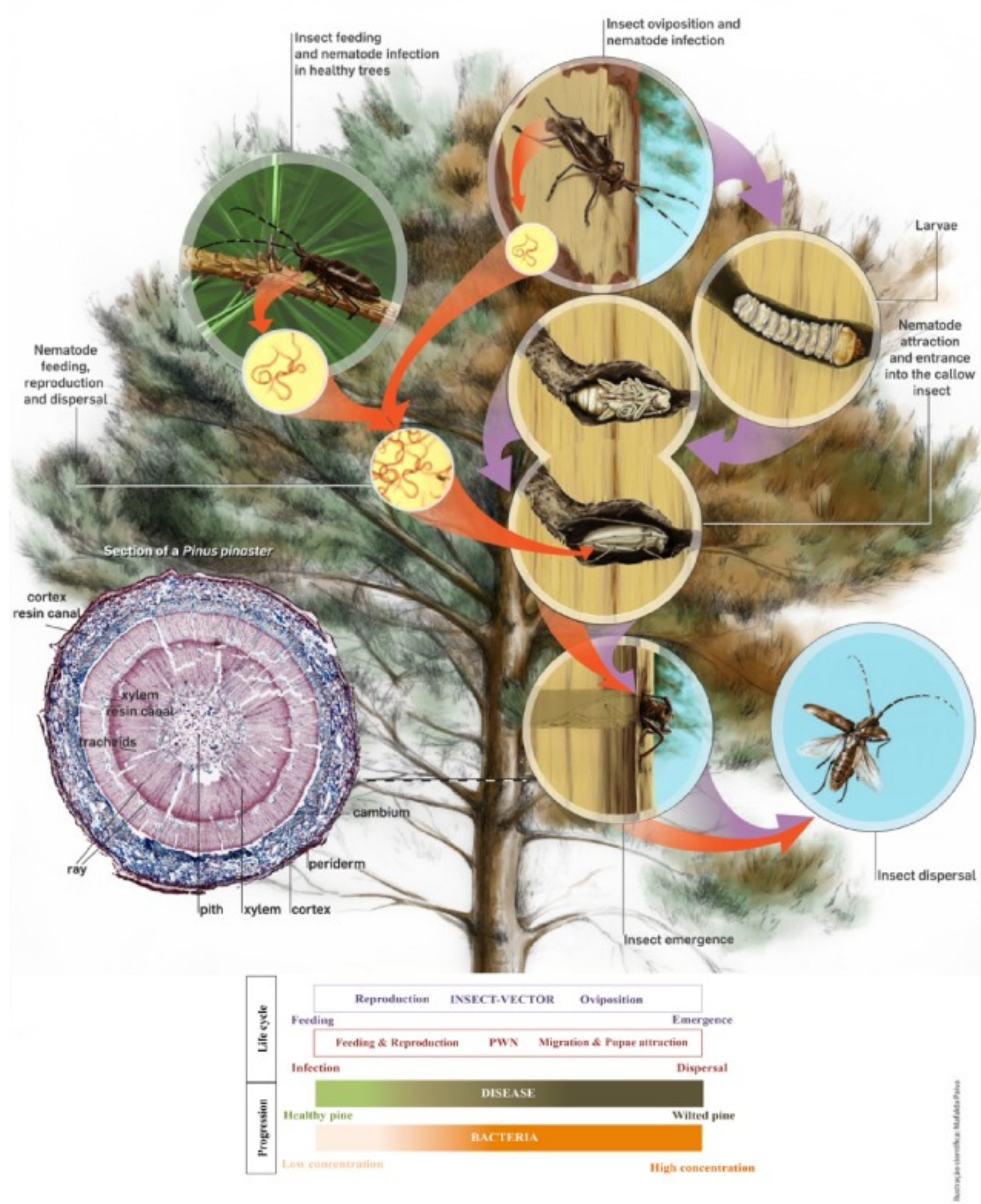
Xylem dysfunction

↓

Blockage of sap ascent

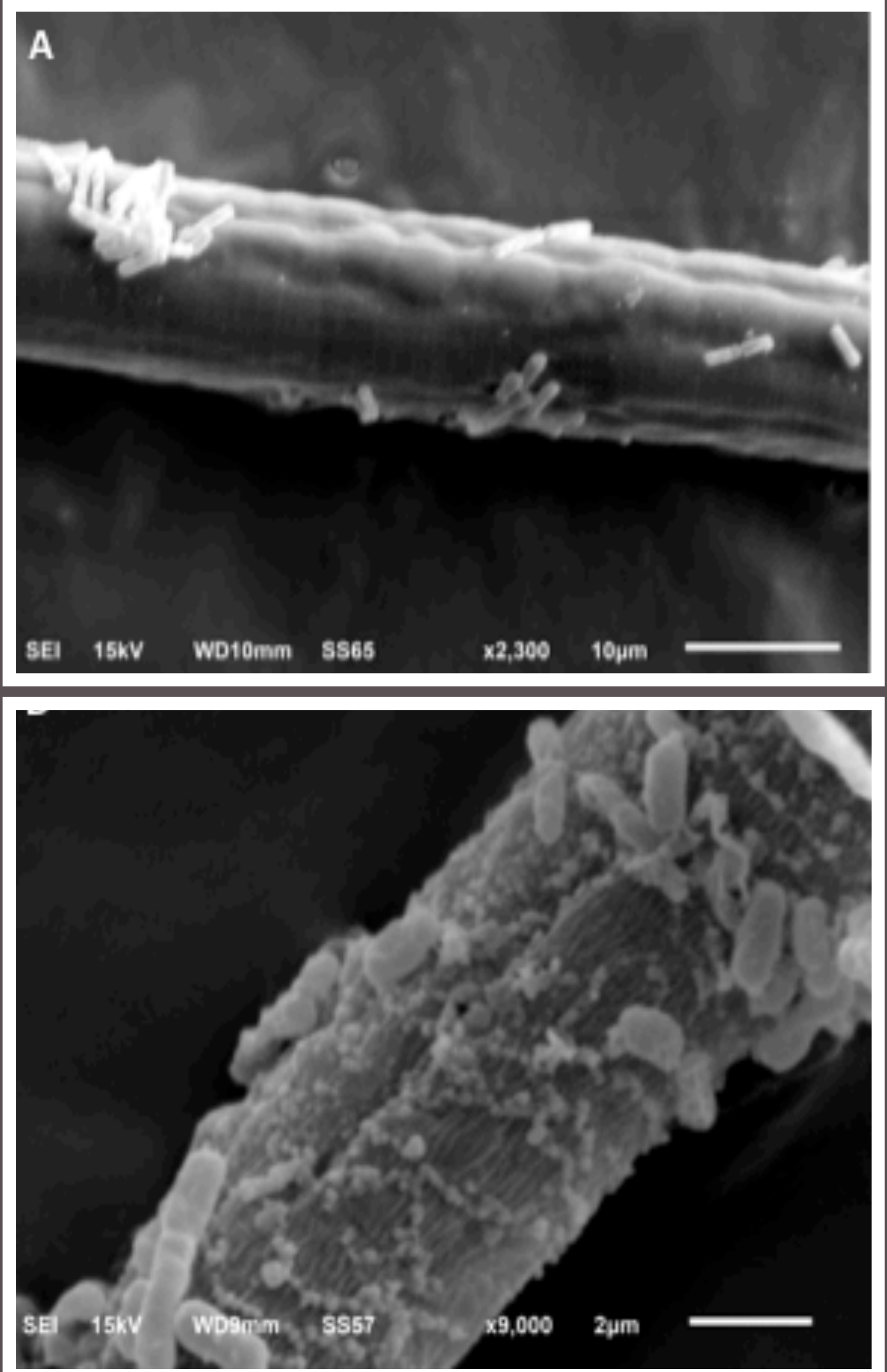
↓

Wilt



Bacterial communities associated with PWN are suggested to play a role in PWD development.

- We believe that these bacteria are carried (in their cuticle) by the nematode from the plant host;
- Several species of bacteria were found associated to the nematode;
- Alone, these bacteria cannot cause disease but in association, they can accelarate the disease development;
- Some can help the nematode to survive in the pine tree – Experiment 1

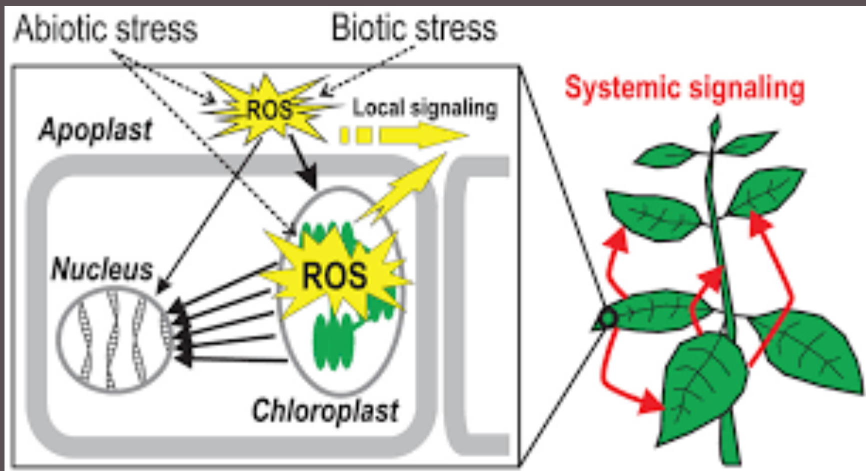


Experiment 1

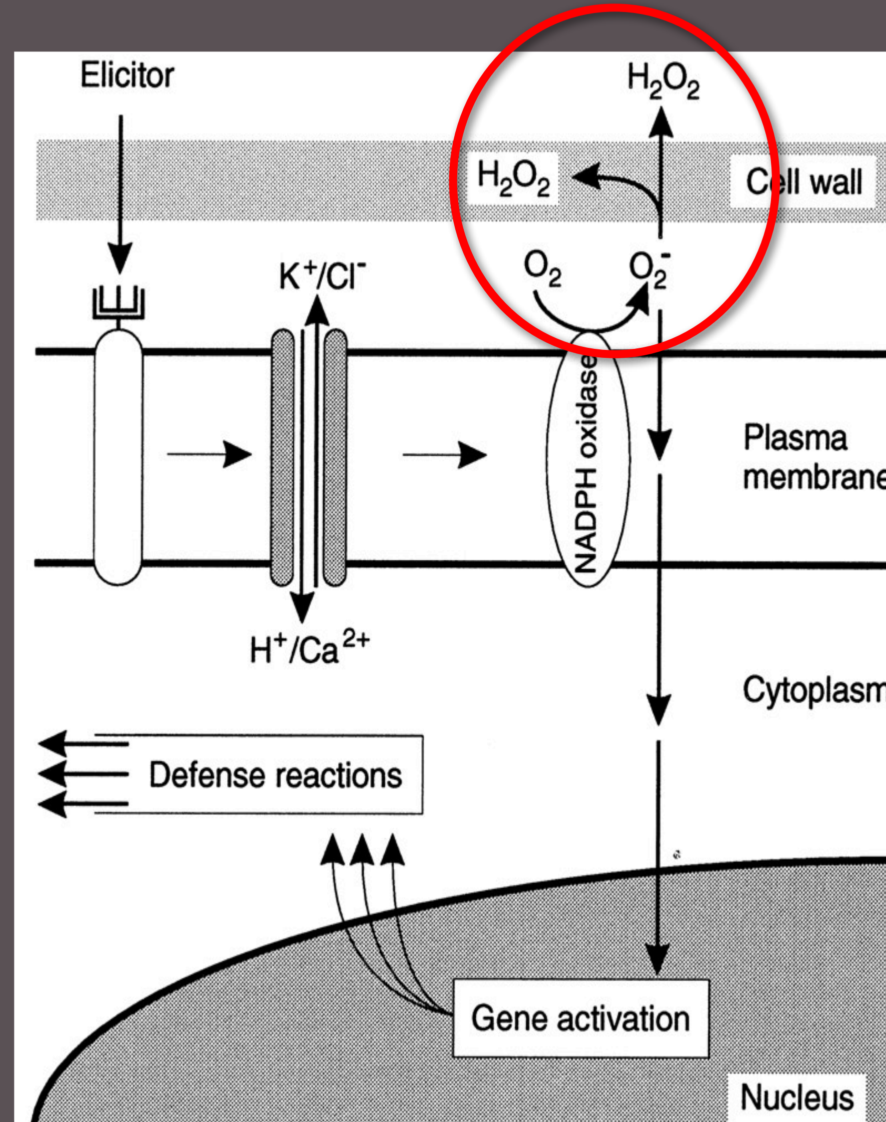
SCIENTIFIC HYPOTHESIS – CAN BACTERIA HELP NEMATODE?

Background
Hypothesis
Experiment
Conclusions

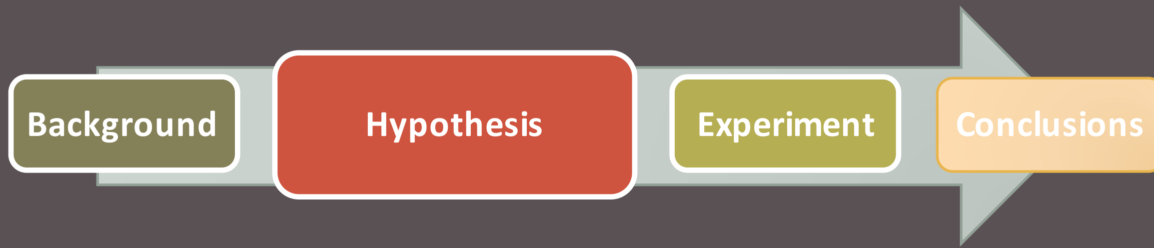
Oxidative stress: an early plant response to pathogen infection



Because it is relatively stable and permeable to the cell membrane, **H_2O_2 is the most predominant ROS in plant oxidative burst.**



Jabs et al., 1997

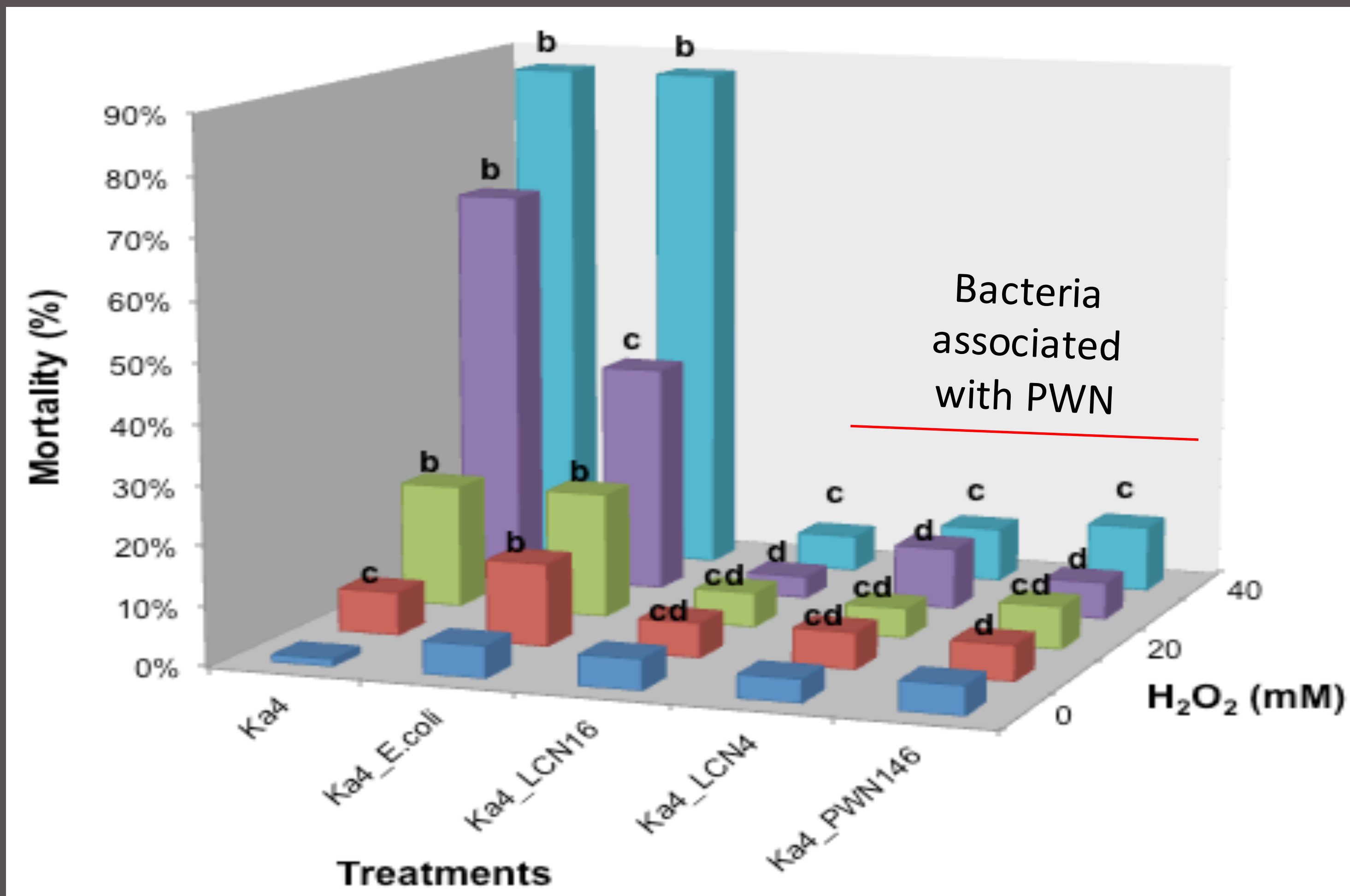


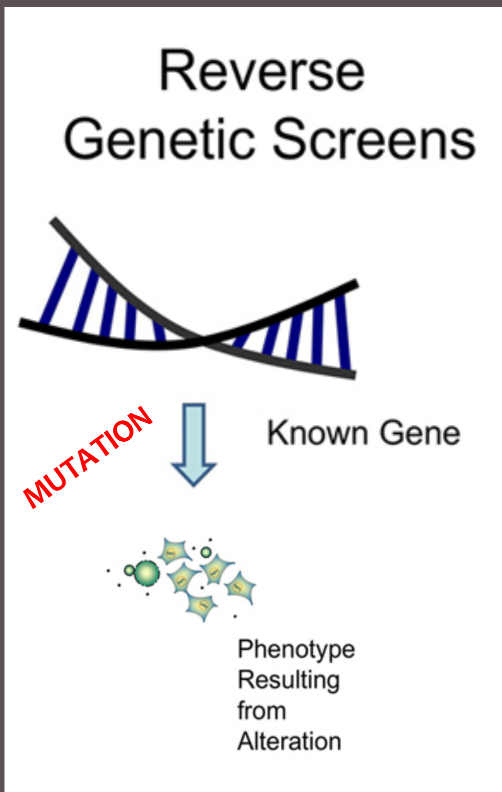
Bacteria are resistant to H_2O_2 stress and can help the nematode to overcome the oxidative stress

1. Testing bacteria and nematode resistance to H_2O_2
2. Using reverse genetics, create H_2O_2-sensitive bacteria to prove that bacteria help nematode in the presence of H_2O_2
3. Testing H_2O_2-sensitive bacteria in association with nematode

Background | Hypothesis | Experiment | Conclusions

1. Testing bacteria and nematode resistance to H_2O_2





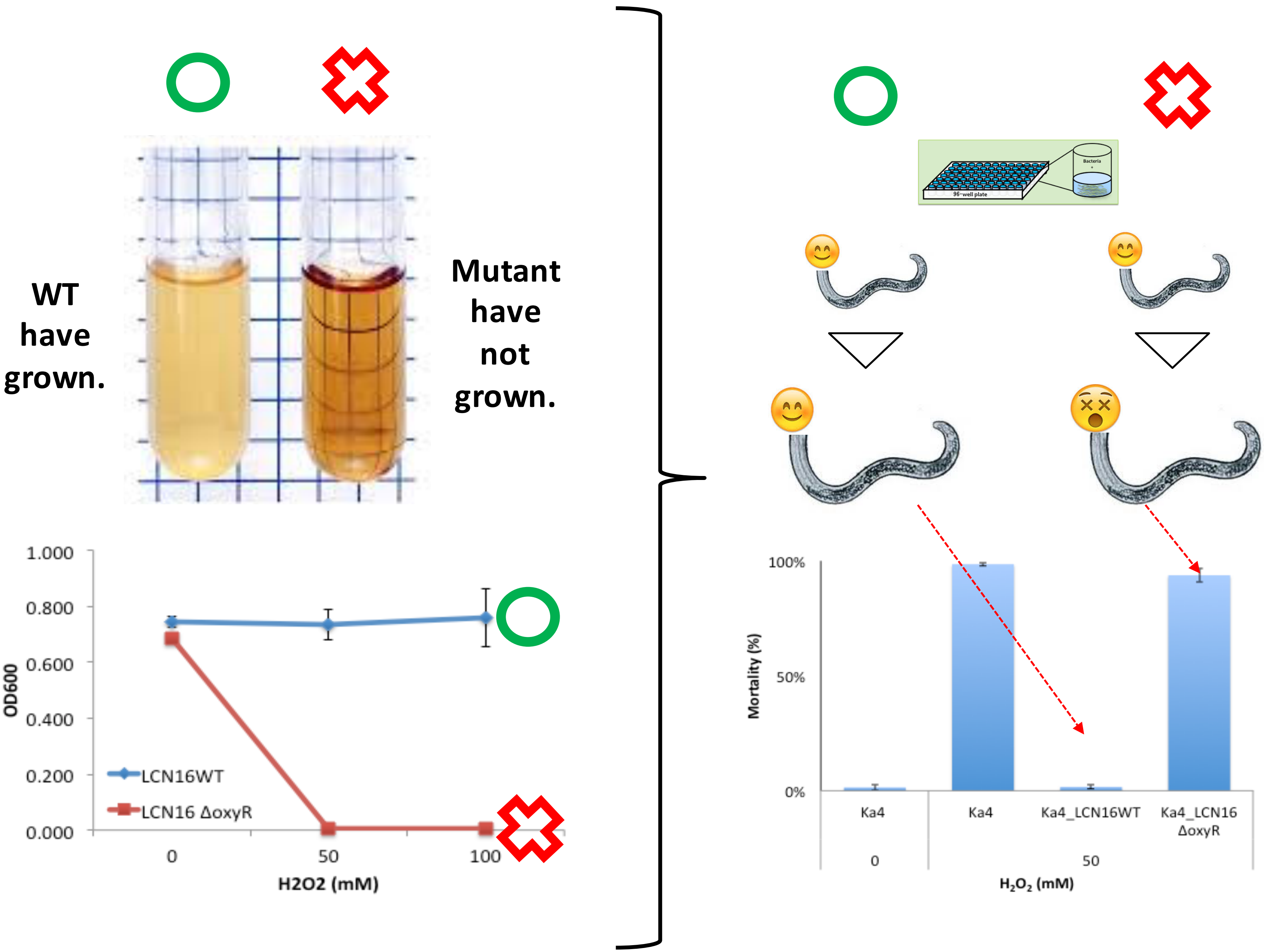
2. Using reverse genetics, create H_2O_2-sensitive bacteria to prove that bacteria help nematode in the presence of H_2O_2

Phenotype

Phenotype	
○ RESISTANT TO H_2O_2	*Serratia proteamaculans* LCN16 WT (wild-type)
✖ NOT RESISTANT TO H_2O_2	*S. proteamaculans* LCN16 ΔoxyR (mutant)

MUTATION

What is a mutation ? Alteration of the nucleotide sequence of the genome of an organism.


WT have grown.
Mutant have not grown.
1.000
0.800
0.600
0.400
0.200
0.000
OD600
0
50
100
H2O2 (mM)
LCN16WT
LCN16 ΔoxyR
100%
50%
0%
Mortality (%)
Ka4
Ka4
Ka4_LCN16WT
Ka4_LCN16 ΔoxyR
0
50
H_2O_2 (mM)

Experiment 2

LET'S LOOK AT *BURSAPHELENCHUS XYLOPHILUS*


© Peter Mullin/2001
Bursaphelenchus xylophilus
male 400X
Pine
East Campus, UNL
20 µm

Microbes interactions

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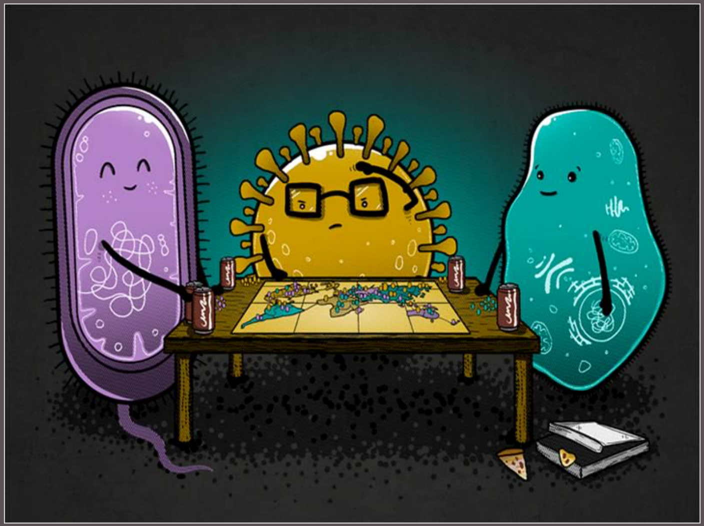


2. Insect – Bacteria interactions
    a. Terms that you will learn:
        1. Microbiome
        2. Parasitism versus Mutualism
        3. Endosymbiont

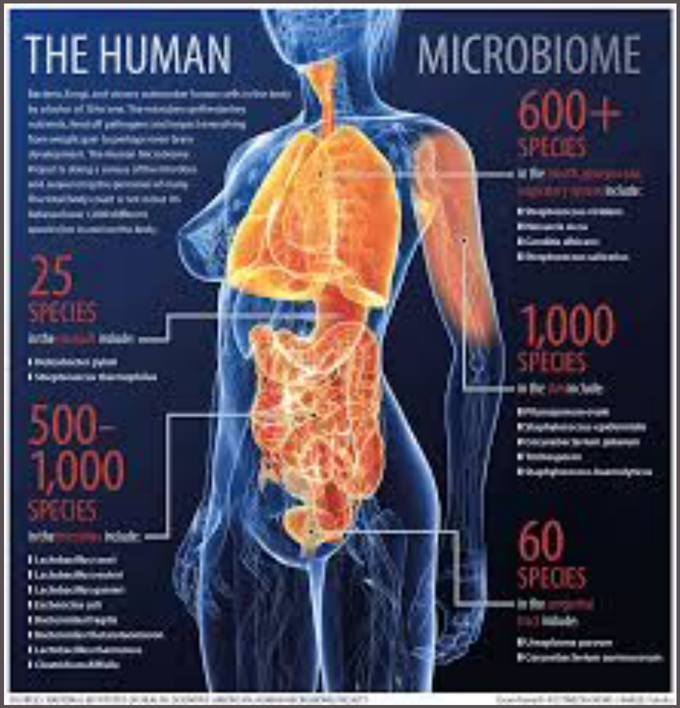
What is the Microbiome?

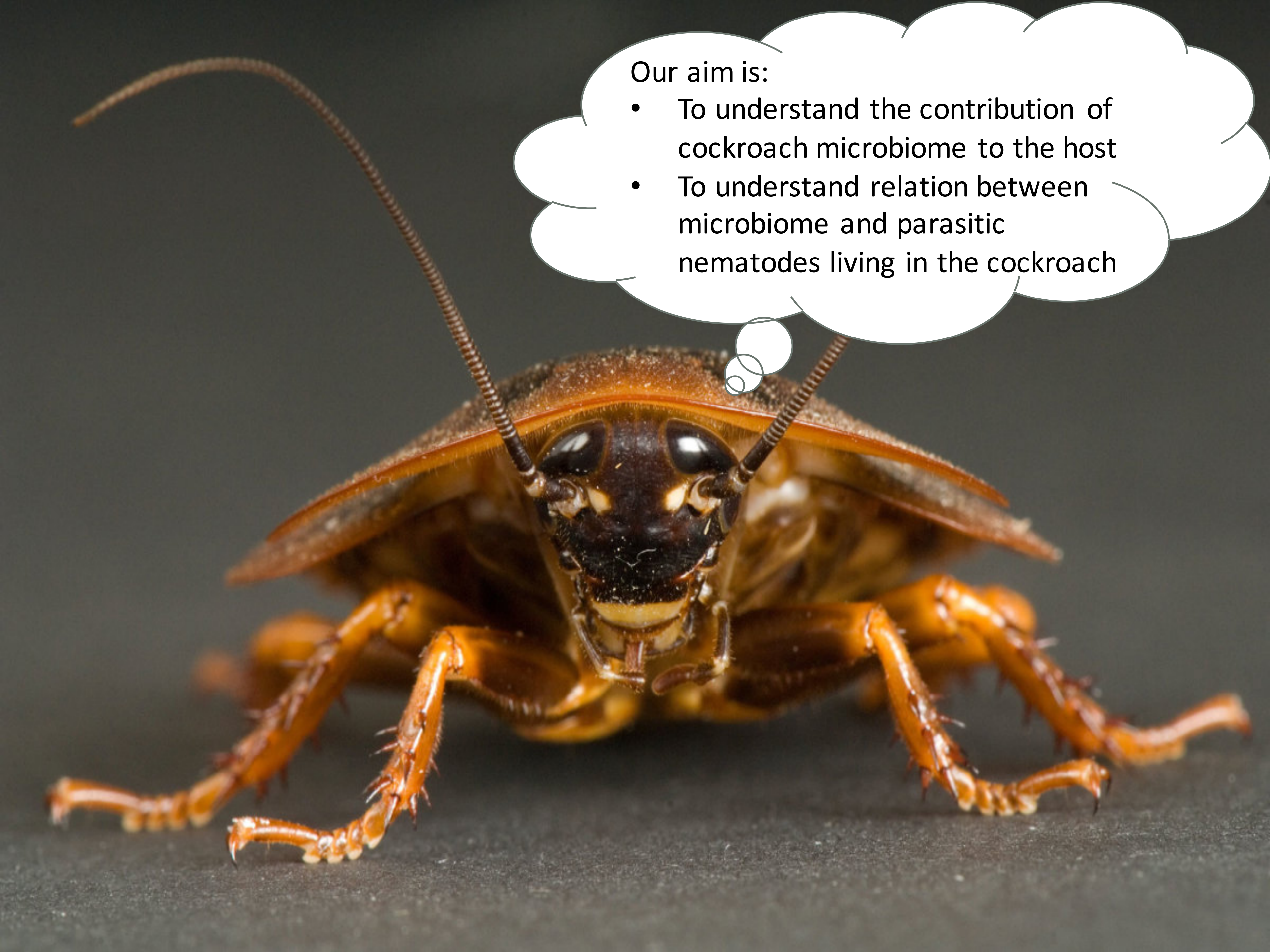
Community of microorganisms (such as bacteria, fungi and viruses) that inhabit a particular environment.

Vital for the well-being of the ecosystem or organism.







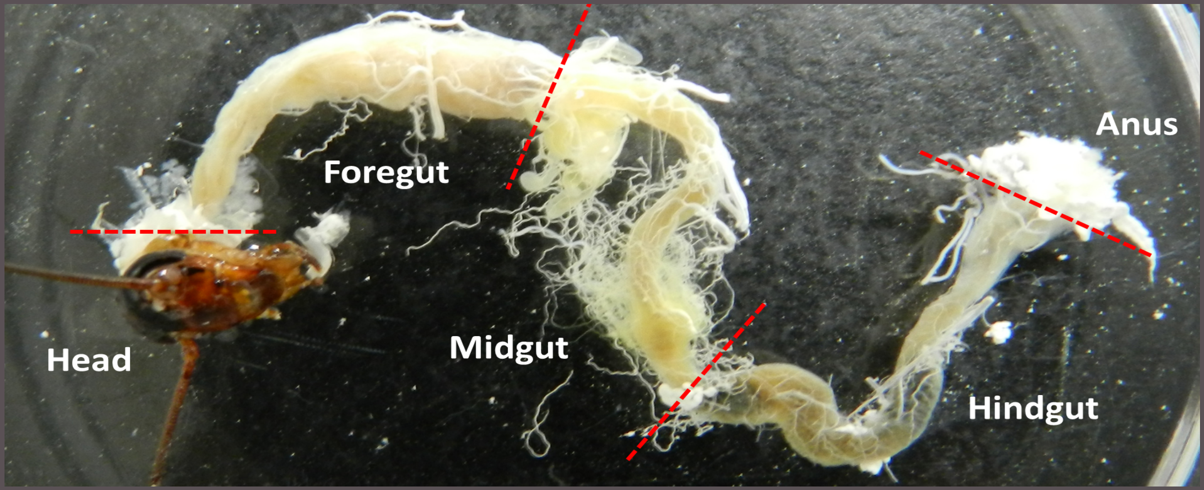

Our aim is:
• To understand the contribution of cockroach microbiome to the host
• To understand relation between microbiome and parasitic nematodes living in the cockroach

Insect – bacteria interactions

Resident bacteria attached to the gut walls.

How they get the bacteria ?

- Environment
- Food
- Social interactions



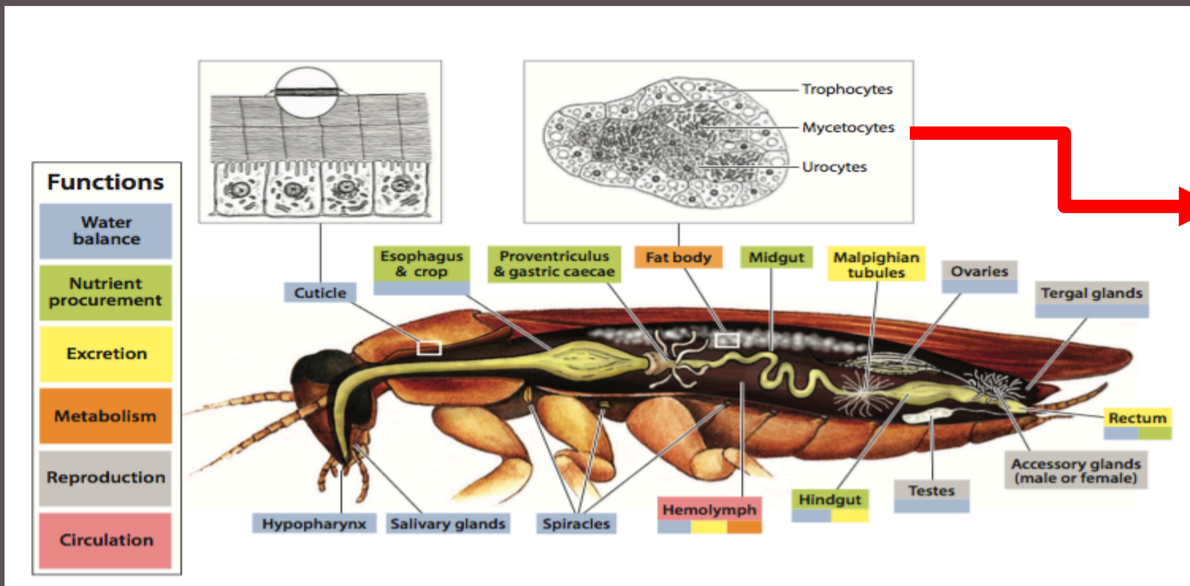
How they transfer bacteria?

- Eggs
- Coprophagy
- Social interactions

Insect microbiome is beneficial to the host because helps in:

- **Dietary supplementation**
- **Tolerance to environmental perturbations**
- **Maintenance and/or enhancement of host immune system homeostasis**

Endosymbiont: *Blattabacterium*



- Endosymbiont = endo (inside) + symbiont (organisms that establishes a close relation with other organism);
- *Blattabacterium* lives inside mycetocytes in the fatbody;
- Helps the host to "clean-up" uric acid (toxic waste) and provides amino acids essential for host survival.

Nematode - bacteria interactions in cockroaches

- We know that cockroaches harbor nematodes in the hindgut.
- These nematodes fed on the end-products of host digestion and, probably the resident/transient bacteria flora.
- The playing-role of these nematodes is not clear.

Types of symbiosis

?

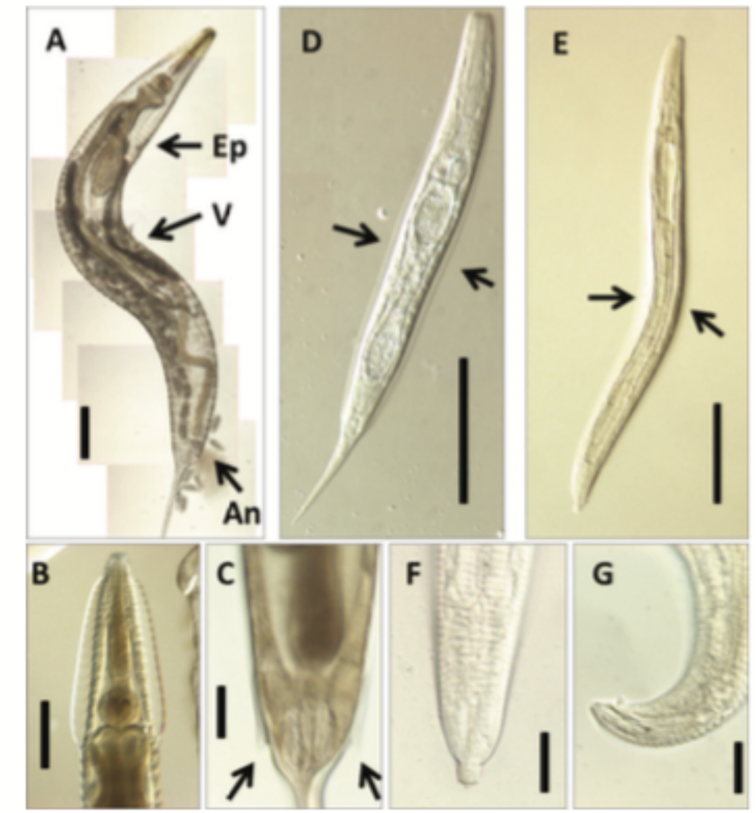


Fig. 3. Nomarski DIC image of *L. appendiculata*. (A) Adult female, right lateral view. Cylindrical and tapered shape toward both anterior-posterior ends. The corpus consists with anterior cylindrical part and posterior bulb. Alimentary system is divided to diverticulum and gut tube. Vulva is located at center but a little closer to the cephalic region. Ep, Excretory pore; V, vulva; An, anus. (B) Adult female head with prominent lateral allae, ventral view. Cephalic alae are prominent. (C) Adult female tail with lateral allae (arrows), ventral view. (D) Juvenile with lateral allae (arrows), ventral view. Lateral alae expand from cephalic to caudal region. (E) Adult male with lateral allae (arrows), ventral view. (F) Male tail, ventral view. Caudal tip has smaller tail with short terminal spine. Three pairs of caudal papillae present. (G) Male tail, left lateral view. Scale bars, (A), 200 μm; (B), (E), 100 μm; (C), (D), 50 μm; (F), (G), 20 μm

Thank you for listening!
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中部大学 Hasegawa Lab, Department of Environmental Biology, Chubu University