Laboratory #2: Bacterial metabolism of chemical signaling molecules

Goals of the laboratory:
- Explore the microbial world
- Learn laboratory methods for microbiology
- Identify and culture bacteria from the environment that have interesting metabolisms

Introduction:

Microbial Diversity
Bacteria are single celled prokaryotes that compose one of the three domains of life (with Eukarya and Archaea being the other domains). Bacteria are believed to be the first life forms on earth 3.5 billion years ago, and they have evolved a tremendous diversity of metabolic capabilities. Today, bacteria carry out a myriad of chemical transformations and are responsible for all of the nutrient recycling on the planet. Bacteria are truly the world’s best biochemists! There is tremendous diversity, both metabolic and morphological, in the bacterial world. It is estimated that there are $10^6$ species of bacteria in a gram of soil (Gans, 2005). Bacteria are the tiny majority on earth and thrive in many diverse niches from hot springs to deep sea vents, to the human gut. However, much of bacterial metabolic and physiological diversity is still unknown. We have relatively few bacteria represented in culture in the laboratory and available for study as compared to estimates of bacterial diversity based on culture independent molecular techniques.

What do bacteria need in order to grow?
There are several basic elements that bacteria need in order to grow. Just like other life on earth, they need a source of carbon, nitrogen, sulfur, phosphorous, trace elements, and vitamins for growth. Bacterial growth is also influenced by pH, temperature, the presence or absence of oxygen, and other abiotic factors in an environment. Bacteria can be enigmatic to culture and grow in the laboratory because we often do not know enough about an environment, and the different interactions that bacteria have within an environment, to replicate proper growing conditions in the laboratory. With the tremendous microbial diversity present in the environment, however, it is likely that there will be a microbe that thrives in any combination of conditions in the laboratory, provided that essential elements for growth are present.

Acyl homoserine lactone signaling molecules: the language of bacteria
Bacteria are not solitary beings, but some can communicate with one another using small chemical signaling molecules called acyl homoserine lactones (AHLs). Bacteria use AHL signaling molecules to control a variety of group behaviors such as the production of light in the light organ of a squid, production of antibiotics, antifungal agents and secondary compounds, exoenzymes that can make your lettuce slimy, virulence factors that can cause illness, and exopolymers that can cause biofilms to form on a variety of surfaces.

![A bacterial AHL signaling molecule: 3OC6HSL](image)
There has been a variety of ongoing research to study methods to interfere with bacterial signaling using AHL molecules because this signaling molecule mediates some bacterial group behaviors that are not beneficial for human society. One method of interfering with bacterial communication is by degradation of the AHL signaling molecule. A variety of bacteria have been found that can degrade AHL signaling molecules, and the enzymes from these bacteria have been used in biotechnology (Dong et al. 2001).

![AHL acylase enzyme](image)

**Figure 2.** One enzymatic pathway for bacterial AHL signaling molecule degradation (Leadbetter et al., 2000)

**Isolation and identification of bacteria with signal degrading metabolism**

In this laboratory, we will examine microbial diversity by culturing bacteria from the environment that have the ability to degrade AHL signaling molecules. This laboratory uses the principles of the enrichment culture for growing bacteria with particular metabolic capabilities. The principles of the enrichment culture, in which bacteria are grown in a minimal medium were pioneered by the microbiologists S. Winogradsky and M. Beijerinck in the late 19\textsuperscript{th} century, but their principles are still in use today. We will use a minimal salts medium to culture the bacteria such that the bacteria that grow will have the metabolism of interest: the ability to degrade AHLS. Unlike Luria Bretani (LB) medium, which is nutrient rich and made of yeast components and “undefined” nutrients, we will use a minimal salts medium that has defined sources of the nutrients N, P, S and AHL signaling molecules as the sole carbon source. This minimal medium will enable us to know whether the bacteria that grow have the metabolism of interest (AHL degrading ability) because the bacteria that do grow up under these conditions must possess the enzymes to degrade and consume AHL signaling molecules. Every group will try a variation of culture medium conditions and we will examine if and how the medium composition affects what bacteria grow in our enrichments.

**References:**


**Laboratory overview:**

In the following 3 weeks, we will use techniques in microbiology to try to culture bacteria from the environment that have the ability to metabolize the AHL signaling molecule: N-(β-Ketocaproyl)-DL-homoserine lactone (3OC6HSL). Bacterial signaling using this AHL is known to mediate bacteria group behaviors from soft rot of vegetables to antibiotics production to light production in a squid symbiosis. The ability to degrade these signaling molecules represents a method for bacteria to interfere with bacterial cell-to-cell communications. This is interesting from the point of view of microbial ecology as well as for applied purposes, such as mediation of unwanted bacteria activities, such a soft rot.

Below is a summary of the lab activities for our 3 week lab. We will go over the procedures in greater detail during the lab. Please be familiar with general flow of the lab when you arrive.
Week 1: Start enrichments for bacterial signal degraders from Lake Waban - 10/7/2010

1. Assemble liquid medium and pour agar plates for bacterial enrichment cultures. Each group will try a different combination of media components.

For liquid cultures:

You will make 2 liquid enrichment cultures that contain 5mls of media each:

A) **Experimental Culture** - test tube that contains bacterial signaling molecule as the sole carbon source

- 4.5ml of your 1X salts medium
- 350ul of Lake Waban water
- 150ul of 100mM 3OC6HSL bacterial signal stock

B) **Control Culture** – test tube that contains hexanoic acid (we will call this C6) as the carbon source. C6 is the break down product of the bacterial signal as a carbon source (Figure 2)

- 4.6ml of your 1X salts medium
- 350ul of Lake Waban water
- 50ul of 1 M C6 hexanoic acid stock

Incubate your cultures in the 30 degrees C shaking incubator in the AC404.

For solid agar plates:

Assemble 2X concentrations of your salts medium to combine with 2X agar for solid agar plates
Autoclave your liquid medium and agar components separately and combine to form 1X agar with salts after the components have cooled. Be sure to add the carbon source C6 to a final concentration of 10mM to your agar before it solidifies. The petri plates will have a final agar concentration of 1.5% agar.
3. Streak samples of Lake Waban water onto agar plates containing C6 and onto yeast extract plates using an inoculating loop to examine what microorganisms grow from Lake Waban on the two types of media.

4. As a class we will extract the community genomic DNA from a sample of Lake Waban water for PCR and sequencing. This will enable us to find out what bacteria are in Lake Waban and compare this inventory to the bacteria that we grow in our enrichment cultures.

5. Test the *Chromobacterium violacein* AHL signal detecting bioassay strain by plating this strain near a wild type *Chromobacterium violacein* strain that has a functional signaling producing gene.

**Week 2: Observe and transfer bacterial cultures for purification**

1. Examine primary liquid cultures and record observations.

2. Examine growth from Lake Waban samples on yeast extract and C6 plates and record observations.

3. If there is growth in either of your experimental or control cultures, transfer 200ul of primary culture to a tube that contains fresh medium with the same carbon source as your primary culture that is being transferred. This Secondary Culture will enable you to begin to purify only the bacteria that have the metabolism that you are interested in because these bacteria should grow up relatively quickly in the transfer.

4. Between weeks 2 and 3 during class: plate growth from secondary cultures onto hexanoic acid plates to obtain pure cultures for sequencing in Week 3.

**Week 3: Identification of Bacteria using 16srDNA PCR and sequencing** (a separate laboratory handout will follow)

1. Pick isolated colonies from hexanoic acid plates and use PCR to amplify the 16srRNA molecule of the bacteria.

2. Determine identity of the bacteria from sequenced 16srRNA and database searches.