Bi 1x, Spring 2009

Week 6

- **Session 1**
    o Plate count anomaly

- **Session 2**
    o Growth curves

Session 1 – Plate count anomaly

This "anomaly" was termed by Staley and Konopka in 1985 to describe the large discrepancy between the number of cells observed in natural environments by microscopy versus those that form colonies on solid media. During the Week 3 activities, you got an idea of the characters that inhabit the pond water through microscopy. Today, you will examine the pond bacteria by growing them on agar plates containing different media.

Materials

- Plates of different types; You will given a selection of the following:
    o Basal medium
    o Freshwater low nutrient medium
    o LB medium
    o Baxter pond water medium
    o BBB pond water medium
    o Beef broth medium
- Liquid media to dilute with
- Plating supplies

Protocol

1. Create serial dilutions of the pond samples according to the instructions given by the TAs (total volume, 1 ml each dilution). To make the dilutions use the liquid form of the same type of media comprising your selected plates (for example, use LB to dilute if you are using LB plates).
2. Spread 100 µl of each dilution on a separate plate using plating beads.
3. Label your plates and place them upside down on your bench to incubate.

Session 2 – Growth curves

Introduction

While there are a multitude of important time scales in biology, arguably one of the most important is concerned with the rate of cell division. In the bacteria *E. coli*, rates of cell division are closely linked with evolutionary fitness and hence our basic understanding of natural selection. To better understand the concepts involved in cell division, we will measure the bulk growth rate of E. coli in LB media by two methods: plate counting and spectrophotometry.

Background - Spectrophotometry

It is often useful to determine how many cells one has in a culture—spectrophotometry is one technique than can be used to do this. As visible light passes through a liquid sample of cells, it will be scattered; the amount of scattering is related to the density of cells in the suspension. At 600 nm, an optical density (OD) reading of 1.0 means there are approximately 10^9 cells in an *E. coli* culture.

The basic physics are as follows:

1. Light, with a wavelength comparable to the size of the object being measured (600 nm), enters the sample at an initial intensity (I_0).

2. Cells, having a different index of refraction as the surrounding medium, randomly reflect and scatter light out of the incident light path (scattering with no change in momentum). The amount of scattering is proportional to the amount of cells at any given point in the sample.

   Caveat: to get an accurate reading, cell density must be low enough that light is only scattered once in its journey through the sample. Often, this means that cells must be diluted to get an accurate reading.

3. Upon exiting the sample, the light intensity is reduced by $I_1=I_0e^{-\alpha lc}$, where α is the wavelength-dependent molar absorption coefficient of the species in question, l is path length – usually 1 cm in modern spectrophotometers, and c is the concentration, this is known as *Beer's Law*. See the figure below:
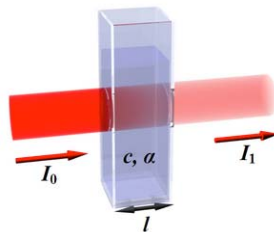
The transmission efficiency of the sample is the ratio of the final and incident light intensity, $T= I_1/I_0$, where the measured quantity, called 'absorbance' is $A=\ln(1/T)=\alpha lc$. The general rule of thumb is that accurate absorbance readings lie in the range *0.01< A <1*.

Materials

- Plating supplies
- LB plates
- Cuvettes (located by the spectrophometers)

Protocol

1. Every 15 minutes, remove a 1 ml sample of your culture (using a sterile pipette) and deposit this sample into a cuvette.

   Note: Please minimize the time that the incubator lid is open to avoid temperature fluctuations.

2. Set your spectrophotometer to measure optical density at 600 nm and blank it using 1 ml of fresh LB media in a cuvette.

   Note: Each of the spectrophotometers operates differently; please ask your TA how to operate the one you plan to use.

3. Insert your sample cuvette into the spectrophotometer and record the resulting OD_{600}.

4. Every 60 minutes, also remove an additional 0.5 ml of culture and deposit it into a sterile tube. You will use this sample for plating.

   Plating:

   1. You will first need to make three dilutions according to the calculations performed in class on Monday.

   2. On three different LB plates, evenly spread the 100 ul with beads or a sterile spreader. Make sure the plates are dry before incubating them (leave them slightly open until they dry up).

   3. Incubate the plates upside-down overnight at 37ºC. You should come in and count your colonies during office hours or free time in the next lab session.

You should continue taking readings until the end of the lab session.