Autoinduction in a Marine Bacterium: Biotechnilights-How Bacteria Talk to Each Other

(adapted with permission from Dr. Boonie Bassler, Princeton University, first published in Shoestring Biotech)

A. OBJECTIVES

At the conclusion of this lab, students will be able to:

- Describe the ability of a dark mutant strain of *Vibrio harveyi* to cause induction of light production in a second dark mutant strain of *V. harveyi*.
- Demonstrate the use of *V. harveyi* as a sensor of marine pollution.
- Learn the basics of sterile technique as required in growing bacterial cultures.

B. Before coming to lab

- Read Parts A through F of this exercise. In your notebook, answer the “Test Your Understanding” questions in Part E.

C. During labs

First lab

1. Work in a group of four.
2. Carry out the experiment as outlined in Part F. Wednesday labs: At the end of lab, check the agar plates of Tuesday lab students to see whether your hypothesis regarding cross-feeding is supported or not.
3. As a group, read Part G and design an independent investigation as outlined in Part G. Your task is to determine the concentration tolerance of *V. harveyi* to a particular marine pollutant of your choice.

Second lab

1. Tuesday students: Check the agar plates of Wednesday labs to see whether your hypothesis regarding cross-feeding is supported or not.
2. Carry out the pollutant test according to your protocol.
3. Record and analyze your data. Compare your results with the results of other groups. Discuss your findings. You should be able to answer the questions in Part H.
4. Dispose of all materials appropriately.

D. After lab

In your notebook, evaluate your experimental design. What worked well? What would you do differently the next time?

E. Background

People have long been fascinated by the ability of fireflies to produce light. The fireflies' ability to produce light, bioluminescence, is due to lux genes. The lux genes code for two sets of proteins. One set is for the subunits of luciferase, the enzyme whose action results in light production. The other set of genes codes for proteins that are responsible for the synthesis of the substrates for luciferase. When ATP is present, luciferase cleaves luciferin, releasing light as a result of metabolic oxidation, as shown in the equation below (Figure 1):

$$\textbf{Luciferin} + \mathbf{O_2} + \textbf{ATP} \xrightarrow{\textbf{Luciferase}} \textbf{Oxyluciferin} + \mathbf{CO_2} + \textbf{Light}$$

Figure 1: Reaction for the release of light by luciferin.

ATP provides the energy to drive the reaction. The lights of fireflies blinks off and on in relation to the level of ATP energy available. When the firefly runs out of ATP, the light goes out while new ATP is generated.

Most people are unaware that many other species also have this talent. One of the most interesting is a free-living species of marine bacterium, *Vibrio harveyi (V. harveyi)*. When its population size senses a critical density level, it lights up an environmental sensing system. Since it is stimulated to express luminescence in response to cell density, the bacterium must measure its population density. This is accomplished by synthesizing, secreting, and responding to small molecules called autoinducers. These autoinducers are analogous to pheromones or hormones of higher organisms. As a population of *V. harveyi* grows, autoinducer accumulates in the extracellular environment. At a critical concentration of autoinducer (which is indicative of the cell density) the bacteria sense and respond to the autoinducer by turning on the genes associated with light production. Autoinducers have recently been discovered in more than 30 regulated genera of bacteria and are believed to play a role in helping bacteria to control the expression of density-controlled functions, such as virulence, plasmid transfer, and secretion of antibiotics. This phenomenon is called **quorum sensing**, and it enables the bacteria to communicate with one another and to behave as a community by performing functions as a population instead of as individuals.

In bacteria such as in a different species of *Vibrio, V. fisheri*, the genes for making luciferase are contained in the lux operon-a segment of DNA that encodes the luciferase-producing genes that can be "turned on and off." In every case, the sequence and organization of the lux genes encoding the luminescence enzymes are very similar. When the local concentration of autoinducer is very high, the protein LuxR binds the autoinducer, and this complex binds to the operator to turn on transcription (see Figure 2). On the other hand, if autoinducer is low, LuxR cannot bind to the operator, and very little luciferase can be produced.

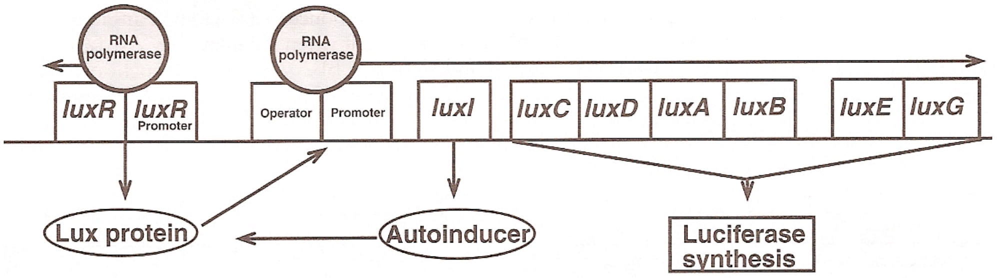

Figure 2: The lux operon for *Vibrio fischeri*.

When the critical amount of bacteria producing the autoinducer is present, the autoinducers cause a quorum response of bioluminescence through bacterial luciferase production. The autoinducer binds to LuxR, which in turn binds to RNA polymerase and the promoter, initiating the transcription of the lux operon.

The gene that encodes the enzyme that synthesizes the autoinducer is luxI. When a bacterium undergoes the transition from not making luciferase to making luciferase, it needs to have the autoinducer present in order to promote the binding of LuxR to the operator. Before the operon is turned on, LuxI must be made, so that there is a continuous level of autoinducer. In general, operons are never completely turned off. There is always some level of transcription occurring, so that autoinducer is continuously made.

The organism used in this activity is *Vibrio harveyi (V. harveyi)* , named after E.N. Harvey, a pioneer in the study of bioluminescence (Bergey's, 1984). *V. harveyi* is a free-living, bioluminescent bacterium that lives in marine waters. Like *V. fischeri*, it possesses autoinducers that enable it to produce light when its population density reaches a critical level. Unlike *V. fischeri, Vibrio harveyi* has two auto inducer systems (see Figure 3).

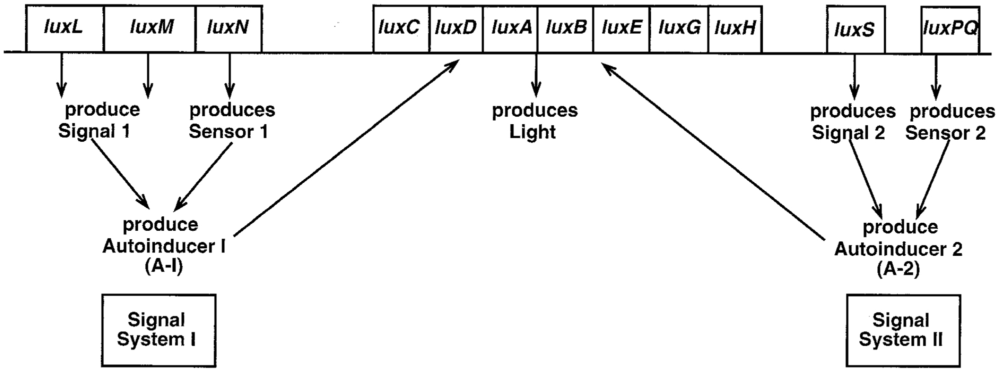

Figure 3: *Vibrio harveyi* lux operon with two signaling systems. Note that in Figure 2 there is just one signaling system.

V. harveyi, which is lux$^+$, expresses bioluminescence. Various lux$^-$ mutants exist. One fails to produce the luciferase enzyme and therefore cannot cleave luciferin and remains dark. Another does not produce autoinducer and cannot self-stimulate, so it remains dark. However, external autoinducers can induce bioluminescence in dark mutants. Once the dark mutants are induced to produce light, they retain the characteristic permanently. You can experiment with these autoinducers and examine their diffusion through agar. As the autoinducers diffuse through the agar, the dark mutant cells will begin to light up. In the following exercise, the mutant that cannot produce luciferase can produce an autoinducer. It could be termed the autoinducer donor. The second mutant cannot produce an autoinducer but can produce luciferase. It could be termed the autoinducer recipient. This stimulation of gene expression is known as intercellular cross-feeding.

Test your understanding by answering the following questions (please explain your answers):

1. Will providing autoinducer to the donor strain allow it to express bioluminescence?
2. Will providing autoinducer to the recipient stain allow it to express bioluminescence?

3. Can you determine which *lux* mutant produces the autoinducer and which produces luciferase? In other words, which is the donor and which is the recipient capable of having its light-producing genes turned on?

F. Procedures First Week

We will apply the scientific method to the following question:

You will be using *V. harveyi* BB151 and *V. harveyi* BB202B. One cannot produce the autoinducer, and the other lacks the luciferase gene.

Questions:

1. Which (if any) of the two strains can glow in pure culture?
2. If grown in close proximity with each other on a Petri dish, can both or either one (which) be induced to glow?
3. Can you find out which of the two lacks the luciferase gene and which lacks the ability to produce the autoinducer?

In your notebook: Write down your hypotheses (explain your reasoning) and predictions.

Protocol:

1. Label a petri dish as shown in Figure 5.
2. Dip a sterile cotton swab into the tube containing an overnight culture of *V. harveyi* BB151. Press the swab against the inside of the culture tube to squeeze out the excess broth.
3. Paint half the plate with the BB151, as shown in Figure 6.
4. Repeat the procedure on the other half of the Petri dish with BB202B and a fresh cotton swab, but leave a small space (2 cm) of unpainted petri dish between the 2 strains as shown in Figure 6.
5. Properly dispose of used swabs.
6. View the dish in a dark room as instructed to see whether any of the cultures can glow (assuming that no cross-feeding has taken place yet) by itself.
7. Tape your plate closed and incubate plates at room temperature in the area designated for your lab section.
8. **Today or next time:** Look at a culture from a previous section in complete darkness, as instructed during lab.
9. Compare results with other students and record result in your notebook. Evaluate your hypotheses and predictions in light of your hypotheses.

G. Investigation of concentration tolerance of V. harveyi to a particular environmental pollutant

V. harveyi is useful as a biosensor to provide important information about the health of the marine environment, because *V. harveyi* responds to environmental pollutants and other changes by abruptly turning off its bioluminescence. The concentration of the pollutant is a critical factor.

In your notebook:

1. Make a list of chemicals likely to be found in oceans as pollutants and their sources.

Your task is to determine the concentration tolerance of *V. harveyi* to a particular marine pollutant of your choice. Check the available chemicals in lab that could serve to simulate environmental changes. Remember that serial dilutions are commonly used to generate different concentrations of solutions. A cheap and easy way to create small lawns to test the effect of chemicals is to incubate bacteria in well-plates. You will have four six-well plates available per group (don't forget that you need replicates). To grow a bacterial lawn in the wells, 0.25 ml of overnight BB721 per well would be a good volume. Cultures need to grow about 48 hours at room temperature to form the lawn. Don't forget labels and your aseptic techniques!

2. Approach this investigation by recording all steps of the scientific method in your notebook, as well as a detailed protocol and a list of materials you will need.
3. Discuss your protocol with your instructor.
4. Carry out the first part of your investigation (preparation of *V. harveyi* lawns and serial dilution of pollutant)
5. Store your materials in the designated lab area, don't forget to label them so that you will find them next time.

H. Review questions

1. Considering your classes' results: Can either BB151 or BB202B bioluminesce?
2. Which mutations would lead to the phenotypes you observed in BB151 and BB202B before incubation?
3. Can either BB151 or BB202B bioluminesce when grown with the other? Why (not)?
4. Could either BB151 or BB202B bioluminesce when grown with BB721? Why (not)?
5. Which chemicals did you use for your independent investigations? Did it affect bioluminescence of BB721? At what concentrations?
6. Is the *lux* operon an example for positive or for negative control of gene expression?
7. Is the *lux* operon inducible or repressible?
8. What is the regulatory gene in the *lux* operon of *V. fischeri* in Figure 2?
9. What happens at low concentration of LuxI (note, if the L is capitalized and the word is not italicized, it denotes a protein. The corresponding gene would be *luxI*).
10. What happens at high concentration of LuxI?
11. When is LuxR produced?
12. How many genes are involved in the synthesis of Luciferase?