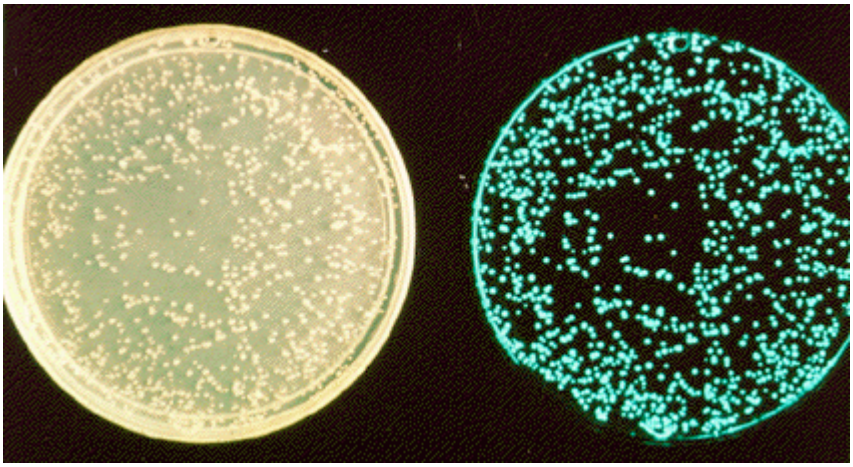
Bioluminescent Bacteria

There are bacteria living in the oceans that actually produce light! These microbes are easily found in seawater, marine sediments, in the guts of marine animals, and on the surface of decomposing fish. There are fish and squid that have evolved the ability to harness the power of these light-producing microbes. These animals have specialized organs that provide bioluminescent bacteria with both a safe place to live and a source of food. In return the animals can use the light that is produced by the microbes either as a means of camouflage, as an aid in hunting, or even as a way of attracting mates. While interesting from the point of view of understanding the complex interactions between animals and microbes the study of luminescent bacteria has also provided unexpected insights on strategies for treating cystic fibrosis and preventing antibiotic resistant bacterial infections.

Over the three days of the course we will isolate luminescent bacteria from seawater and we will make some basic observations about the nature of bacterial luminescence. This exercise will introduce you to some basic techniques that are required for growing microorganisms. In addition, we hope to be able to send you home with your own culture of luminescent bacteria and the ability to grow them on your own.



Bioluminescent Bacteria:
The picture at left shows how bioluminescent bacterial colonies appear on a petri plate under normal light. The image on the right is what you will see when you look at the same plate of bacteria in a dark room

Luminescent Bacteria

Who are they?

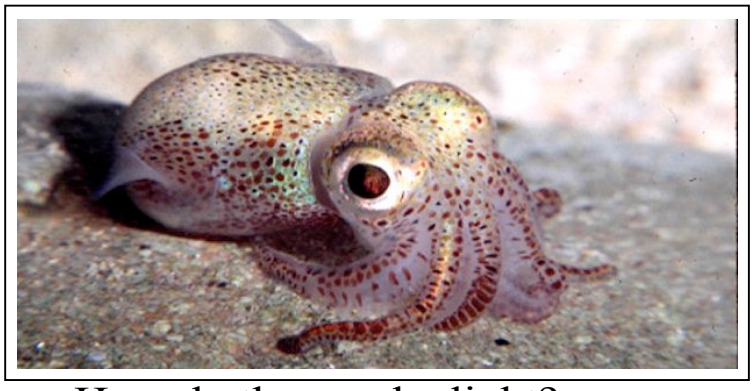
-Genera *Vibrio* and *Photomicrobium*

What do they look like?

-typically curved rods 1 to 3 microns long and motile by flagella

Where are they found?

-Seawater (1 to 100 cells per ml)
-fish digestive tracts
-on the outside of decaying fish
-Symbiotic relationships:
Angler Fish
Flashlight Fish
Bobtail Squid



The flashlight fish *Photoblephron palpebratus*. The comma-shaped,light organ is visible under the eye. The photograph was taken with a strobe light that was reflected off the reflective material at the back of the light organ.

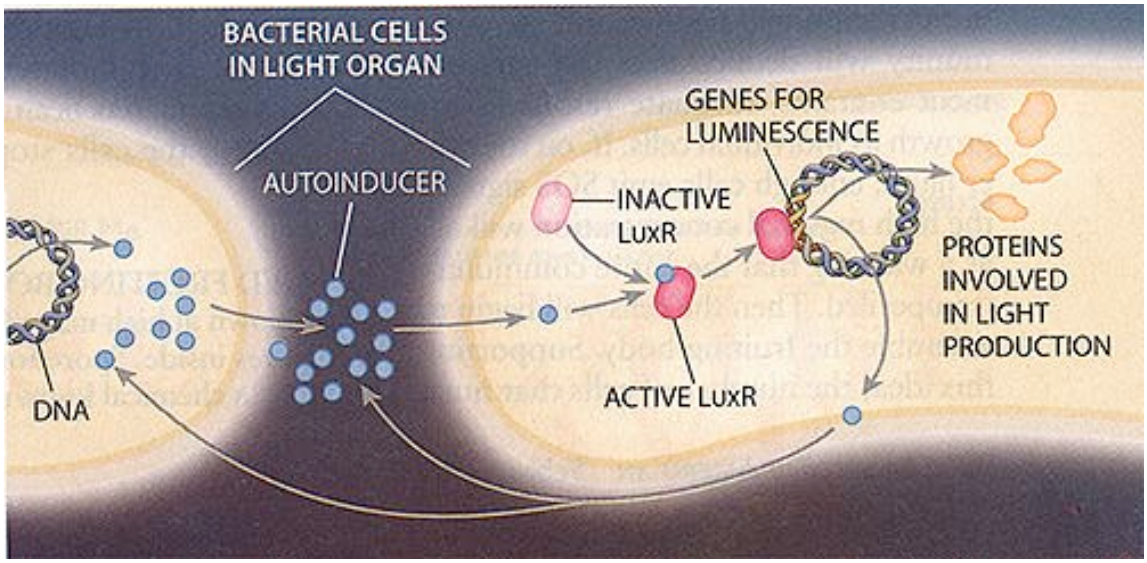
How do they make light?

-by a chemical reaction that consumes oxygen
($FMNH_2 + O_2 + RCHO \rightarrow FMN + RCOOH + H_2O + Light$)
-the reaction is catalyzed by an enzyme called luciferase

When do they make light?

-Light is only produced when the organisms are present at high cell densities

Quorum Sensing: the ability of bacteria to regulate gene expression in response to cell density



Gene	Gene Product	Function
LuxI	autoinducer	always expressed, this is the signal
LuxR	receptor	always expressed, the receptor detects the signal (autoinducer) and in response turns on expression of luciferase genes
LuxAB	luciferase	makes light, only expressed when the receptor is active

Why is Quorum Sensing Important?

-involved in Biofilm formation:

Psedomonas aeruginosa and cyctic fibrosis

Antibiotic resistant infections

Luminescent Bacteria as Teaching Tools

Potential Topics

- Basic microbiological techniques
- Symbiosis
- Genetic regulation

Advantages

- Easily captures student interest
- Grow at room temperature
- Grow rapidly
- Easy to identify without a microscope
- Does not require expensive equipment

Isolation of Luminescent Bacteria

1) Using a clean container collect some seawater
2) Get 2 plates of SWC agar medium (see "Making seawater complete media")
3) Pipette onto one plate 0.1 ml of your seawater sample and onto the other 0.2 ml of your seawater (0.1 ml is about 1 drop from an eyedropper).
4) Spread the samples thoroughly over the surfaces of the plates with a glass spreading rod (see "Making a glass spreading rod").
5) Allow the samples to absorb into the agar (about 5 minutes), then invert the plates and incubate at room temperature.
6) Examine the plates after 18-36 hours. How many different types of colonies can you see? Look for luminescent colonies by going into a dark room and allowing your eyes time to adjust to the dark. How many colonies were on the plate in total and how many of those were luminescent?
7) Using a felt-tip pen mark the location on the plate where you see the luminescent colonies.
8) Read the section entitled "Streaking a Plate" that can be found on the following pages.
9) Sterilize an inoculating loop over a bunsen burner. Touch the end of the loop to the colony you are interested in and then use the loop to streak a fresh SWC plate (as described in "Streaking a plate). Incubate your fresh plates at room temperature.
10) Observe your plates after 18-36 hours. If you only touched one colony on your original plate there should only be one colony your streak plate, however it is common for microbiologists to have to streak a colony several times before a pure culture is obtained. How many different colony types do you see on your plate? Are the colonies bioluminescent? (note: bioluminescence only occurs when the organisms have been grown fresh, if plates have been on your bench for several days then the organisms may no longer be producing light, however if you re-streak you will be able to observe luminescence when the culture has grown on your fresh plate).

Safety Issues: The luminescent bacteria that you will obtain in this exercise are not pathogenic and so are safe to grow. However, organisms such as Vibrio vulnificans and Vibrio cholerae can be found in seawater and these organisms can grow on the media used in this exercise. These two organisms are known to cause food poisoning and so basic precautions should be made to avoid touching the bacteria that are growing on your plates. You should also wash your hands with soap and clean your work area with a disinfectant after any lab exercise as a basic safety precaution.

Alternatives:

If you don't have access to fresh seawater to use as a source of luminescent bacteria you can also use fresh seafood (note: frozen seafood or freshwater fish rarely produce good results). Luminescent bacteria can be found on the surfaces of dead fish, shrimp, and squid. In fact people have observed that fish start to glow in the dark after they have been around in the kitchen for a while! You can collect bacteria off of the surface of some fresh seafood and then use this material for your exercise. Alternatively, if you cover a dead marine fish with a thin layer of seawater or artificial seawater and keep the fish in a cool spot for a few days you will likely observe that the spots on the surface of the fish will begin to glow (beware you will also notice that dead fish really start to stink after a few days).

Additional Useful Information

Websites to Find Info on Luminescent Bacteria:

whyfiles.org/022critters/light.html
lifesci.ucsb.edu/~biolum/index.shtml
www.bioart.co.uk/lux
www.biology.pl/bakterie_sw/index_en.html

Sources for ingredients: Companies such Carolina Biological (www3.Carolina.com) of Fisher (www3.fishersci.com) can provide you with all of the ingredients that are mentioned below as well as inoculating loops.

Expenses: For approximately $240 or less (prices vary depending on the quantities you order) it is possible to purchase sufficient materials to make somewhere in excess of 500 SWC agar plates. This number of plates would be sufficient to perform this exercise with about 125 students (figuring 4 plates per student).

Making an inoculating loop

(inoculating loops can be purchased for about $2.00 each or made as below)

materials: pencil, about 8 cm of thin wire, tape, needle nose pliers

1) Tape the end of the wire to the pencil so that about 3 cm of the wire stick out beyond the pencil.
2) Use the needle nose pliers to curl the end of the wire into a small loop (the smaller the loop the better, the optimum size is about 1-2mm diameter).

Making a glass spreading rod

materials: glass rod about 15 cm long (can use glass pasteur pipettes as a substitute), bunsen burner (a propane torch can substitute for a Bunsen burner)

1) Hold the glass rod over the bunsen burner so that about 5 cm stick out beyond the flame, make sure the glass rod is perpendicular to the flame.
2) After a few seconds the glass will soften and the end of the rod will swing down, remove the glass from the flame and hold still for a few seconds so that the glass hardens into an "L". Use caution: The glass will be hot for several minutes.

Making Seawater Complete (SWC) media

Materials: natural or artificial seawater (you can get instant ocean salt mix from any pet store that sells marine fish), tap water, agar, yeast extract, peptone, glycerol, 20 sterile petri plates, container suitable to boil 1 liter of liquid, heat source to boil water

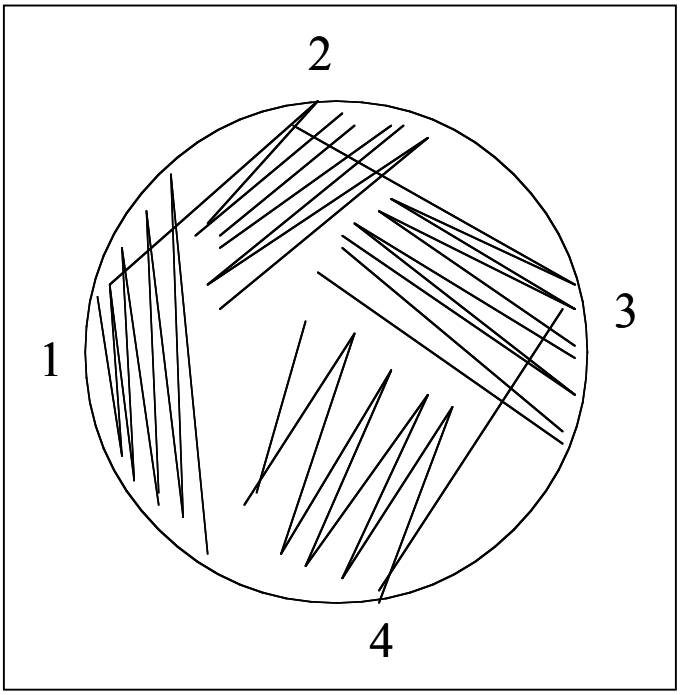
SWC agar recipe:

- 750 ml seawater
- 250 ml water
- 5 g peptone
- 3 g yeast extract
- 3 ml glycerol
- 15g agar

If you have an autoclave:
Mix all of the above ingredients together and autoclave for 20-40 minutes. After media has cooled to the point where you can just stand to touch the container (about 50 –60 degrees C) and pour into sterile petri plates. Be aware that the agar will solidify once the temperature falls below 40 degrees C.

If you don't have an autoclave:
1) Mix the media ingredients in a container sufficient for boiling 1 liter of media.
2) Cover and boil media for at least 30 minutes. Be careful not to boil over.
3) Allow media to cool to the point where you can just stand to touch the container (about 50 –60 degrees C) and pour into sterile petri plates. Be aware that the agar will solidify once the temperature falls below 40 degrees C.

Streaking a plate



1) Remove the lid of your petri plate and using your inoculating loop touch the surface of a colony on the plate.
2) Close the first plate.
3) Do not touch the inoculting loop to any surface, do not set down the inoculating loop.
4) Open a fresh petri plate.
5) Gently rub the end of your inoculating loop that you used to touch the colony back and forth over one end of the plate. Be careful not to push down too hard on the loop or you might gouge the agar surface.
6) Close the plate and use a flame (from a Bunsen burner or plumbers torch) to sterilize your loop (let it get red hot).
7) Holding the loop in your hand allow it to cool for several seconds.
8) Turn your plate 90 degrees so that the area you streaked last time is on the left.
9) Open the lid of the plate and touch your loop to a sterile region of the plate, if you hear a sizzle the loop is still too hot, wait a second and try again. If you don't hear a sizzle then run your loop once across the area of the plate that you streaked last time. Proceed to rub your loop back and forth over a fresh corner of the plate.
10) Repeat steps 6 through 9 until you have streaked all four quadrants on the plate (the figure above shows what your streak pattern should look like).