“Something’s Glowing In My Petri Dish!”
Bacterial Fluorescence

Science in the Real World
Microbes In Action

“Something’s Glowing In My Petri Dish!” is a curriculum unit developed as part of the *Science In The Real World: Microbes In Action Program*. The curriculum units were developed with support from the National Science Foundation, The Coordinating Board for Higher Education, Sigma Chemical Company, Pfizer Foundation and the Foundation for Microbiology.

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SOMETHING'S GLOWING IN MY PETRI DISH

AT A GLANCE

SOMETHING'S GLOWING IN MY PETRI DISH

Description: This lab will investigate the effect of iron on the fluorescence of several species of *Pseudomonas* bacteria. The student will also gain experience in various microbiological techniques

Time Requirements: This lab, from start to finish, is designed to be completed in 1 1/2 to 2 periods (50 minutes). Students should be given the lab to read the day before the lab is to be performed. Students should be familiar with basic principles of sterile technique.

Curriculum Placement: This activity could be used in an introductory biology course as an interesting lead-in to the study of bacteria. The phenomenon of fluorescence in living organisms is intriguing to students at all levels. This unit could be used as part of a microbiology course in a unit on the nutritional requirements and strategies of bacteria. It could also be used to illustrate the example of cell membrane receptors aiding cells in the acquisition of nutrients (i.e., iron). Finally, the role that the siderophore plays in the survival of pathogens within the host leads into ways the immune system may counter invading bacteria.

The science process skills used in this unit include the following: observation, recording data, analysis of data, interpretation of data, microbiological technique, hypothesizing, making inferences, and critical thinking.

Equipment: Many school labs have accumulated most of the equipment listed below. Check around chemistry or physics departments or items such as hot plates, UV lights and goggles.

balance
autoclave or sterilizer
4 beakers (250 ml)
2 Erlenmeyer flasks (500 ml)
scoop
3 to 4 hot plates
autoclavable test tube rack
8 test tube racks
8 inoculating loops
8 bunsen burners
safety goggles (1 pair/student)
UV light source (long wave) and UV goggles **or**
UV light box with viewing panel

Materials:

Bacterial cultures:
Pseudomonas fluorescens
Pseudomonas fragi
waterproof markers
nutrient agar
ferric ammonium citrate
petri dishes (disposable)
culture tubes (disposable)
culture tube caps
distilled water
masking tape

Time line for lab:

- Order bacterial cultures
- 2- 4 days before the lab: Teacher prepares 8 agar slant stock cultures of *Pseudomonas fluorescens* and *Pseudomonas fragi*.
- 1 to 10 days before the lab: Teachers prepares nutrient agar
- Day one of the lab: Students will prepare petri dishes by pouring the agar plates and then streaking the plates with the appropriate bacteria.
- Day two of the lab (24 to 48 hours later): Students will view plates, interpret results and complete questions.

PREPARATIONS

I. Materials Set-Up

For groups of 3 students:
- 2 tubes (15 ml) of sterile nutrient agar, melted
- 2 tubes (15 ml) of sterile nutrient agar + iron, melted
- test tube rack
- 4 sterile petri dishes
- stock slant of *Pseudomonas fragi*
- stock slant of *Pseudomonas fluorescens**
- inoculating loop
- bunsen burner
- safety goggles (one pair/student; wear when bunsen burner is on)
- masking tape
- waterproof marking pen
- 10% bleach solution (or other disinfectant to clean up)

Per Class:
- UV light source (360-400 nm)
- UV goggles (3 pairs) or
- UV viewing box with 360 nm light

II. Preparing Nutrient Agar Slant Stock Tubes

Materials Needed:	Bacterial Cultures of:	inoculating loop
	Pseudomonas fluorescens	bunsen burner
	Pseudomonas fragi	goggles
	8 sterile nutrient agar slants with caps or cotton plugs	

One tube of *Pseudomonas fluorescens* and one of *Pseudomonas fragi*, should be prepared for each lab group. Purchase one tube of each of these species. See Sources of Supplies, page 7. Label the 8 sterile nutrient agar slants for each of the above species. Using aseptic technique, and an inoculating loop, transfer bacteria from the supply house tube to the students' stock tubes. Repeat for second species. Incubate at room temperature 24 to 48 hours (an extra day or two is okay). Distribute these tubes to each lab group when the lab is started. Store all nutrient agar slants in the refrigerator at the end of the lab if you want to use these species for other microbiological activities.

III. Preparing Nutrient Agar

The label on the nutrient agar bottle lists the amount needed for one liter. You need to prepare 250 ml. So, add 1/4 the amount of nutrient agar listed on the label of the bottle to a 500 ml flask. Add 250 ml distilled water. Stir well. Cover with foil. Bring to a boil on a hot plate, stirring occasionally.

- Preparing Nutrient agar + iron:

Prepare a second flask of nutrient agar the same as you did above. To this flask, add 3 ml of ferric ammonium citrate solution (1000X stock). If preparing 1000 ml of nutrient agar, add 12 ml of ferric ammonium citrate solution. The small volume doesn't affect the solidification of the agar. Stir well. Cover with foil.

- Preparing 1000X stock solution:

Add 1 gram of powdered ferric ammonium citrate to 167 ml distilled water. Mix well. Refrigerate after use.

IV. Setting Up Test Tubes

Set up the autoclavable test tube rack, with the 32 autoclavable glass test tubes, for which you have either microbiology tube caps or aluminum foil squares. Using a waterproof marker, place an "Fe" or "I" on each of the 16 tubes to which the Nutrient Agar + Fe will be added.

V. Autoclaving the Test Tubes

Autoclave both racks of tubes at 15 psi, 250° F, for 15-20 minutes. Cool. Refrigerate until ready to use.

VI. Distributing Agar Solutions

To melt the agars, set up 3 to 4 hot plates with 250 ml beakers, half full of water. Put 8 to 10 tubes of cold, solid agar in this hot water bath for 20 to 30 minutes. The sterile agar will liquefy. Distribute to students to pour into their petri dishes while still liquid.

SOURCES OF SUPPLIES

Carolina
2700 York Road
Burlington, NC 27215
(800) 334- 5551

Description	Stock Number	Quantity	Cost
Pseudomonas aeruginosa	F6- 15- 5250	1 tube	$7.00
Pseudomonas fragi	F6- 15- 5260	1 tube	$7.00
UV absorbing goggles (use with pocket black light from Frey)	F6- 64- 6706	3 pair	$22.50
inoculating loops	F6- 70- 3060	12	$16.50
petri dishes, polystyrene (sterile)	F6- 74- 1350	100	$20.00
culture tubes, Kiman (20 x 150 mm)	F6- 73- 1426	72	$39.60

Sigma
P.O. Box 14508
St. Louis, Mo. 63178
(800) 521- 0851

Description	Stock Number	Quantity	Cost
Ferric ammonium citrate (brown form)	F 5879	100 g	$6.55
Nutrient agar	N 0394	250 g.	$29.00
Culture tube caps (20 mm)	C5791	100	$15.25

Frey Scientific
95 Hickory Lane; P.O. Box 8101
Mansfield, OH 44901- 8101
(800) 225- FREY

Description	Stock Number	Quantity	Cost
UV darkroom cabinet	F23775	1	$138.30
Compact UV light **or**	F14063	1	$87.00
Pocket black light (use with Carolina UV goggles)	F16008	1	$24.95

TEACHER HINTS & TROUBLESHOOTING

1.Carolina Biological lists both *P. fragi*/and *P. aeruginosa* as non-pathogenic bacteria. Most microbiology books list *P. aeruginosa* as an opportunistic human pathogen. *P. aeruginosa* is commonly found in soil and water, and can be isolated from wound, burn, and urinary tract infections. Treat both species of *Pseudomonas* as potential pathogens. Follow proper aseptic and sterile technique.

2. Have students wear goggles when using the bunsen burner.

3. Set up lab team equipment in trays before class to minimize the need for students walking around the room.

4. Borrow equipment from chemistry and physics teachers.

5. Autoclave media 15 to 20 minutes. Autoclaving too long can cause some breakdown of nutrients in the media.

6. The production of the siderophore, pyoveridine, occurs best at temperatures between 20-25° C. Using a warmer temperature actually decreases production, so incubate at room temperature.

7. Monitor the use of the UV lights.

ANSWER KEY

Data Table
Record your observations after examining your petri dish with the UV lighting.

NA/ *P. fluorescens*	**NA +Fe/ *P. fluorescens***
Fluorescent	*Not Fluorescent*
NA/ *P. fragi*	**NA + Fe/ *P. fragi***
Not Fluorescent	*Not Fluorescent*

1. In which petri dish(es) was fluorescence noticed?

Fluorescence should be noticed in the P. fluorescens plate grown on nutrient agar.

What causes the fluorescence?

The fluorescence occurs because P. fluorescens secretes the siderophore, pyoveridine, which absorbs the UV light and re-radiates light back to our eyes. This light has a greenish-yellow glow to it.

2. What is a siderophore?

A siderophore is a compound secreted by bacteria which binds with iron in the environment. The siderophore then delivers the iron to the bacterial cell, which uses the iron to carry out respiration and other necessary processes.

3. What is the siderophore receptor?

The siderophore receptor serves as the location where the siderophore-iron complex "latches" onto the bacterial cell.

4. Based on your reading of the introductory material, explain your observations.

P. fluorescens grown on nutrient agar secretes pyoveridine to help capture iron and therefore fluoresces under UV light in the 360 nm range. P. fluorescens grown on

nutrient agar + iron gets enough iron, does not secrete pyoveridine to capture iron, and therefore does not fluoresce. P. fragi is a Pseudomonas species, which does not secrete the siderophore, pyoveridine. It therefore will not fluoresce on either plate.

Critical Thinking

1. Some bacteria that are human pathogens produce siderophores. What role might these bacterial siderophore play with respect to the ability of the bacteria to cause an infection in the host organism (such as a human being)?

   Microbial siderophores help the bacterial cell compete with the host for available iron. Much of the success of an infection appears to be due to the ability of the bacterial cell to "steal" some of the host's iron by means of the siderophore. In experimental infections of lab animals, bacterial strains that don't secrete siderophores have markedly reduced virulence (ability to cause infection).

2. How might a host organism counteract bacteria that secrete siderophores?

   A host organism might secrete a substance which ties up available iron in the environment, which limits the availability of iron to the invading microbe. (One example of a compound found in humans, which does this is lactoferrin. It is found in secretions such as saliva, nasal discharge, tears, seminal fluid, urine, hepatic bile, and bronchial mucus. Since these fluids bathe "ports of entry" for disease causing microbes, this is part of the body's external, chemical barrier system to prevent infection.

3. Why should distilled water always be used when preparing agars and various media in a microbiology lab?

   Distilled water, unlike certain tap water or well water, usually has little or no iron in it to contaminate the media.

SOMETHING'S
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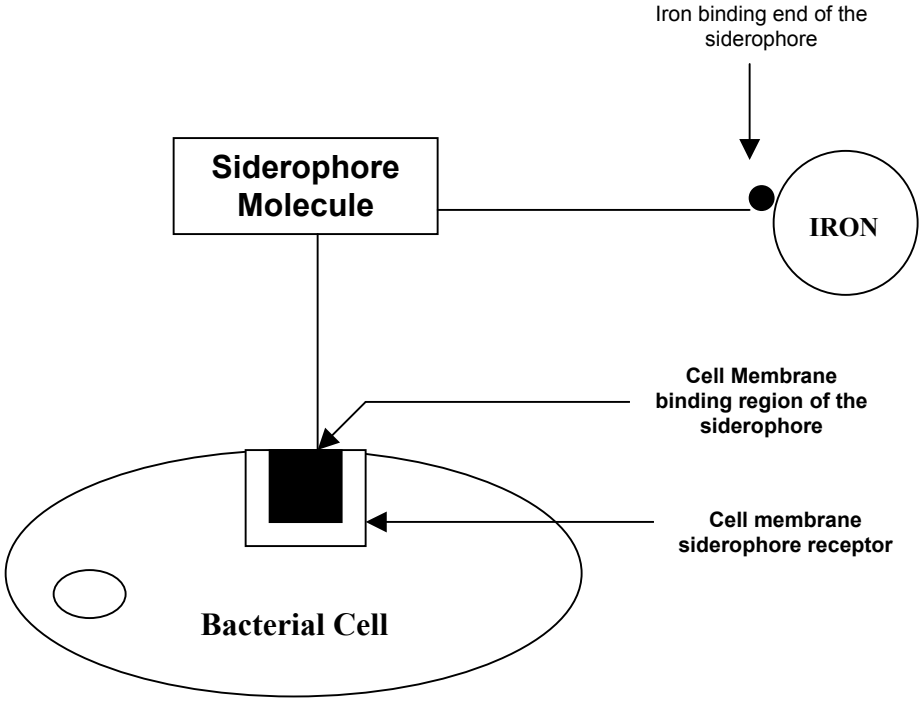
IN MY PETRI DISH!

PURPOSE: This lab will investigate the phenomenon of fluorescence in several species of *Pseudomonas* bacteria and the effect that iron has on this fluorescence.

BACKGROUND

Iron plays a major role in cellular respiration, DNA and chlorophyll biosynthesis, and many other important enzyme systems in living organisms. Bacteria, like other living organisms, need iron to survive. Many bacteria have a special way of obtaining iron. They secrete a chemical compound known as siderophore (sideros=Greek for iron, phorus=Greek for carrying). The siderophore binds to iron in the environment and carries it back to the bacterial cell. The siderophore-iron complex actually interacts with a receptor on the bacterial cell membrane when "delivering" the iron to the bacterial cell. See diagram.



Some siderophores (particularly one known as pyoveridine, which is secreted by several species of Pseudomonas), are fluorescent under UV light wavelengths around 360 nm. Energy of this wavelength is absorbed by the pyoveridine and re-radiated in the visible part of the spectrum. The fluorescence stops when the source of the energy (i.e., the UV light) is removed.

If bacteria are growing in a medium or environment where iron is scarce, the bacteria secrete large amounts of the fluorescent siderophore to capture and deliver iron to the bacteria. Conversely, if bacteria are growing in a medium or environment rich in iron, the bacteria do not secrete the fluorescent siderophore to capture and deliver iron. An abundance of iron represses the formation of siderophore-iron receptors on the bacterial cell membrane.

MATERIALS PER GROUP OF 3 STUDENTS

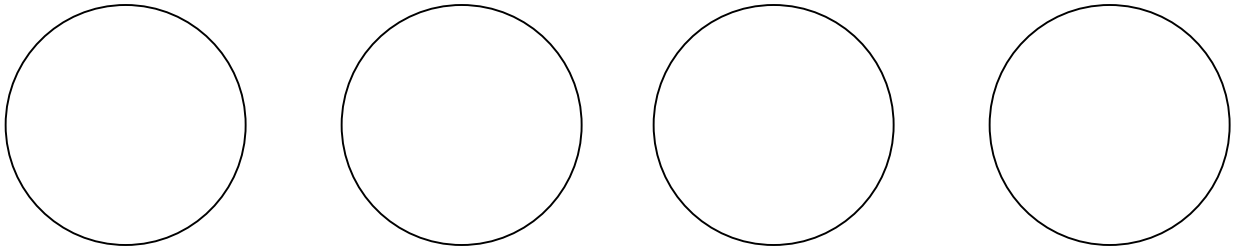
2 tubes (15 ml) of sterile nutrient agar, melted
2 tubes (15 ml) of sterile nutrient agar + iron, melted
test tube rack
4 sterile petri dishes
stock slant of *Pseudomonas fragi*
stock slant of *Pseudomonas fluorescens**
inoculating loop
bunsen burner
safety goggles (1 pair/student; wear when using bunsen burner)
masking tape
waterproof marking pen
10% bleach solution (or other disinfectant to clean up)

One set-up per class:

UV light source (360-400 nm)
3 pairs UV goggles or
UV viewing box with 360 nm light

PROCEDURE

1. Label 4 petri dishes:



NA/P. aeruginosa Name, Date	NA/P.fragi Name, Date	NA+Fe/P. aeruginosa Name, Date	NA+Fe/P. fragi Name, Date

2. Obtain 2 tubes of melted nutrient agar and 2 tubes of nutrient agar + iron. Pour the agars into the appropriately labeled dishes: NA=nutrient agar; NA+Fe=nutrient agar + iron. Swirl the agar to cover bottom of dish; allow to solidify 5 to 10 minutes.

3. Obtain the stock culture tubes of P. fragi/and P. aeruginosa. Put on your safety goggles. Light your bunsen burner. Sterilize your inoculating loop. Streak the appropriate bacteria into each petri dish. Sterilize your loop between each transfer. Tape your petri dishes shut. Incubate the four dishes upside down at room temperature for 24 to 48 hours.

4. Clean up your work area with 10% bleach solution or other disinfectant supplied by your teacher. 24 to 48 hours later

5. Your teacher will help you examine the plates using the UV light. DO NOT look directly into the UV light. Wear UV goggles if you are using one of the small hand held UV light sources. The light should be directed downward into the petri dishes. Look for a glowing, yellow-green border around the bacterial colonies. Record your observations in the data table.

Name _____

Date _____

Data Table

Record your observations after examining your petri dish with the UV lighting.

NA/ *P. aeruginosa*	**NA +Fe/ *P. aeruginosa***
NA/ *P. fragi*	**NA + Fe/ *P. fragi***

1. In which petri dish(es) was fluorescence noticed?

   What causes the fluorescence?

2. What is a siderophore?

3. What is the siderophore receptor?

4. Based on your reading of the introductory material, explain your observations.

Critical Thinking

1. Some bacteria, which are human pathogens produce siderophores. What role might these bacterial siderophore play with respect to the ability of the bacteria to cause an infection in the host organism (such as a human being)?

2. How might a host organism counteract bacteria, which secrete siderophores?

3. Why should distilled water always be used when preparing agars and various media in a microbiology lab?