EXPERIMENT 12

Applications in Bacterial Pigments

INTRODUCTION

So far we have witnessed several useful applications of microbes including applications in food and the bioremediation of the environment. Besides consuming the desired substrate (oil) and producing antibiotics, microbes produce multiple valuable compounds suitable for use in human medical, food, and industrial applications. Countless **bioactive compounds**, those with biological effect, are produced separately by yeast, algae, cyanobacteria, fungi, and bacteria and for simplicity's sake we will focus only on bacterial pigment compounds.

Bacterial metabolic processes result in a myriad of compounds that could be either beneficial or harmful for human use. The compounds produced by bacteria depend primarily on bacteria type, environment (temperature, pH, light, dark, depth, oxygen, pressure, etc.) and substrate (nutrients). Some of the most useful products produced by bacteria are pigments. Microbial pigments have numerous beneficial properties including antioxidant, anticancer, immunosuppressive, and antibiotic. Bacterial pigments also have wide applications including fluorescent probing, organic food and clothing dyes, vitamins (B-carotene), and even industrial paints.

Pigmentation is a common bacterial trait that is involves the light-absorbing compounds and is responsible for the display of colors observed (Figure 1). Pigmented bacteria are termed **chromobacteria**. Bacterial pigments can be either **water soluble** or **water insoluble**. A good way of discerning bacterial water soluble pigments from insoluble pigments involves observing an agar plate with colored growth, and looking for diffused colors within the agar medium. Bacterial pigmentation is not restricted to aerobic or anaerobic bacteria, as oxygen-deprived hot springs or sulfur ponds can house colorful bacteria such as purple sulfur bacteria, green sulfur bacteria, and red and green filamentous non-sulfur bacteria.

Cyanobacteria pigments and those of purple and green sulfur bacteria differ from those found in other non-photosynthetic Proteobacteria. Cyanobacteria contain

phycobilisomes that are part of the **photosynthetic**, light-harvesting complex wrapped within the bacterial thylakoid cell membrane. Cyanobacteria phycobilisomes are made of pigments termed phycobiliproteins. Examples of phybiliproteins include phycocyanin (blue color) and phycoerythrin (red color). On the other hand, purple and green sulfur bacteria contain bacteriochlorophyll, bacteriorhodopsin, and proteorhodopsin (similar to plant chlorophyll). Besides photosynthesis in autotrophic bacteria, non-photosynthetic bacteria contain non-light harvesting pigments of various colors within their membranes.

Pigments play an important role in bacterial survival. UV radiation from sunlight is highly damaging to cellular structures, especially DNA, and pigments play a role as antioxidants. Pigments produced by bacteria absorb damaging UV radiation and quench oxygen radicals and prevent oxidation. Some pigments have shown antibiotic activity against pathogenic microorganisms. *Serratia marcescens*, for example, produces a deep red pigment prodigiosin that is a potent antibiotic pigment.

The color of a pigment is the result of the light absorbed and reflected by that pigment molecule. When identifying a pigment, scientists often use a spectrophotometer in order to observe the absorbance spectrum of a pigment. An **absorbance spectrum** is a graph that displays the portion of light radiation absorbed by the molecule over a range of wavelengths frequencies (nm) (Figure 2a). Where the molecule peaks on the spectrum is representative of the light wavelength the molecule absorbs, and the corresponding complimentary color your brain will perceive. For example, actinorhodin, a blue water soluble pigment produced by the bacterium *Sterptomyces coelicolor*, absorbs yellow-orange light which peaks at 600nm on the absorption spectrum and results in the complimentary blue color on the color wheel that we observe (Figure 2b). Table 1 lists a few common pigments produced by bacteria and their corresponding colors and wavelengths. Figure 3 shows an array of pigments extracted from various bacteria.

Extraction of the various pigments that bacteria produce depends on the compound's solubility. **Solubility** refers to the ability of a given substance to dissolve into a solvent. If the pigment of interest is water soluble or **polar**, alcohols and water are the best solvents in order to extract that pigment. Conversely, if the pigment in question is water insoluble or **nonpolar**, hydrophic solvents such as acetone or DMSO (Dimethylsulfoxide) are used. Figure 4 indicates the increasing polarity of the solvents used in this lab.

In this lab, you will isolate your own pigment-producing bacteria. Additionally, you will be given an unknown pigment-producing bacterium and you will have to

tentatively determine the pigment being produced by extracting the pigment in varying solvents of increasing polarity.

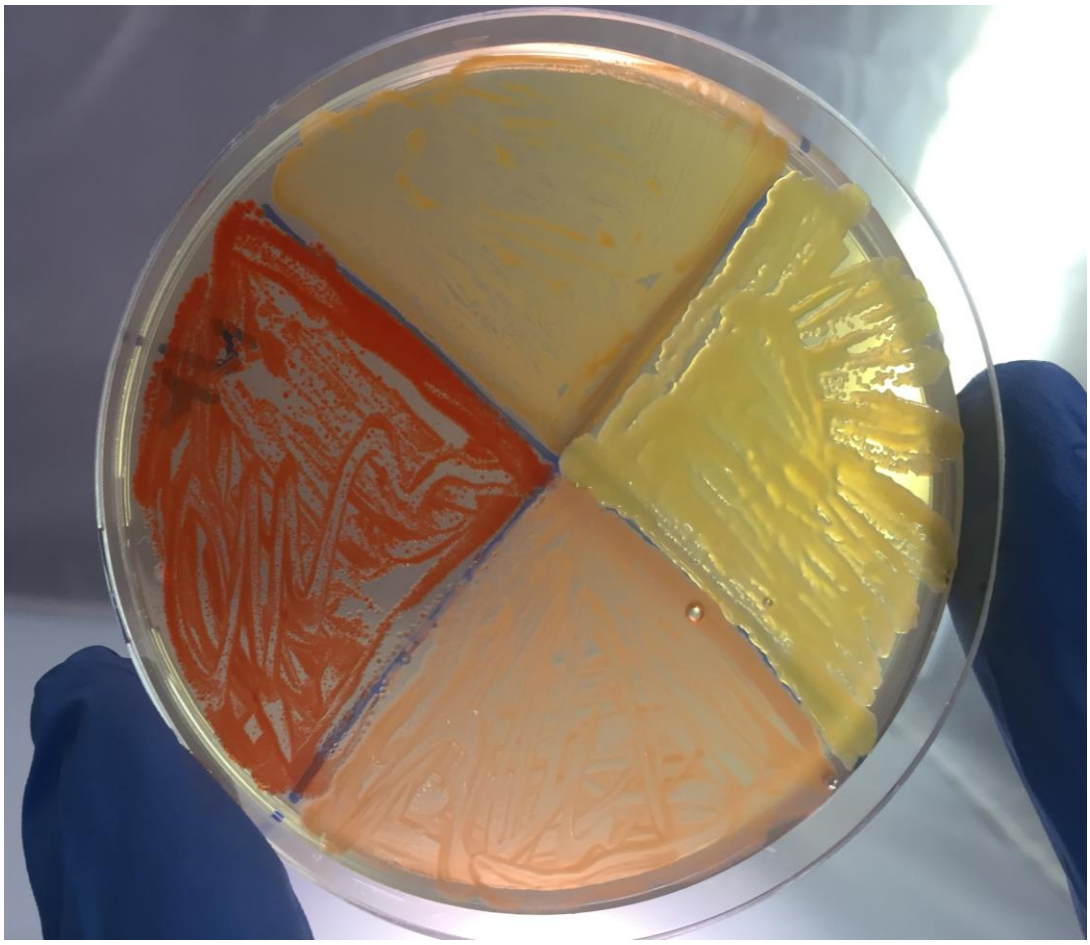
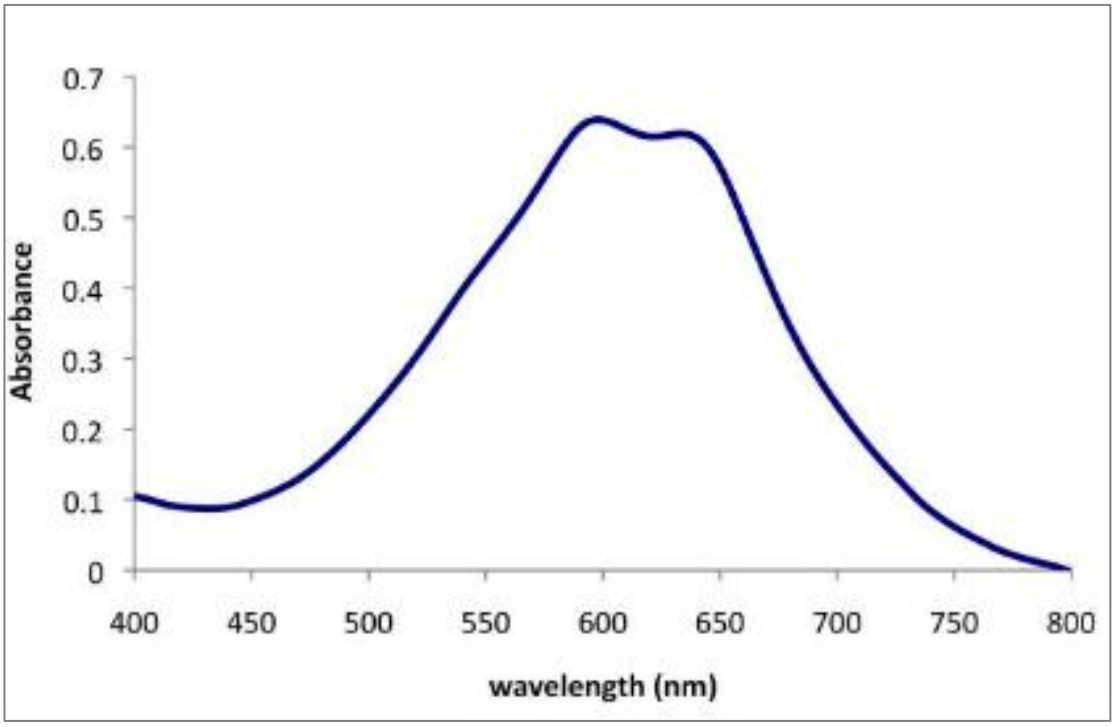


Figure 1. Nutrient agar plate inoculated with four various pigmented bacteria.

a)



b)

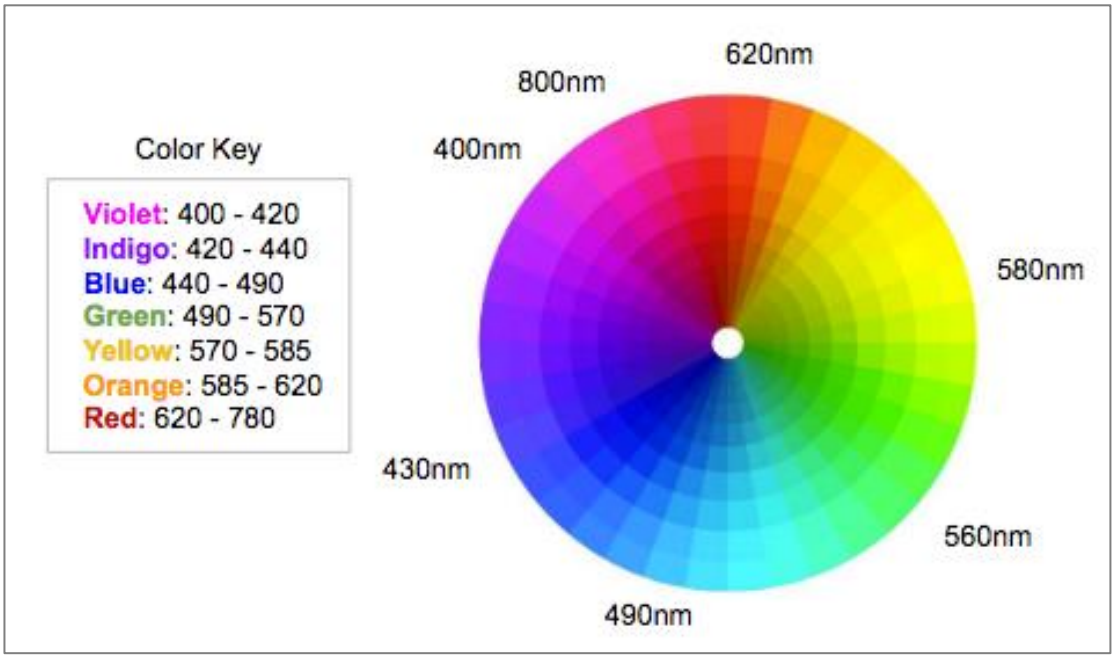


Figure 2. Properties of pigments in relation to light wavelength and corresponding color. a) Absorption spectrum of Actinorhodin, b) relationship between light wavelength (nm) and observed color.

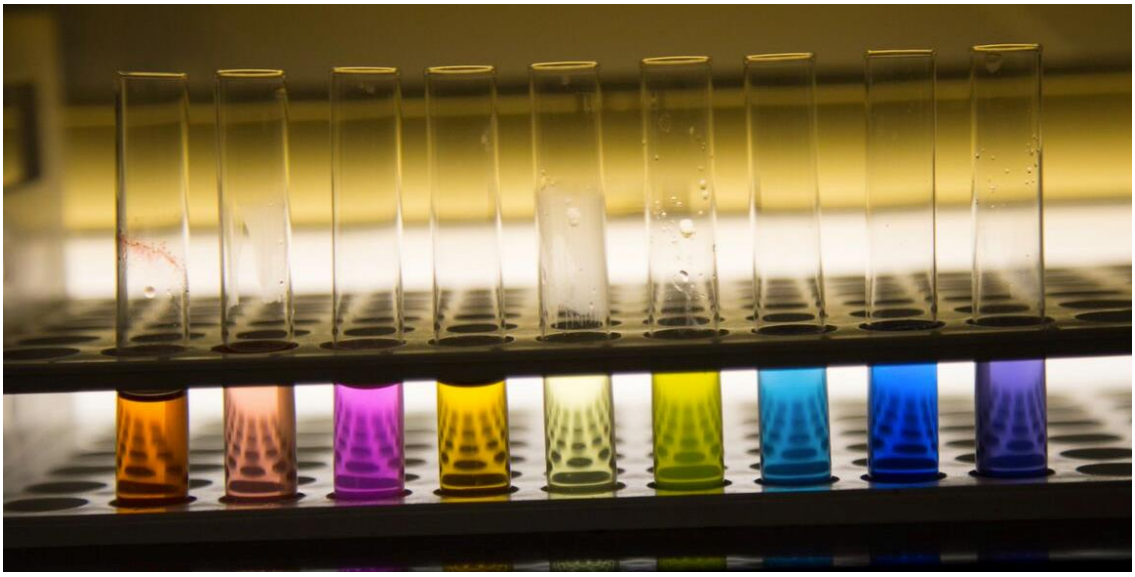


Figure 3. The variety of pigments produced by various photosynthetic and non-photosynthetic pigmented bacteria.

POLAR

NONPOLAR

Figure 4. Solvents used in this experiment in increasing order of polarity from acetone, a nonpolar solvent, to water, a very polar solvent.

Materials:

1. **(4) Nutrient agar plate**
2. **(3) Sets of Small test tubes with 2ml each of Acetone, DMSO, Vinegar, Ethanol, and deionized water. (15 total)**
3. **Unknown sample of chromogenic bacteria**
4. **Spectrophotometer (Optional; not required), cuvettes**
5. **(100-1000ul) Pipet and tips**
6. **1ml Sterile Peptone solution (0.1%).**
7. **Gram Stain Kit**
8. **Oxidase and Catalase**
9. **UV lamp**

Procedures for WEEK 1:

Part I. Isolation of Chromogenic bacteria

1. Grab a sample of marine or brackish water. Alternatively, bacteria from Table 1 may be purchased and given as an unknown.

2. Place 0.1 ml of the sample liquid onto a Nutrient Agar plate.

3. Using a sterile spreader, smear the sample over the entire plate.

4. Incubate plate at 25-30°C for 48-72hrs.

Procedures for WEEK 2:

Part I Isolation of Chromogenic bacteria

1. From your previously inoculated Nutrient Agar plate, locate TWO colonies with different colors and shapes and mark them on the back of the petri dish with a marker. Obtain your chromogenic bacterial sample as well.

2. Using a pipette, place 0.1ml of sterile peptone solution onto each nutrient agar plate.

3. With a loop, grab the first colony and place or smear it carefully onto the peptone drop. Spread the bacteria onto a plate using a sterile plate spreader.

4. Repeat this step for the second chromogenic bacteria and the unknown sample given.

5. Incubate plate at 25-30°C for 48-72hrs.

Procedures for WEEK 3:

Part II Extraction and characterization of bacterial pigments

1. Obtain your Nutrient agar plates, and observe your growth.

2. **Label** each of your solvent tubes with the Bacteria ID and the solvent used within the tube.

3. Using sterile techniques, inoculate each bacterial colony using proficient amounts of bacteria into each of the tubes containing the different solvents.

4. Remove the bacteria from the loop by making a twisting motion.

5. Do not shake the tube! Flick the bottom of the tube gently, or stir slowly to break up the colony. Set aside for one hour.

6. During the extraction period, Gram stain, oxidase, and catalase each chromogenic bacteria you isolated and the unknown you were provided. Record your results in the worksheet provided.

7. After one hour of extraction time, observe each of your tubes with the varying solvents, and record the most colorful tube found for each set of bacteria and corresponding solvent. Record the color of your pigment for each isolate and match them with to the pigments found in Table 1.

OPTIONAL

8. Using a pipette, draw 1ml of the highest pigmented tube for each colony and place into a plastic cuvette.

9. Indicate what solvent the highest pigmented tube is composed of in order to correctly zero or “blank” out the spectrophotometer.

10. Using the spectrophotometer, perform a spectral analysis of each of your samples ranging from 300 nm to 800 nm.

11. Place your cuvette near a UV lamp and observe for any fluorescence.

12. From the absorbance spectrum, determine the absorbance peak of your samples, and using the chromatogram wheel in Figure 2b to determine the complimentary color, and confirm the color you observe. Record all data in your worksheet.

Part I. Extraction of Bacterial Pigments
Directions: Record your results from your various extractions with your corresponding bacterium. Score your results using the intensity of the bacterial pigment in solution using +++, ++, +, -. **(3 points)**

Solvents	Bacteria 1	Bacteria 2	Unknown
Acetone			
DMSO			
Acetic Acid			
Ethanol			
Water			

Part II. Characterization of Bacterial Pigments *(Optional)*
Directions: Record the peaks (in nm) found from bacterial pigments extracted in the optimal solvent. Use the color wheel in Figure 2b to check the resultant color. ***Not all samples will have multiple peaks or be fluorescent.

Bacteria	Peak 1	Peak 2	Peak 3	Fluorescence	Resultant Color
1					
2					
Unkwown					

Part III. Bacterial Characterizations
Directions: Record the results of your gram stains below. **(2 points)**

Bacteria	Colony Morphology	Cellular Morphology	Gram	Pigment Color
1				
2				
Unknown				

Part IV. Questions

Directions: Answer the following questions in full sentences. Refer to internet searches or Figure 2 for assistance. **(5 points)**

1. If your unknown sample extraction appears red, what wavelength do you expect to be absorbed by that microorganism? And if it appeared Green? **(1 point)**

2. Why do you think it would be difficult and expensive to produce enough of your pigments for commercial use? **(1 point)**

3. According to your data (gram stain, cellular morphology, color, absorbance spectrum, pigment solubility) what do you think your pigment can possibly be? Are there there any real world applications of each of your pigments? **(2 points)**

4. Why do you expect it to be easier to work with soluble pigments rather than insoluble? **(1 point)**