Bacterial Teamwork: The Interplay between Bioluminescent and Light-Harvesting Bacteria

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Introduction:

Light can come from various places. Although the sun is a major light source, many organisms can produce light such as jellyfish, fireflies, and ostracods. Even bacteria can be bioluminescent. Bioluminescent bacteria (BB) create light by transcribing and translating two proteins which combine together to form luciferase (Berglund et al. 2013). In order to produce luciferase a certain of cells is required (Quorum sensing). In the vast diversity of the ocean some bacteria are known to harvest light. Light harvesting bacteria do this using a protein called, proteorhodopsin (PR). This membrane protein, in the presence of retinal uses light in order to create an ion gradient across the cell's membrane (Figure 1) (Béjà 2000). These ions are pumped into or out of the cell in order to facilitate various cellular activities. This may include creating ATP (Phototrophy).

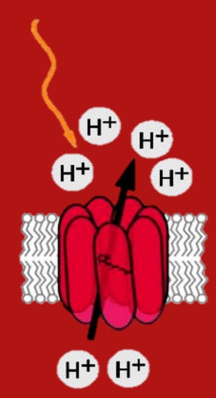

Figure 1. Ion gradient created by rhodopsin.

Our goal was to see if there can be an interplay between bioluminescent and light harvesting bacteria. The experiment would test if BB provided favorable conditions and promote the growth of the light-harvesting bacteria.

Methods:

- **Sampling** the Mediterranean Sea (Figure 2)
- **Use** 0.22µm filter to collect bacteria
- **Plate** filters to grow the bacteria
- **Isolate** bioluminescent strains (Figure 3)
- **Compared** growth of bacteria containing rhodopsin cultured with bioluminescent bacteria and without
- **PCR** on the 16s region and cloning to determine the species of bioluminescent bacteria collected

Figure 2. Mediterranean Sea sample site (32°48'21.2"N 34°57'17.9"E).

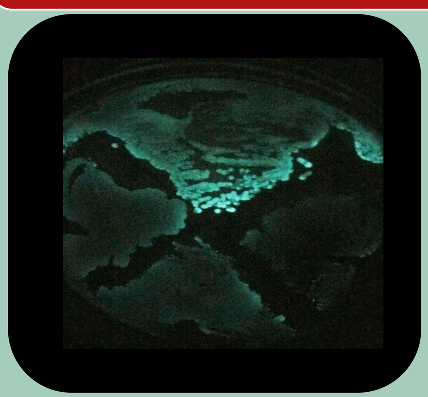

Figure 3. Isolated strain.

Results and Discussion:

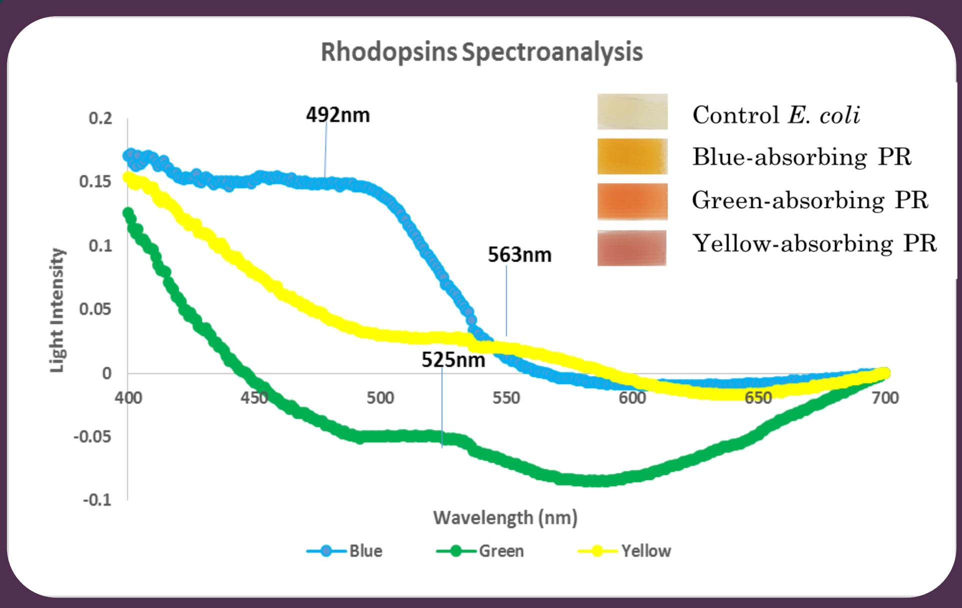

Figure 4. Wavelength absorbance of three types of proteorhodopsin spanning the visual spectrum. The average wavelength emittance of bacterial luciferase is 485nm (Molinari 2008). Blue PR was chosen for the main experiment since its peak absorbance (492nm) is closest to the luciferase emittance.

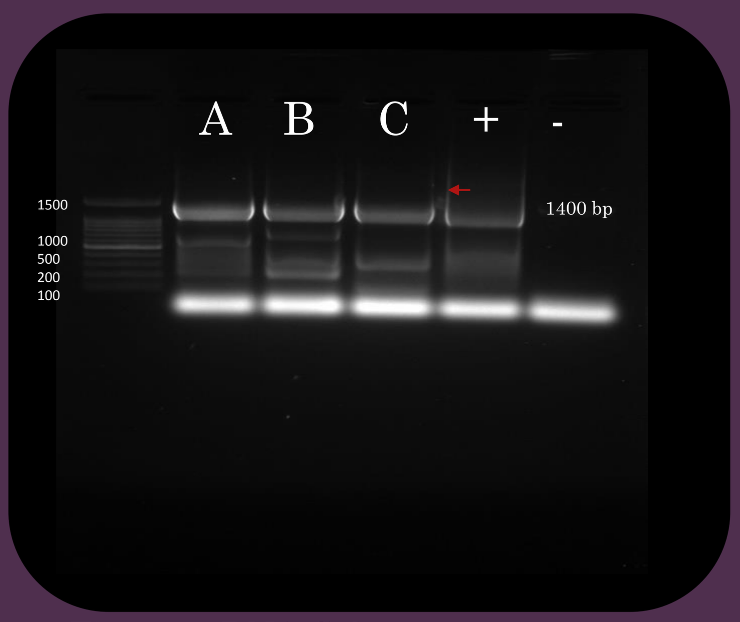

Figure 5. DNA Gel Electrophoresis of three isolated strains of BB. Strain B and C were chosen since they were the brightest.

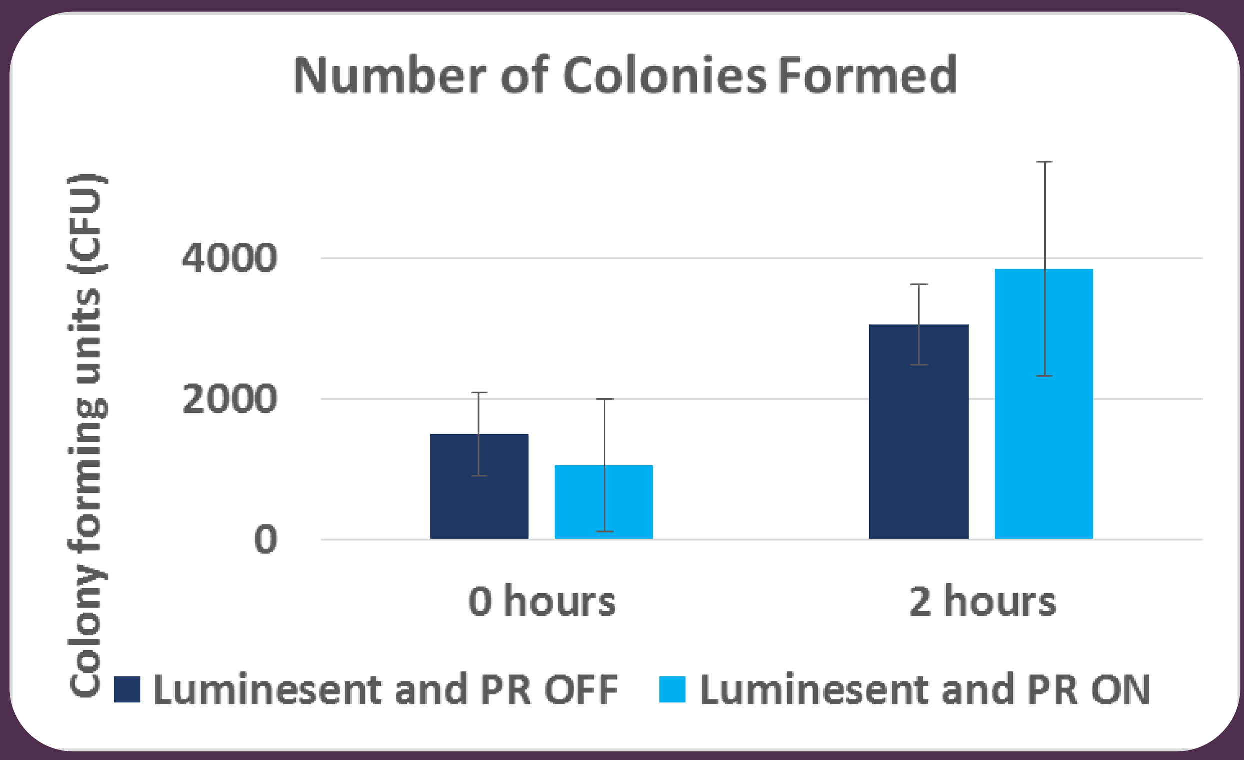

Figure 6. The number of colony forming units based upon time of collection.

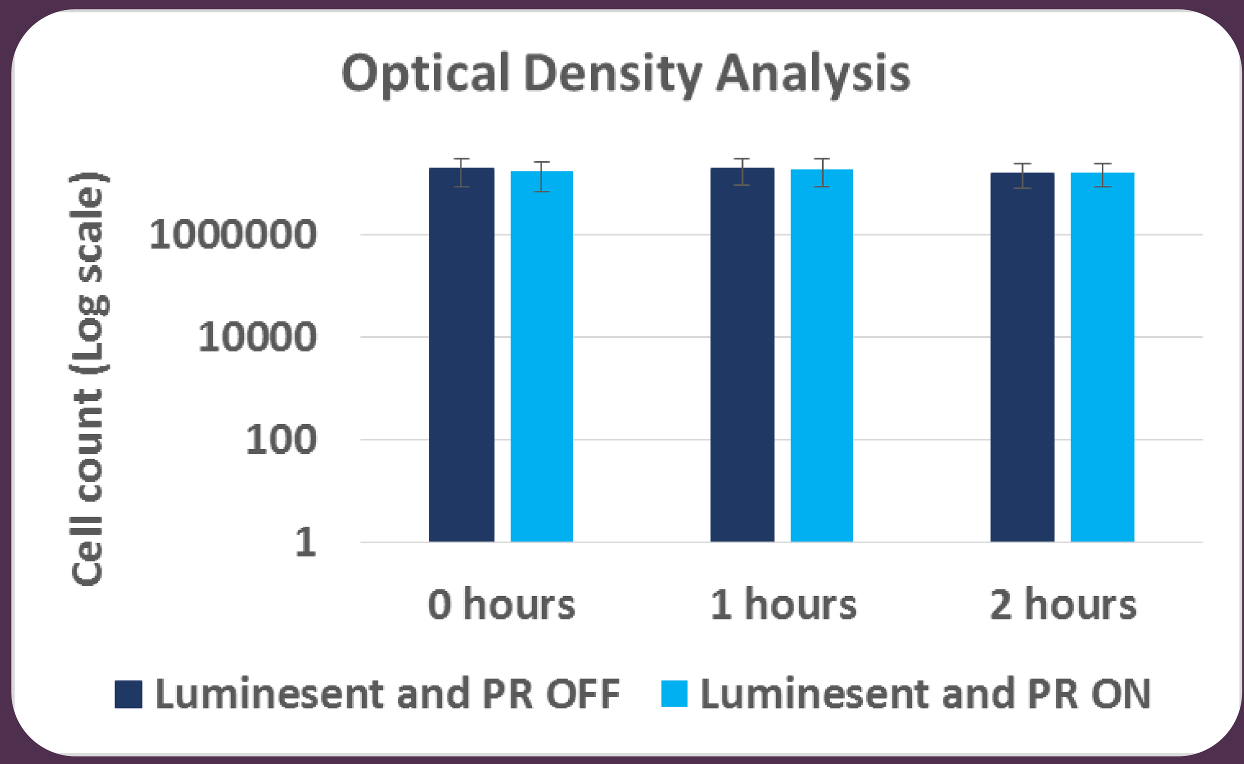

Figure 7. The number of cells based upon time of collection.

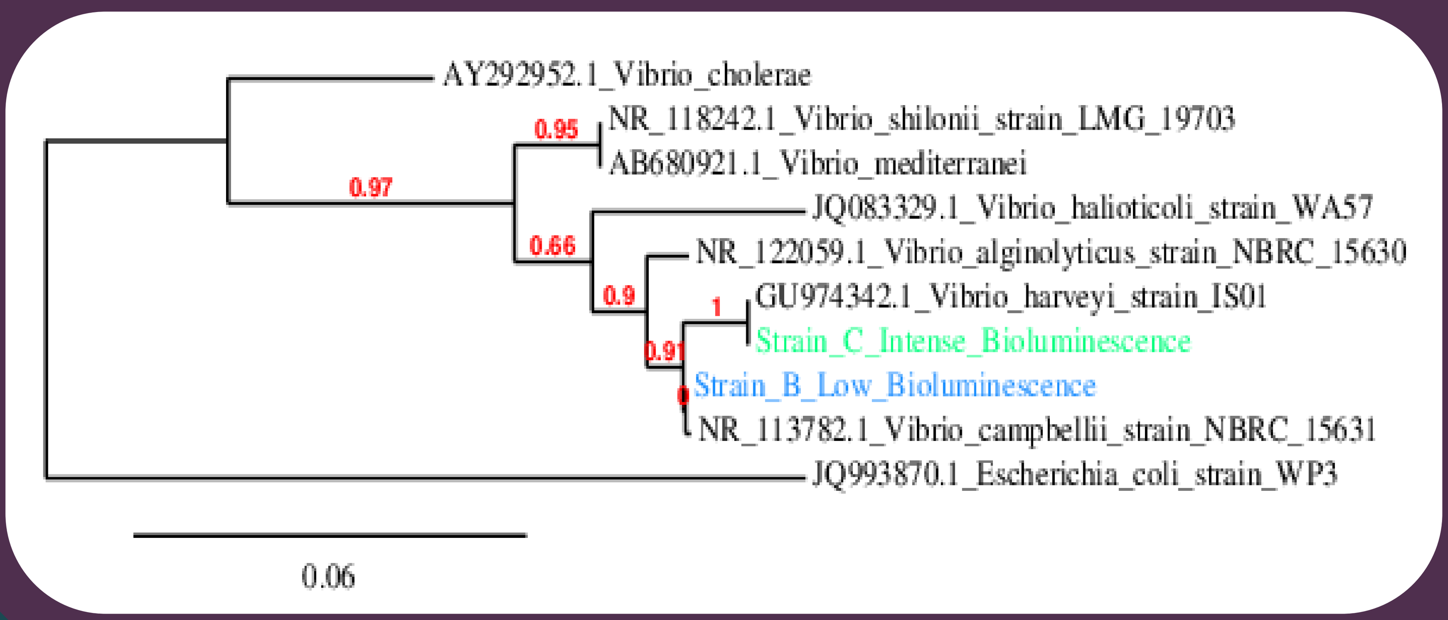

Figure 8. Phylogenetic tree used to show the evolutionary relationship between bioluminescent bacteria.

The 16s region is unique to every bacteria and can be sequenced in order to identify colonies. The results of the 16s sequencing of the strain C we had isolated revealed it to be *Vibrio harveyi* and strain B to be *Vibrio campbelli* (Figure 8). Figure 6 shows the number of bacterial colonies containing BPR observed in the presence or absence of retinal in the culture. Figure 7 shows the number of cells counted from 96-well plates. This was calculated by using the optical density (OD) which was obtained from analysis by the TECAN robot. The time points are based upon the amount of time after the sample was taken from the culture.

Future Directions:

- Grow the bioluminescent bacteria and BPR in cultures side by side
- Use other types of PR that absorb different wavelengths of light
- Use other bacteria such as photosynthetic bacteria or carotenoids which can also absorb light as a substitute for PR
- Attempt to create a single strain which can harvest light and emit light

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Conclusions:

- **The strains isolated were unable to undergo transformation with proteorhodopsin**
- **Bacteria containing PR and Luminescent Bacteria are able to grow together in co-culture**
- **BPR is able to absorb wavelengths of light similar to what is emitted by bioluminescent bacteria**
- **Co-culturing did not yield significant advantages to bacteria containing PR or BB**

References:

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