BIOLUMINESCENCE IN MARINE ORGANISMS AND TERRESTRIAL FLOWERS

Submitted for the course: Microbiology (BIT1007) - J Component

B. TECH

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CERTIFICATE

This is to certify that the project work entitled "Bioluminescence in Marine Organisms and Terrestrial Flowers" that is being submitted by "**Mohith M, Arunkumar R.K., Pranavi J, Aishwarya Magesh and Sathyanarayanan Venkatakrishnan**", for **Microbiology (BIT1007)** is a record of bonafide word done under my supervision. The contents of this Project Work, in full or in parts, have neither been taken from any other source nor have been submitted for any other CAL course.

Place: Vellore

Date: 3rd May, 2017.

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ABSTRACT

Bioluminescence is a phenomenon of nature where light is emitted by a living organism due to some chemical reactions occurring within or on the surface of its body. Bioluminescence ranges from bright to dim glow in shades of blue, green, yellow and red. While blue glow is more common in the ocean life, green, yellow and red are common in the terrestrial life. Our aim of the project was to isolate bioluminescent bacteria from marine organisms and identify the genus by a series of biochemical analysis. Moreover, we wanted to find out if terrestrial flowers showed the phenomenon of bioluminescence.

Further research of the phenomenon of bioluminescence if utilised efficiently can be extended in providing alternate sources of light energy which is environmentally friendly and affordable.

INTRODUCTION

Bioluminescence:

Bioluminescence is the production and emission of light by a living organism as the result of a chemical reaction during which excess chemical energy is converted to light energy.

The etymology of bioluminescence comes from two Greek words, bios –living and lumen – light. Bioluminescence occurs in a wide range of organisms, including vertebrates, invertebrates, microorganisms, and some fungi. Luminescent bacteria that produce light have a symbiotic relationship within other organisms. Bioluminescence is a type of chemiluminescence due to the production of light by a chemical reaction. There are two molecules that are produced by the organisms, luciferin (a pigment) and luciferase (an enzyme). The chemical reaction that produces luminescence can occur within or outside the body of the organism.

Bacterial Bioluminescence:

Luminous bacteria are the most widely distributed light-emitting organisms with the majority existing in seawater and the remaining living in the terrestrial or freshwater environment. While most species of luminescent bacteria are capable of living free, the majority are found in nature living symbiotically with host organisms (i.e., fishes, squids, crabs, nematodes, etc.).

In symbiosis, the bacteria are nourished with readily available food sources for growth, and at the same time the host utilizes the adopted illumination to communicate, to attract prey, and to masquerade itself from predators.

There are three major genera, into which most luminous bacteria are classified; *Photobacterium, Vibrio,* and *Photorhabdus*. Species existing in the marine environment are mainly categorized into the *Photobacterium* and *Vibrio* genera, and the terrestrial species are classified into the *Photorhabdus* genus. Species within the *Photobacterium* genus are generally light organ symbionts of marine animals, whereas the *Vibrio* species exist as free-living forms as well as symbionts in the sea.

Reaction Mechanism – Luciferin-Luciferase Reaction:

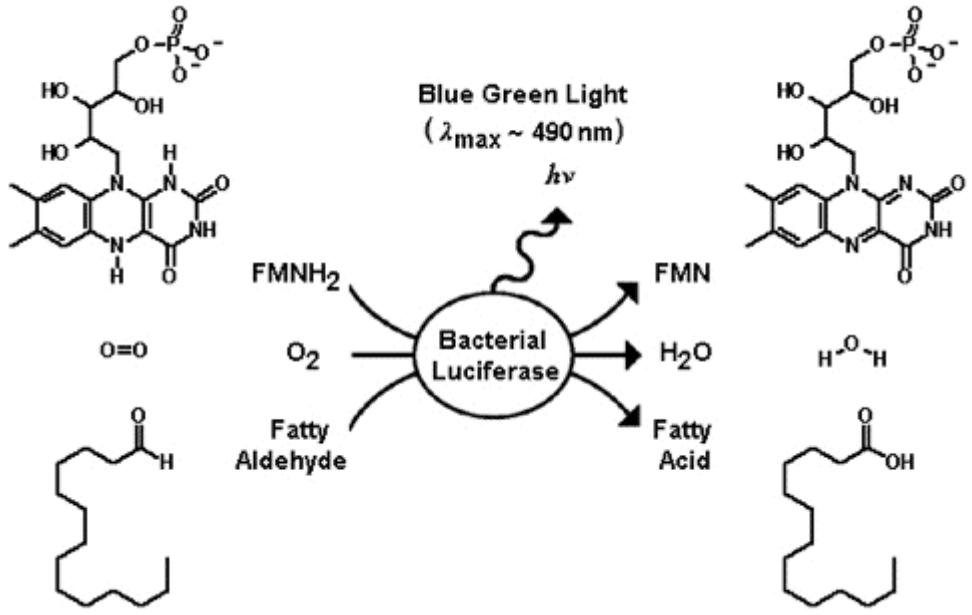


Figure: The net chemical equation of the bacterial luciferase catalyzed reaction.

Luciferases are oxidative enzymes that catalyze the bioluminescence reaction in light-emitting organisms. Bacterial luciferase is made up of two subunits, α and β. Cofactors calcium ions or ATP can mediate luciferase. The luciferase-catalyzed reaction involves the oxidation of a long-chain aldehydes and FMNH2 and results in the emission of a blue-green light. The conversion of the aldehyde to fatty acid provides most of the energy for the light emission. The oxidation of the FMNH2 provides the remaining energy in the reaction.

Luciferin reacts with oxygen to create light. Luciferin consists of a long-chained aldehyde and as a reduced riboflavin phosphate (FMNH2). Long-chain aldehydes are essential for the luminescence reaction. The bioluminescence reaction is highly specific for the reduced riboflavin phosphate (FMNH2). The excess energy, which is liberated from the oxidation of FMNH2 and aldehyde concomitant with the reduction of molecular oxygen, is released as blue/green light emission (λ_{MAX} ~ 490 nm). The characteristic color indicates the energy level of the photon that was produced when the excited electron on the flavin chromophore returns to the ground state.

A critical component in the biochemistry of bacterial bioluminescence is molecular oxygen, which is supplied from the external cellular environment. Without the input of molecular

oxygen, luminous bacteria cannot emit light. In the bacterial luciferase-catalyzed reaction, the energy expenditure on the reduction of molecular oxygen to a per oxy reaction intermediate, and then ultimately to water serves as a trigger for releasing the potential energy from the oxidation of both FMNH2 and fatty aldehyde in the form of photon emission.

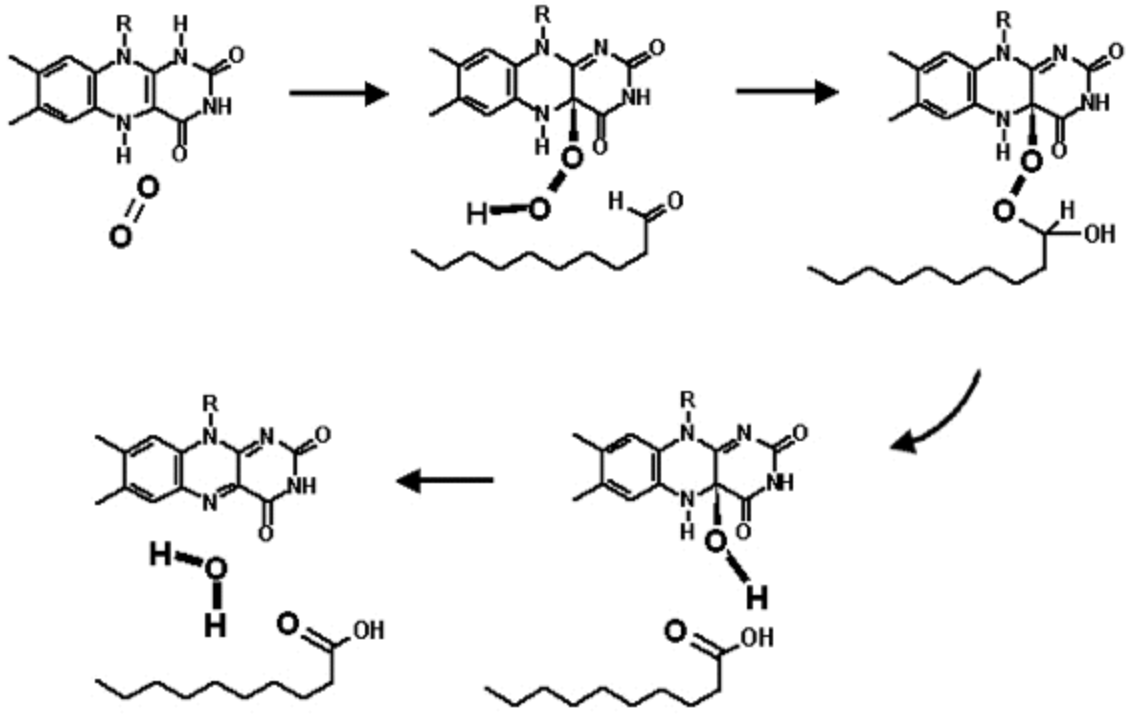


Figure: The chemical mechanism of the substrate-substrate interactions in the luciferase catalyzed reaction.

Factors Affecting Bioluminescence:

There are many factors that influence the intensity of light emission from luminescent marine bacteria. These factors include the nutritional state of the cell, the oxygen and salt concentrations, the temperature, and the level of catabolites and cAMP (intracellular signal transduction). Light emission per bacterial cell is still very low at early stages of cell growth, even with optimal conditions. Changes in intensity also occur due to the availability of luciferase or the substrates needed for the reaction. The consequence of autoinducers unable to accumulate in a concentrated area (ex. ocean or media) is repression of luminescence. These bacteria in an

open environment produce very low levels of light compared to bacteria in confined environment.

METHODOLOGY:

We tested for bioluminescence in terrestrial flowers as well as in marine fishes. For fishes serial dilution followed with streak plate technique was done and for flowers pour plate was done.

The following fishes were used for streaking:

- Threadfin bream (Sankara fish)
- Indian mackerel (Aiyla fish)

The following flowers were used for pour plate technique:

- Nelumbo nucifera (Indian white lotus)
- Lilium longiflorum (Easter lily)
- Helianthus anuus (Sunflower)

Fish:

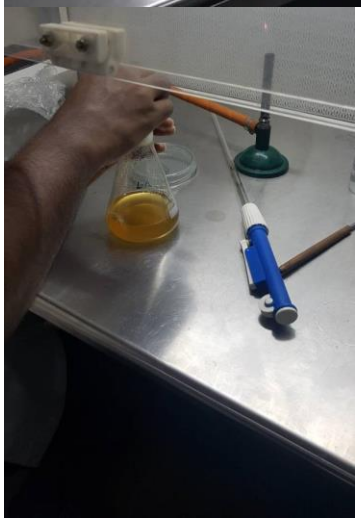
- BOSS medium and Luminescent Agar medium were prepared.
- Both Aiyla fish and Sankara fish were gutted.
- Streaking was done with gut material as well as the shiny scale part of the fish in the already prepared BOSS medium and Luminescent Agar.
- It was kept in the incubator for 24 hours and the results were observed.

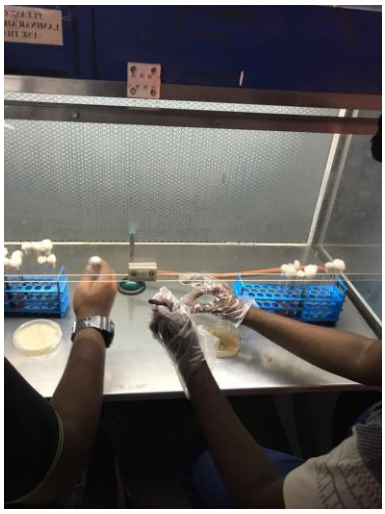
Flowers:

- Flower petals were cut into small parts.
- Serial dilution was done with three flowers up to the concentration of 10^{-3}.

- After serial dilution, pour plate technique was carried out on 1ml of the three flower samples using BOSS medium and Luminescent Agar.
- It was kept for incubation for 24 hours and the results were noted.





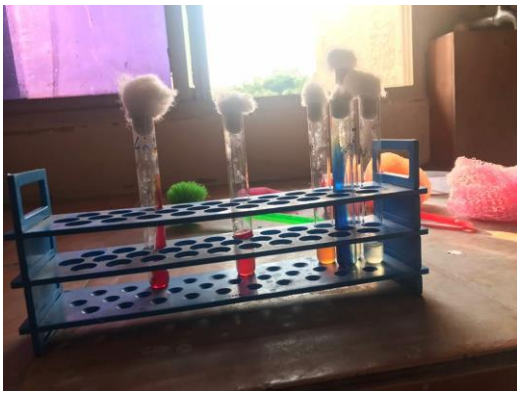


OBSERVATIONS

- No bioluminescence was observed in terrestrial plants.
- No bioluminescence was observed in the guts of Aiyla or Sankara fish.
- Slight bioluminescence was observed in the medium which used sample from the shiny scale part of the fish for both Aiyla and Sankara fish.

Biochemical Test:

- We conducted a test for identification of Gram positive or negative bacteria. For the isolated bacteria, we conducted mannitol salt agar test which showed negative result.
- For the all the series of tests from IMVIC, the bacteria isolated showed negative result.
- Mannitol motility test was also carried out which yielded positive result.
- Oxidase test was also conducted and gave a positive result.



RESULTS AND DISCUSSIONS

We observed slight blue green coloured bioluminescence in two plates one with the thread fin bream and Indian mackerel scales. So exclusively biochemical tests were performed with the thread fin bream fish and the results were observed. No bioluminescence was observed in the

streak plate done from the gut material of Indian mackerel. Even terrestrial flowers such as petals of lily, lotus and sunflower were checked for the presence of bioluminescence, but it did not yield positive results. We chose the flowers specifically because lightly coloured flowers can be expected to show luminous character in night times.

From our observations, we can conclude that the species that we had isolated is of *Vibrio* genus and it could be *Vibrio fischerii* which is Gram negative, oxidase-positive, motile and composed of a cell wall that consists of an outer membrane containing lipopolysaccharides.

CONCLUSION

Therefore, we can conclude that bioluminescence is a phenomenon commonly present in marine organisms but not present in terrestrial flowers. Moreover, it has been found out that bioluminescence was observed due to the bacterial genus Vibrio, which is in a symbiotic relationship with the fish species.

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