Isolation and Characterisation of Bioluminescent Bacteria

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Abstract- *Bioluminescent bacteria are widespread in natural environment. Over the years many researcher have been studying the physiology, biochemistry & genetic control of bacterial bioluminescence. So far many problems have been experience in cultivating these bacteria in labs & maintaining their luminescence with the use of simple laboratory level microbiology practices. This project related to isolation of bioluminescent bacteria from various sample sources such as Shrimp, Squid, Octopus & fish like Raja-Rani (vernacular name) & their characterization. Bioluminescent bacteria are symbiotically associated with these organisms. These bacteria can be grown on specially designed media such as BOSS medium, NaCl complete medium. These bacteria can be used in area of environmental Microbiology using bioluminescent genes as biosensors for environmental studies. These bacteria glow in dark so they are easily identified by observing in dark..*

Keywords- Bioluminescence, BOSS medium, Octopus, Squid.

I. INTRODUCTION

Bioluminescence is the emission of visible light by biological systems, which arises from enzyme-catalyzed chemical reactions. It is from of chemo luminescence, that it occurs in living organisms & requires an enzyme catalyst. Luminescent species are found among marine & terrestrial bacteria , annelids or segmented worms (e.g., fire worms), beetles (e.g.,fireflirs, click beetles, railroadworms), algae(e.g., dinoflagellates), crustaceans (e.g., sahrimp, ostracod), mollusks(e.g.,squid,clams), coelenterates (e.g., jellyfish, sea pansies, hydroids), bony fish (e.g., hatchet fish, flashlight fish, pony fish), & cartilaginous fish(e.g., sharks)

We are interested in bioluminescent bacteria because following reasons:

- Glow at room temperature.
- Grow rapidly.
- Easy to identify without microscope.
- Does not require expensive equipment for maintenance of culture.

Application of Bioluminescence:

- Landmine Detection using bioluminescent bacteria is a current line of research at Johns Hopkins University, Baltimore, MD. Present problems in method for landmine detection are slow, inefficient, & hazardous. Research is being conducted using bioluminescent bacteria to detect NO_2 gas, one of the main chemicals released as TNT decomposes underground. This sensitive technique could provide an early warning system & therefore increase safety in landmine detection.

Some fun applications & ideas exist such as the prospect of luminous Christmas Trees & walkways in addition to luminescent beer & champagne. Luminous jewellary is already in use by tribal communities & could make their way to our shop & malts.

II. MATERIAL & METHOD

SR. NO.	GLASSWARES	MATERIAL FOR DESSECTION	CHEMICALS	SPECIAL INSTRUMENT
1.	Conical flaks(50ml,100ml,250ml)	Sharp blade	NaCl(fisher scientific)	Autoclave
2.	Measuring cylinders(10ml,50ml,100ml)	Forceps	Peptone(Bacteriological granules)	Incubator
3.	Beakers	Pointer	Yeast extract(LobaChemie Pvt Ltd.)	Shaker incubator
4	Petriplates	Scalpels	Beef extract	Deep Refrigerator
5.	Test-tubes(bumper tubes,dilution tubes)	scissor	Glycerol(98 percent pure)	
6.	Pipettes(1ml,2ml,5ml)		Tryptone(Pancreatic digestion of casein)	
7.			Absolute Ethanol	
8.			Agar	

ISOLATION OF BIOLUMINESCENT BACTERIA FROM SQUID

Squid is a marine organism; belong to phylum mollusc having character like soft body. Bioluminescent bacteria are symbiotically associated to this organism and are presents in its ink sac. Ink sac is specialized organ of squid having ink like substance inside it which function is in defence mechanisms against predator. Now we are trying to isolate bioluminescent bacteria from squid hence we purchased squid from fish- market of camp-area, Pune but major problem ahead us was availability of squid because squid is not

common in Indian food culture but finally we got squid. This time we searched on internet about Squid's anatomy and we collected information about squid anatomy after knowing sufficiently about squid we inoculated its ink sac into Boss medium and kept for incubation in shaker incubator at 27°C overnight.

Fig: A photograph of a squid

ISOLATION OF BIOLUMINESCENT BACTERIA FROM WOODENSTICK

We got a sample from a region 'ambolighat'. It was a wooden stick. When observed it in dark, it showed luminescence. It has some growth on its surface which was showing luminescence. We scrape the glowing part from stick and transferred it to BOSS medium. We incubated it in shaker incubator at 27°C for 24 hrs.

Fig: Ink sac of squid

ISOLATION OF BIOLUMINESCENT BACTERIA FROM RAJA-RANI FISH-

While doing literature survey, we found a paper published in journal of microbiology published by University of Mumbai. The journal mentions that bioluminescent bacteria present on the scales of Raja-Rani fish (vernacular name). we collected its scales and inoculated into BOSS broth but this time is not used controlled temperature for incubation we kept it on shaker incubator at room temperature.

Fig: A photograph of a Raja-Rani

ISOLATION OF BIOLUMINESCENT BACTERIA FROM OCTOPUS-

The arms of octopuses are often distinguished from the feeding tentacles found in squid and cuttlefish. These bacteria can be grown on specially designed media such as BOSS medium, NaCl complete medium.

Fig: A photograph of a Octopus

SR NO.	SOURCE MATERIAL	MEDIA	INCUBATION TIME (IN Hrs)	INCUBATION Temp.°C
1.	Squid (2 squids were used)	BOSS	18-24	25°C,Room temperature
2.	Wooden Stick	BOSS	18-24	27°C
3.	Raja-Rani fish	BOSS	18-24	Room temperature
4.	Octopus	BOSS	18-24	Room temperature

III. RESULT & DISCUSSION

We succeeded in isolation of bioluminescent bacteria from squid in two attempts. However, we are still trying to find out the reasons behind the reduction of glow after 24hrs.comparing the conditions of squids, which we used to for isolation of bacteria; the condition of sample such as freshness, storage condition, etc plays an important role.

There is less chances of getting the bioluminescent bacteria's when we used older specimen. As these bacteria are symbiotically associated with squids. As squid starts decaying, the bacteria may also dies. We remain unsuccessful in process of isolation of bioluminescent organisms from the sample (WOOEN STICK) which we got from the region 'ambolighat'. After getting another such sample we will inoculate it in different mediums to get luminescence. We were unsuccessful in isolation of bioluminescent organisms from octopus and Raja-Rani fish. No any glow was obtained. Temperature is another factor which important role. These bacteria show luminescence when temperature is between 20-30^{o}C. if temperature increases beyond this range the growth of bacteria is hampered which affect property of bioluminescence. The media also plays crucial role in getting bioluminescence. The media we used has higher concentration of salt. Also carbon source has a crucial role. So a new media with different carbon source can be formulated so as to get bioluminescence for longer duration.

IV. CONCLUSION

We succeeded in isolation of bioluminescent bacteria from squidbutwe remain unsuccessful in process of isolation of bioluminescent organisms from the sample Wooen Stick. Media also plays crucial role in getting bioluminescence. Optimization of carbon source is very important factor in this study.

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