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Screening and Selection of Bioluminescent strains from Marine fishes

[1]Sushma M,[2]Kalpana Ramamurthy,[3]Sahana R. K, [4]Mariraj Murugan, [5]MuthukrishnanP, [5]Vijayaraghavan R and [6]Kalidoss Rajendran*

Author's Affiliation:

[1]Department of Microbiology, Jaya College of Arts and Science, Chennai, Tamil Nadu.

[2]Former faculty,Department of Microbiology, Jaya College of Arts and Science, Chennai, Tamil Nadu, Currently, PGT Biology, Arsha Vidhya Mandir, Guindy, Chennai.

[3]Department of Biotechnology, SRM Institute of Science and Technology, Tamil Nadu, India

[4]Department of Botany, Sri Kaliswari College, Sivakasi. Tamil Nadu, India.

[5]Associate Professor,Department of Microbiology, PSG College of Arts & Science, Coimbatore.

[6*]Research faculty, Department of Microbiology, PSG College of Arts & Science, Coimbatore.

ABSTRACT:

Bioluminescence is truly an amazing natural phenomenon. It plays a very large role in nature and if we humans can uncover its secrets, bioluminescence could become an incredible resource with thousands of many more important applications. This studytargets at the screening and selection of Bioluminescent strain from squids and shrimps. Four kinds of samples – squids, Deep sea shrimps, prawns and shankara were processed for the isolation of Bioluminescent bacteria. These bacteria were biochemically characterized into different species for which various tests were performed. All these species exhibited blue-green colour luminescence. The luminescence faded from bright to dim light after 24-58 hrs. Again when the culture was inoculated freshly, bright luminescence was seen. Boss medium produced bright luminescence when compared to that of luminescence medium. Plasmid curing was performed using ethidium bromide in order to prove that the luminescence is due to gene products coded by chromosomal DNA and not plasmid DNA. The chromosomal DNA was isolated and run on Agarsoe Gel Electrophoresis which showed a large band near the well. This indicates that the chromosomal DNA as a whole was having a high molecular weight and hence migration was not efficient.

Keywords-Shrimps, squids, Plasmid curing etc.

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1. Introduction

When organic chemicals (luciferns) undergo oxidation, which is facilitated by the enzyme luciferase, living things emit visible light. This phenomenon is known as bioluminescence. Squid, algae, insects, fish, fungi and bacteria are examples of luminescent species found in both marine fresh water and terrestrial environments. Bacteria are the most common and numerous luminous organisms in nature. Although their elementary habitats are found in the ocean and most of them are with free living, "symbiotic," "saprophytic," or "parasitic relationships" some of the "luminescent bacteria" are found in "terrestrial and freshwater habitats [1]. The

important luminescence components (lux) system is the "enzyme luciferase" and the "corresponding lux genes." These data are directly gathered from the extended studies from bacteria of marine such as "*Vibrio* and *Photobacterium"* and from the "terrestrial bacteria" named "*Xenorhabdus* genus," in particular the "*Vibrio harvei, Vibrio fischeri, photobacterium phosphoreum, photobacterium leiognathi* and *Xenorhabdus luminescens"* species [2]. The light-emitting reactions of diverse species have been discovered to be very distinct, with the only shared component being the structure of luciferase and related genes from one luminous organism to the next.

The mutual interaction among "squid and *V. fischeri"* provides an important instance of "specific cooperatively" during the establishment of both organisms [3]. For instance, the premature squid gets a bacterial infection that initiates the early development of the light organs. When hatching squid are nurtured in sterile sea water, the pouches that form the fully developed organ do not expand, according to studies. Thus, the symbiotic interaction between the Squid and *V. fischeri* has a direct impact on the physical growth of the squid's light organ.

The "luminescent bacteria" create a beneficial atmosphere for the squid to survive a "nocturnal forager", by enhancing the incidents of light that would generally be called "moon's rays struck" through vertical incidence upon the organism, thus ensuring the survival of the squid from the "predators below". In return, the squid offers the bacteria a safe refuge and a consistent supply of nutrients. Moreover, the regulation of luminescence by microbes makes sure that the maximum dose of autoinducer signifies that the microbial load has reached a sufficiently high load that the energy spent in illuminating the squid is probably going to be reimbursed in the form of protection and food.

In the luciferase-catalyzed bacterial luminescence reaction, a reduced flavin mononucleotide (FMNH2) and a long-chain alphatic aldehyde are oxidized [4] and [5], releasing a blue-green light at 490 nm.

The agents involved in this reaction are comparatively simple molecules that share a close relationship with the fundamental metabolites of the cell.

"Genes (*lux*AB)" encoding for the sub-units of the luciferase instigates this reaction. The "lux CDE" encodes for "fatty acid reductase" and G for the synthesis of "flavin reductase" [6]. They were numbered from the "lux operons" of "luminescent bacteria" from three types: "*Vibrio, Xenorhabdus* and *Photobacterium*. "Despite the presence of twenty-one "lux genes" reported in 3 organisms, five genes are common in all "illumine scent bacteria."

Light generated by a chemical reaction inside an organism is known as "bioluminescence". "Phosphorescence" and "fluorescence" are not synonymous with bioluminescence. Fluorescence refers to energy emission from a light source that gets established and extracted as the main particle of a photon. In "bioluminescence" or "chemiluminescence," the "excitation energy" is provided by the emission of energy thorough the chemical reaction occurring within the body of a living organism. Two types of chemical compounds act as the main ingredients for luminescence. One of them, which creates the light is named as "luciferin," and the other one acting as the bioenzyme enhancing the speed of the reaction is known as "luciferace". The luciferase develops the "oxidation of luciferin" that emits light and generates a by-product named "oxyluciferin," which is typically inactive. The lucifering-luciferace bond is known as a single unit named a "photoprotein". The photoprotein molecule is exercised through light production by typical ionization and is mixed with calcium for frequent system development.

Numerous luminous bacteria have had their luciferase studied, purified, and isolated, including five different types of marine bacterial species such as *Beneckea harveyi, Beneckea splendid, Photobacterium fischeri, Photobacterium phosphoreum* and *Photobacterium leiognathid*, and two fresh water terrestrial species, namely "*Vibrio cholera"* biotype "*albensis* and *Xenorhabdus luminescence*. The β and α subunits of bacterial luciferases have a molecular weights of 40 and 35 KDa, respectively [7]. The action of luciferase is specific for the aliphatic aldehyde moiety and FMNH2.

2. Objectives

To isolate symbiotic bacteria from squids, deep sea prawns and deepsea mud shrimps.

To biochemically characterize the bioluminescent bacteria isolated

To confirm the bioluminescent character due

to chromosomal DNA and not plasmid DNA by plasmid curing
To isolate genomic DNA
To run the isolated DNA on agarose gel electrophoresis and to determine its molecular weight
To estimate the isolated chromosomal DNA.

3. Scope and Methodology

Bioluminescent bacteria have risen to be an interesting organism with varying applications in the field of Molecular biology. Dealing with this organism always raise interesting queries and is also an enchanting experience. The squids have been purchased from the following fish markets, Kasimedu landing centre and Royapuram fish market. Prawns and deep sea shrimps were obtained from Kasimedu fish market. The media chemicals were obtained from Himedia Laboratory Limited.

Specimens

A total of three squids, four deep sea mud shrimps, four deep sea prawns and three shankara fish were collected and analyzed for study (Table 1). The specimens were placed in a container with sea water, surrounded by ice and transported to the laboratory. The prawns and shrimps were half immersed in sea water and kept in the refrigerator for 2-33 days undisturbed.

Table 1 shows the samples used for the isolation of bioluminescent strains

S. No	**Name of Sample**	**Zoological Name**	**Vernacular name**	**Total**
1	Common squid	*Loligo Duvancelli*	Oosikanavai	2
2	Bobtail squid	*Eurpymna scolopes*	Kanavai	1
3	Deep sea mud shrimp	*Solenocera hextii*	Aalkadaleraal	4
4	Deep sea prawn	*Aristeus antennatus*	Kadaleraal	4
5	Shankara	Lutjanus campechanus	Shankaraa	3

Isolation techniques

The squid sample was transferred to a clean tray. The organ was dissected and placed on Boss medium and LM medium [8]. The plates were incubated at 18-22℃ for luminous colonies. The prawns were half immersed in sea water soon after their collection and stored in the refrigerator for 2-3 days. Using sterile toothpicks, the body surface of the prawns were swabbed and inoculated on Boss and LM media. The shrimps were half immersed in sea water soon after their collection and stored in the refrigerator for 1-2 days. The organisms on the body surface were aseptically inoculated in Boss and LM media using sterile tooth picks. Gram's staining, motility, Nitrate reduction, Indole, Methyl red, Citrate Utilization, catalase, oxidase, ONPG, sugar assimilation tests were done for the isolates according to the procedure described by [9]. Gelatin test was done by the procedure of [10].Aminoacid decarboxylation test was done by the procedure of [11].Antimicrobial test was carried out according to the procedure described by [12]. The plasmid curing was done by the procedure of [13]. The genomic DNA was isolated and separated in an agarose gel using the procedure described by [14]. The concentration ratio of DNA at A260/A280 was done using the spectrophotometer. The amount of DNA was quantified using the general rule that

1 unit of A260 = 50 µg of DNA/mL

Literature Review

[15] have described members of at least 11 teleost genera-based aquatic bodies that include specific organs for emission of light. It is in these organs where bacterial infection results in the generation of bioluminescence. [16] have analysed the bacterial symbiont colonizing the light emitting organ of a

number of marine squid and fish species. [17] explains if luciferase synthesis is low, explains the ability of *invivo* acting based on decreased luciferace syntheisis and substrate generation lowering.

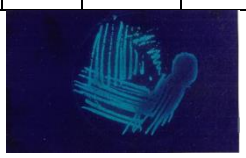
4. Result and Discussion

A total of 11 squids and prawn samples were analyzed for this study and five different luminous bacteria were isolated (Tables 2 and 3, Figure 1). All the species were found to be Gram negative rods and actively motile. The species were identified by performing biochemical tests (Tables 4 and 5). All the organisms produced bioluminescence and it can be observed in dark conditions while it was normal in a day light (Figure 1). All the species produced blue green colour luminescence. *V. harveyi* showed the maximum incidence over all other luminous organisms among the four kinds of samples tested. *V.splendidus*, *V.orientalis* and *V.logei* had the lowest incidence. From the antibiogram studies, *V.harveyi* was found to be resistant to both Ampicillin and Penicillin. Figure 2 shows the plasmid curing and the luminescence encoded by chromosomal DNA. Chromosomal DNA was isolated and separated (Figure 3). The concentration ratio of DNA in the spectrophotometer A260/A280 was found to be 1.044. The amount was calculated to be 205 microgram /mL.

Table 2. Shows the bioluminescent variants isolated

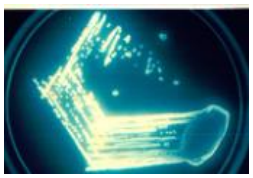
Sl. No	***Tests***	**Bioluminescent strains**				
		V. logei	***V.harveyi***	***V.fischeri***	***V.splendidus***	***V.orientalis***
1	Arginine	Negative	Negative	Negative	Positive	Late positive
2	Lysine	Positive	Positive	Positive	Negative	Positive
3	Ornithine	Positive	Positive	Negative	Negative	Negative
4	Gela	Neg	Positi	Neg	Positi	Posit
	tinase	ative	ve	ative	ve	ive

***V.harveyi* in day light**

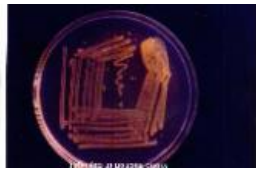


***V.harveyi* in dark**

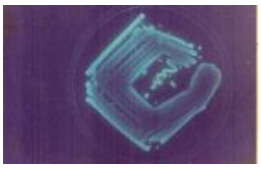
***V.logei* in day light**



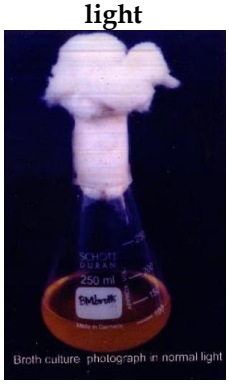
***V.logei* in dark**



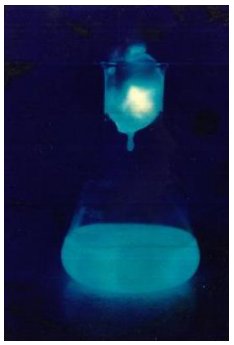
***V.fischeri* in day light**



***V.fischeri* in dark**



Broth culture in normal light

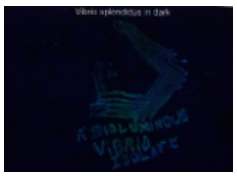


Broth culture in dark

***V.orientalis*in day light**

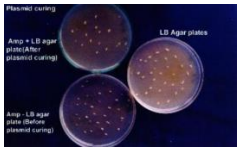
***V.orientalis*in dark**

***V.splendidus* in day light**



***V.splendidus* in dark**

Figure 1 shows different luminous bacteria isolated from the different samples



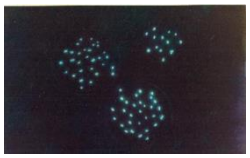


Figure 2 shows the plasmid curing doesn't affect the luminescence of isolates to prove the luminescence is chromosomal DNA encoded

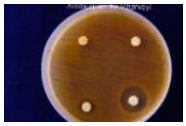


Figure 3 shows the chromosomal DNA separated in an agarose gel and antibiogram of an isolate.

Table 3 shows five different luminous bacteria isolated from the different samples of squids and prawn

S.No	Name of Sample	Name of organism isolated
1	Common squid	*V. harveyi, V.fischeri*
2	Bobtail squid	*V.harveyi, V.splendidus*
3	Deep sea mud shrimp	*V.harveyi, V.logei, V.orientalis*
4	Deep sea prawn	*V.fischeri, V.splendidus, V.orientalis*
5	Shankara	*V.harveyi, V.logei*

Table 4 shows the species identification using the biochemical tests

Sl. No	Organism	Mot	Ox	Cat	Ind	VP	Glu
1	*V.harveyi*	+	+	+	+	-	-
2	*V. fischeri*	+	+	+	+	-	-
3	*V.logei*	+	+	+	-	-	-
4	*V.splendidus*	_	+	+	-	-	-
5	*V.orientalis*	_	+	+	-	-	d

Ox – Oxidase Cat – catalase Ind – Indole test VP – Voges Proskauer test Glu – Glucose

Table 5 shows the species identification using the biochemical tests

Sl. No	Organism	Lac	Suc	Man	Xyl	ONPG	Nit
1	*V.harveyi*	-	-	+	-	+	+
2	*V. fischeri*	-	-	+	-	-	+
3	*V.logei*	-	-	+	+	-	+
4	*V.splendidus*	-	+	+	+	-	+
5	*V.orientalis*	d	+	+	-	+	+

Lac – Lactose Suc –sucrose testMan – Mannitol test Xyl – Xylose test ONPG – O-Nitro-phenol B galactose Nit –Nitrate reduction test

5. Discussion

Bioluminescence has been observed more in marine environment than in terrestrial. Bioluminescence is used in nature for a variety of purposes, ranging from communication to defense against enemies. Bacterial bioluminescence is confined in the genera of Vibrio and photobacterium. Among these, Vibrio genus showed maximum distribution as free-living forms, symbiotic with marine organisms like squid and even parasitic or saprophytic on many fishes and crustaceans and it was well in agreement with [18].*V. fischeri* was isolated from *Eurpymnascolopes*as a pure culture as stated by [19]. All the luminous species produced blue-green colour luminescence. *V.logei*, *V.orientalis* and *V.harveyi* were found saprophytic on shrimps and prawns as stated by [20].*V.harveyi* showed maxium incidence rather than any other species.

The luminescence was due to gene products coded by chromosomal DNA rather than plasmid DNA. This finding is in accordance with [21]. The Chromosome-based DNA was isolated and purified. It was run on agarose gel electrophoresis. Pure DNA was obtained. The molecular weight of isolated DNA was found to be 43,100 bp.

6. Limitations and Research Gaps

Due to constraint of time and resources, a pause is created at this point in the venture of bioluminescence.

7. Conclusion

The chromosomal DNA isolated can be further processed for restriction analysis and can be investigated for "lux genes". The past 50 years have witnessed great progress in all facets of our knowledge based on luminous bacteria. Of particular importance are the applications of bioluminescence in almost all the fields like Medicine, Agricultural science, environmental science or molecular biology. For ex. "Lex genes" are used as molecular reporters which are non-hazardous, unlike "radioactive isotopes." There should always be a place in life for an organism which lives in its own distinctive way. "Bioluminescent" systems thus offer the possibility for studying evolutionary adaptive processes by following a readily identifiable trait, the genetic, biochemical and physiological parameters.

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