Isolation and Identification of Bioluminescent Organism From Marine Water Sample

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Abstract- *Bioluminescence is the emission of light, through a chemical reaction, by living organisms. Around 700 genera are found to be luminescent in whole world. Most of the organism that emit light share the chemical components involved in light production, these are referred as luciferin and luciferase. In present study, five bioluminescent bacterial strains were isolated from 3 marine water samples collected from beaches of Goa, Pondicherry, Kashid. Enrichment of bioluminescent bacteria was done in artificial sea water broth (SWB) and Luminescent Broth (LB). Purification of the bacteria was done on LA (Luminescent Agar).The isolated pure cultures were then identified by MALDI-TOF MS, where they showed similarity with two well-known bioluminescent bacterial species viz. Vibioharveyi and Vibrio parahaemolyticus.*

Keywords- Bioluminescent bacteria, MALDI-TOF MS.

I. INTRODUCTION

Bioluminescent organisms with their attractive beauty and ease of detection have drawn interest among scientists to study about them. It is the ability of organisms to release visible light by using natural chemical reaction. The emission of light is a result between the enzymatic activity and biochemical of the living organism (Widder*et al.*, 2012).

There are a group of genes that are responsible for this known as the *lux* operon found in the luciferase enzymes (Heba*et al.*, 2016). The reaction of bioluminescence involves the oxidation of a long-chain aliphatic aldehyde and reduced flavin mononucleotide (FMNH2). This mechanism needs oxygen and is catalyzed by enzyme luciferase. In this process, the excess energy is liberated and emitted as a luminescent blue-green light at 490nm (Robin T., and Jan R. M., 2008).

Therefore, the reaction of bioluminescence emission of light can be written as follows:

$FMN+H_2$ + RCHO + O_2 ----- FMN +H_2O+RCOOH+ light
(490 nm)

The *lux* operon basically contains the genes *lux ICDABEG*. *LuxA*gene and *LuxB*gene are the, respectively. On the other hands, lux CDE genes are of contaminants, measurement of pollutant toxicity, and monitoring of genetically engineered bacteria released into environment. Other application of biosensor is as the indicator of cellular metabolic activity and for detection of pathogen. In the food industry, the application of bioluminescence was found to have started a few centuries earlier (Katherine J. B., and Edward G. R., 1995) where the bioluminescence was used to detect pathogens in food spoilage.

1.2 Mechanism

It is the process of production of light through chemical reaction. The development of method to isolate the luciferin-luciferase complex from tropical insects belonging to family Lampyridae offered a possibility for biochemical companies to commercially produce testsdetecting high-energy cell components, such as e.g. ATP. Most of the organism emits the share the chemical components' involved in luminescence emission these are referred as luciferin and luciferase. Luciferin recycles as a specific activity remains same for years. Not only luciferins there are other compounds which are recycling to maintain the light emission phenomenon. Luciferin is a heterodimer which consist of two polypeptide chains alpha and beta of different molecular weight encoded by different genes *luxA, luxB(*Robinson and Tonks 2011).

Luciferin + O2Oxyluciferin+ Light

II. METHODS

2.1 Sample collection

Marine water samples from two beaches on the western costs viz, Kashid beach and Goa beach the third sample was collected at Pondicherry beach situated on the East coast of India. Approximately one liter of water sample was collected in presterilized plastic bottles and brought to laboratory under ambient conditions and stored at 4°C till further use.

2.2 Enrichment of Bioluminescent bacteria

One ml of the water sample was added to 50 ml (in 150 ml flask) of artificial sea water agar broth and incubated at room temperature for overnight. After the incubation the flasks were observed for the luminescence in dark room and used for isolation of bioluminescent bacteria.

2.3 Isolation and purification of bioluminescent bacteria

A loopful of the enriched sample was streak inoculated on Boss Agar/Bioluminsecent agar plates and incubated in darkroom at room temperature for 24 hrs. After the incubation, the plates were observed in darkroom, and the bacterial colonies showing light production (bioluminescence) were marked. The plates were brought to culture room and the marked colonies were suspended in sterile saline and streak inoculated on respective media plate. The procedure was repeated for several times to get pure isolated colonies of bioluminescent bacteria. The isolates were appropriately coded and preserved in 20% Glycerol and stored at -80^{o}C.

2.4 MALDI TOF MS

Identification of Isolates was done by MALDI TOF MS.

III. RESULTS AND DISCUSSION

3.1 Isolation of Bioluminescent Bacteria

The enrichment, isolation and purification method resulted in five isolates of the bioluminescent bacteria. The cultures were appropriately coded and preserved and autoclaved in 20% Glycerol stock for long time storage at -80 C freezer.

Table No.1

Sr. No	Sample	Culture code
1	Goa	AVMC-GA
2	Pondicherry	AVMC-PD
3	Kashid	AVMC-KS1,KS2,KS4

3.2. Morphological Study of Isolates:

All the isolates were studied for their morphological characters. The Gram reaction and motility behavior is noted in Table.No.2

Isolate code	Gram character	Motility
AVMC-GA	Gram negative	Motile
AVMC-PD	Gram negative	Motile
AVMC-KS1	Gram negative	Motile
AVMC-KS2	Gram negative	Motile
AVMC-KS4	Gram negative	Motile

3.3 Identification of isolates:

MALDI Identification:

The identification of the isolate was carried out by MALDI-TOF-MS. Among the five isolates for identification by MALDI-TOF-MS by direct transfer and ethanol extraction method around 2 isolates were identified to closest match. Secure identification upto species level was achieved in most of the isolates.

(Table No.3)

Isolate	Identified organism	Score value
AVMC-GA	*Vibrio harveyi*	2.108
AVMC-PD	*Vibrio parahaemolyticus*	2.141
AVMC-KS1	*Vibrio parahaemolyticus*	2.018
AVMC-KS2	*Vibrio harveyi*	2.133
AVMC-KS4	*Vibrio parahaemolyticus*	2.098

IV. DISCUSSION AND CONCLUSION

Biodiversity is resource for Biotechnolgy. Looking to the depth of microbial diversity there is a chance of finding microorganism producing the novel biomolecules with better proerties and suitable for commercial exploitation. The physiochemical diverse habitats has challanged nature to develop equally numerous molecular adaptation in microbial world.

In the current project out total five isolates of bioluminescent bacteria were isolated from marine water (Goa, Pondicherry and Kashid beach). All the isolates were found to be Gram negative and motile rod shaped. According to MALDI-TOF MS result all the isolates were identified as *Vibrio parahemolyticu* and *Vibrio harvery*). Out of the five isolates two isolates from Kashid beach showed similarity with *Vibrio parahaemolyticus.* One isolate from Pondicherry beach was also found to be *Vibrio parahaemolyticus* and the other one from the same site was identified as *Vibrio harveyi.* While the isolate from Goa beach also showed similarity with *Vibrio harveyi.*

4.1 Significance of study

The luminous bacteria are becoming more and more popular in several fields, including medicine, pharmacology, biochemistry, bioprocessing and environmental engineering. In the recent years, microorganisms are being used as biosensors, the light emission character of this group of bacteria will be a good tool for production of biosensors.

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