Bioluminiscent bacteria as future torch lights-Screening, Characterisation.

Ved Prakash, Dipeshsingh Rajpurohit, Dr.Suneetha V.

Abstract-Bioluminescence is a phenomena in an organism which produces luminescence on quorum sensing and this phenomenon has been explored in many fields like invivo diagnostics, marker technology, protein engineering etc. and has immense potential as future torchlight too with the aid of technologies. Present study aims at the screening and identification of bioluminescent bacteria from different marine sources like fish, lobsters and crabs from the eastern coastal region of India. Total of about 10 different species of fish and some lobsters were screened for their symbiotic bioluminescent bacteria out of these 2 species of fish showed predominant presence of bioluminescent bacteria associated with them, they were namely rani fish and sukum puri. 16srRNA studies revealed bacteria to be belonging to pshychrobacter species associated with Rani fish which has been reported for the first time for showing bioluminescence..The strain sequence has been submitted in NCBI with Accession number LK931862 was named psychrobacter sp.RDP1.

Keywords: Bioluminiscence, quorum sensing , Pshychrobacter, 16SrRNA sequencing.

1. INTRODUCTION

Bioluminiscence is a kind of luminescence that occurs in wide range of organisms including bacteria, protozoa, dinoflagellates, molluscs , insects, sponges and fish. However , majority is confined to organisms found in sea, that is fish. Almost 60 to 80% of fishes in deep sea give bioluminiscence. [1] Three genera are observed under the bioluminiscent bacteria being photobacter, vibrio and photorhabdus. Vibrio and photobacter genera are confined to marine environments and account for maximum bioluminiscence found in nature. Photorhabdus species are terrestrial.[1,2] These bacteria establish a symbiotic relationship with their host and obtains nutrients and support from host for survival. In return, the host uses the luminescence provided by these organisms for its benefits which may be for defence. The bacteria forms a symbiotic relationship with the host organism, where the host provides a nutrient rich environment for the growth of the bacterium and in return benefits from the luminescence such as camouflage or protection from its predator. A basic observation done in case of these marine bacteria was the time of bioluminiscence. It showed sharp increase in luminescence when the culture reached mid-logarithmic phase. This phenomenon is termed quorum sensing and is used today on a large scale to study processess resembling the same and the cell to cell and biochemical pathways involved it.

- *Ved Prakash has completed masters degree program in Biotechnology in VIT University, India, Mob. 7845793794. E-mail: ved.prakash2012@vit.ac.in*
- *Dipeshsingh Rajpurohit has completed masters degree program in Biotechnology in VIT University, India.*

Quorum sensing is the ability of bacteria to regulate gene expression and respond to cell density and has been used to study antibiotic resistance mechanisms of pseudomonas aeruginosa and cystic fibrosis.[3] The basic observation in newly inoculated cultures of a luminescent marine bacterium like *Vibrio fischeri*, showed that the onset of exponential growth occurs without a lag but bioluminescence does not increase until mid-logarithmic phase, when it literally shoots up[4].It's induced when the individual bacteria release chemical autoinducers to alert others to its presence, and when the level of autoinducers reaches a certain density the bacteria turns on genes that react with proteins to emit the light.[5]The bacteria uses quorum sensing to coordinate their gene expression according to the local density of their population [6]. The bacterium *V.fischeri* is in single cellular form in planktonic habitats and doesn't show bioluminiscence, production of light seems a waste and hence the bacterium does not emit light.It only produces light in symbiotic relationship . The quorum sensing system of *V.fischeri* employs two genes, *luxI* and *luxR* which activate the expression of the *lux* structural genes.Bioluminiscence bacteria have found large application in the field of diagnostics or detection of pollutant. A freeze dreid recombinant bioluminescent bacteria was used to develop a toxicity detection biosensor. It had three main components, the strain, a small light proof test chamber and an optic fibre connected between sample chamber and a luminometer. The reaction giving luminescence is catalyzed by luciferase enzyme. However, other enzymes are also responsible for the luminescence generating the various substrates responsible for the emission of luminescent light. This phenomenon was recently explored for the production of light as light bulbs bearing methane with these bioluminescent bacteria. New technological platforms will still help in the usage of this

phenomenon. New bioluminescent bacteria have to be explored for their bioluminescent potential.

2. MATERIALS AND METHODS

2.1 Collection of sample

Coastal regions of Tamil nadu were explored for the sample which could be used as source of bioluminescent bacteria. Different species of fish and lobsters were collected from a local fish market of old bus stand of Vellore, Tamilnadu. The samples were stored at -20 degree Celsius or were used fresh..Fish varieties having indigenous names Rani, sukumpari, marol, pomfret, tilapia, tengapara and a few species of shrimps were used as samples.

2.2 Isolation

The fish body is first segmented and its gills, fins and gut region are taken and dipped in sea water obtained from Pondicherry coastal area. These were incubated in cold room having a temperature of about 14-16 degree celcius. After certain time mostly overnight the colonies start growing on the surface of the fish body. These are scraped and pour plate technique is performed using BOSS medium(Fig 1).The colonies are then observed in dark room ,those showing luminescence were then streaked to get the pure cultures. The composition of BOSS medium comprises of NaCl(30 gm),Glycerol (1 gm), peptone(10 gm),beef extract(3 gm) for 1 litre media to be made in distilled water with final pH of 7.3.Other than BOSS medium, luminescent agar was tested but it was not as efficient as BOSS medium.[7]

2.3 Visualization and gram nature.

Colony characteristics were studied along with gram staining to determine the gram nature of the bacteria.[8,9]

2.4 16 S rRNA sequencing and BLAST alignment

16 S rRNA gene of the bacteria was isolated and sequenced. This was followed by alignment of the sequence with other bacterial species using BLAST tool to identify the isolated bioluminescent bacteria[10].

3. RESULTS AND DISCUSSION

3.1. Isolation

Different species of fish were used for isolating the bioluminescent bacteria associated with them. The following table gives the account of presence of bioluminescent bacteria in each of the tested variety.

Marine source/fish (indegenous names)	Bioluminiscent bacteria
Sukum pari	Yes
Rani	Yes
Marol	No
Pomfret	No
Tilapia	No
Tambusa	No
Tenga para	No
Para kola	No

Apart from these a few indegenous shrimp varieties were also tested for bioluminescent bacteria but only two fish vaireities namely sukum pari and rani were able to show the luminescent bacteria associated with them. Pure cultures were streaked on the BOSS medium(Fig 2).Bacteria from rani fish(*Nemipterus japonicus*) was studied further.

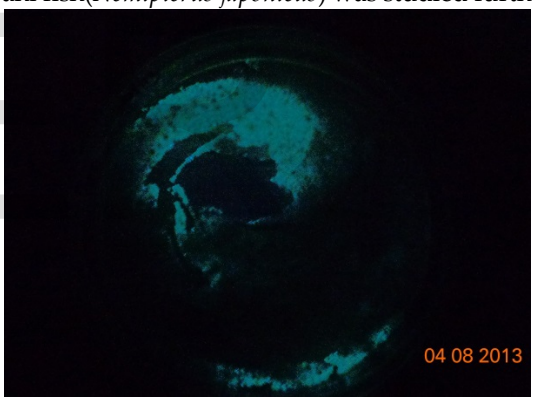

Fig 1:Pour plate in BOSS medium of the bioluminescent bacteria from Rani fish

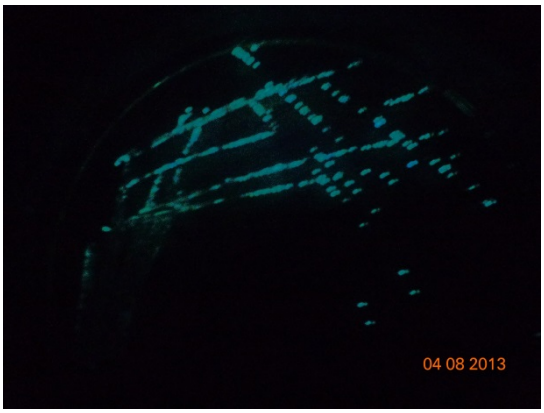

Fig 2: Streaking of the pure culture of bioluminescent bacteria from Rani fish.

3.2. Visualization and gram nature

The colony morphology of the bacterial colony isolated from rani fish was further studied and gram nature was found out to be negative with coccobacilli cells. The colonies were non pigmented, off white in colour, flat and smooth in appearance.

3.3. 16 S rRNA sequencing

The 16 S rRNA gene was sequenced and the resulting sequence was aligned using BLAST tool to get the homolous sequences which was done by Acme Progen biotech Pvt. Ltd. Salem, Tamil Nadu.

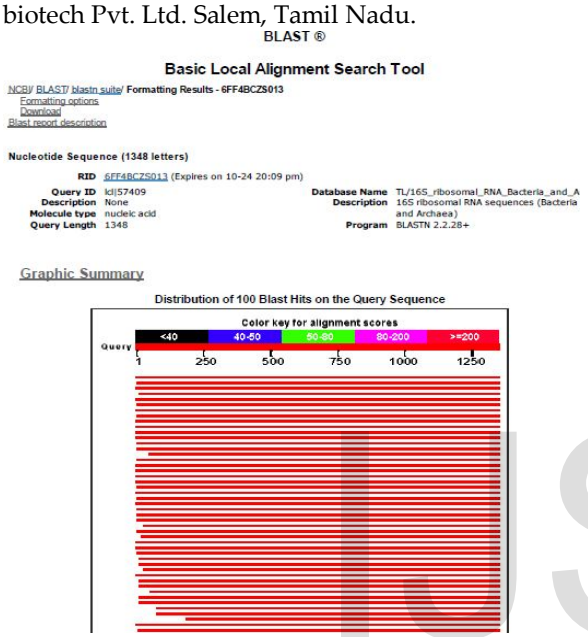

Fig 3:BLAST tool result for local alignment of 16SrRNA sequence of isolated bacteria.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Psychrobacter sp. PRwf-1 strain PRwf-1 16S ribosomal RNA, complete sequence	2484	2484	100%	0.0	99%	NR_074709.1
Psychrobacter arenosus strain R7 16S ribosomal RNA, partial sequence	2370	2370	99%	0.0	99%	NR_027204.1
Psychrobacter faecalis strain Iso-46 16S ribosomal RNA, partial sequence	2309	2309	100%	0.0	98%	NR_028966.1

Fig 4:The bacterial isolate found to be as psychrobacter species.

The results suggest the bioluminescent bacteria to be belonging to the psychrobacter genera and having 99% similarity with *Psychrobacter arenosus*.[11,12].

4. CONCLUSION

The coastal region of India shows a good promise for the presence of bioluminescent bacterial strains which could be studied further. The presence of luminescence in a psychrobacter species has been reported for the first time with no existing record of luminescence in this genera. This psychrobacter specie was found symbiotically with the rani fish(*Nemipterus japonicus*).Further studies have to be done on these bioluminescent bacteria which shows a good potential for application in varied fields like he in vivo diagnostics or imaging,biosensor development for toxicity testing and for future torch lights and many more.

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