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Original Article

Isolation and characterization of novel marine luminescent bacteria from Diu beach, India

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ABSTRACT

Aim: To carry out the isolation and characterization of novel marine luminescent bacteria from Diu beach, India.

Materials & methods: In the present study attempt has been made to isolate luminescent bacterial species from marine water sampled from Diu beach, India, using standard plating method on nutrient medium. Obtained isolate was analyzed susceptibility against antibiotics and heavy metals in order to use them in bio-sensing system. Isolated bacterium was amplified for 16S rRNA gene by PCR and further identified by aligning through BLAST and MEGA software.

Result: The *Vibrio rotiferianus* when growing prominently at 22 °C shown tolerance to low concentration of heavy metals & resistance to few antibiotics which makes isolate, a potential bio-sensing agent. Further, molecular characterization including 16S rRNA gene and its phylogenetic analysis confirmed that the isolated strain as *V. rotiferianus*.

Conclusion: The use of pharmaceuticals products is on the rise and in turn its pollution is rising in an ecosystem. The isolated strain may act as a potential biosensing agent as it responded to change in luminescence for even nanomolar quantities of several heavy metals/antibiotics. In conclusion, study highlighted *V. rotiferianus* isolated from Diu beach could be utilized in detection and quantitative estimation of pollutant by change in luminescence level of *V. rotiferianus*.

1. Introduction

Bacteria are one of the prominent able-bodies among bioluminescent organisms.[1] Bioluminescence is usually generated through oxidation of a light-emitting molecule commonly known as the luciferin in combination with a vital catalyzing enzyme a luciferase.[2] Luminescent bacteria subsist as symbionts within several larger organism, includes the deep sea squids, lantern fish, the angler fish, jelly fish, clams and the eel.[3,4] In luminescent bacteria around 5% of total cellular protein is luciferase and it also utilizes 10% of cellular energy to execute the light emission during bioluminescence reaction. These facts signify the highly regulated system behind amazing bioluminescence phenomenon.[5,6] The lux operon, a genetic element responsible for light production will surely be of great help to explore numerous biotechnological

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applications.[2,3,7–9] Bioluminescence is a collective activity of five closely linked structural genes which involves, *luxA* and *luxB* genes, responsible for the alpha and beta subunits of luciferase, and *lux C*, *D*, and *E* genes, encode the fatty acid reductase complex.[4,5,10] In recent times, the bacterial bioluminescence genes (*lux* genes) have been employed in the field of molecular biology and in environmental biotechnology as genetic reporters and contaminant biosensors, respectively.[11]

The luminescent system is highly sensitive to even micro quantities of pollutants which make it one of the most promising methods for monitoring the environmental pollution. Bioluminescent bacteria based bioassays and biosensors offer an imperative way for the estimation of water toxicity and recurrently go beyond other known bioassays in speed, accuracy, sensitivity and simplicity.[1] The bioluminescent properties of *Vibrio rotiferianus* for development of bioluminescent bacteria based bioassays and biosensors are yet to be studied in detail. The present investigation is a key step toward investigating role of *V. rotiferianus* in pollutant detection system.

2. Materials and methods

2.1. Water sampling

In December, 2012 water samples were collected from the surface water layer of varied locations of Diu beach, Diu district, India (Asia) through dissolution system in sample bottles. After collection, the bottles were sealed and transported at 4 °C in cool boxes to the laboratory and processed further.

2.2. Screening, isolation & purification of bioluminescent bacteria

About 300 μl of water samples were plated on nutrient agar medium by spread plate method along with several additives like 3% glycerol and 50% sea water. Plates were incubated in a dark room at three different temperatures 15 °C, 22 °C and 37 °C for 24 h. The sample's prevalence for luminescent colonies was performed after incubation period was over. Selected strains were further tested for the bioluminescence assay as explained.

2.3. Bioluminescent intensity assay by media formulation

The growth and luminescence pattern of bacterial isolates were further tested on Nutrient agar (NA) media enriched with addition of artificial sea water with (8.25, 16.50, 24.75, 33.00 g sea salt/1000 ml) as 25%, 50%, 75% and 100% respectively with various pH such as 6, 7 and 8 and incubated at 4 °C, 22 °C, 37 °C, and 45 °C to determine the optimum medium constitute, pH and temperature at which the culture show prominent growth.

2.4. Bacterial DNA extraction, PCR amplification, sequencing and identification

Bacterial genomic DNA was extracted using the Axyprep bacterial genomic DNA Miniprep Kit (Axygen). PCR was performed to amplify the 16S ribosomal gene locus using universal primers as 8F: 5′ AGA GTT TGA TCC TGG CTC AG 3′ and 1492R: 5′ ACG GCT ACC TTG TTA CGA CTT 3′. Amplification cycle was kept as follows: an initial denaturation of 94 °C for 3 min, 30 cycles of 94 °C 30 s, 52.7 °C 30 s, and 72 °C 1.30 min. Amplicon was resolved on 1% Agarose Gel and further sequenced using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The sequence was checked against the microbial nucleotide databases using BLASTN search algorithm and identified for genus and species. Best scored sequences were further aligned to obtain the phylogram using the MEGA software.

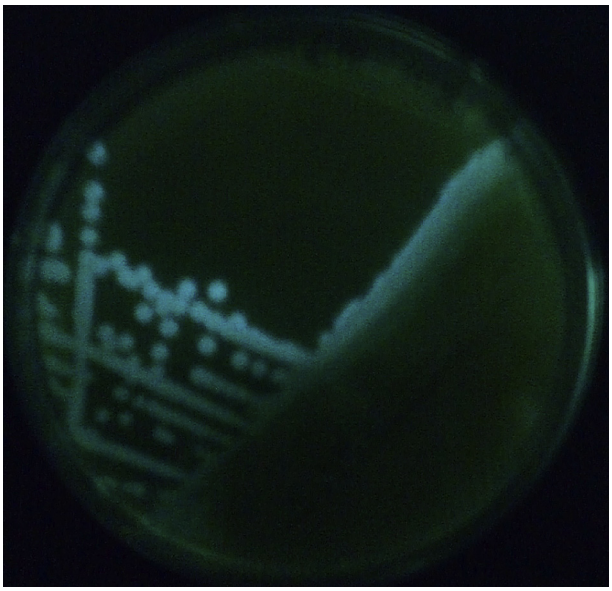

Fig. 1 – Isolated colonies of luminescent bacteria on modified nutrient agar plate.

2.5. Antibiotic and heavy metal tolerance study

V. rotiferianus was also characterized for its antibiotic susceptibility against nine antibiotics (Hi-media) along with

Table 1 – Evaluation of different media for their effect on the growth & luminescence patterns of strain DB1 (*V. rotiferianus*).

Media	Diu bacterial isolate (strain DB1)	
	Growth	Luminescence
NA (3% glycerol + 25% sea water)	+++	+++
LM	++	++
SWC medium	++	+
BOSS medium	+	+
Photobacterium agar medium	+	+
Glycerol chalk medium	+	+

+ = Low; ++ = Medium; +++ = High.