

RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF LUMINESCENT BACTERIA FROM MANGROVE AREAS IN PARANGIPETTAI COASTAL AREAS, SOUTHEAST COAST OF INDIA

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ABSTRACT

The present study was carried out to isolate luminescent bacteria from the sediment sample collected from mangrove areas in Parangipettai. The luminous bacteria density was found to be 5×10^4 CFU/g in sediment samples. Totally 10 luminous bacteria selected for biochemical analysis and genus level identification. The results shows all ten bacterial isolates were belonging to the genus *Vibrio* sp. *Vibrio* sp are the good indicator of the environmental conditions. Hence, it is useful to assess the environmental conditions of an aquatic environment.

Keywords: Luminescent bacteria, mangrove, *Vibrio* sp..

1. INTRODUCTION

Microorganisms are essential parts of all ecosystem which includes viruses, bacteria, fungi, diatoms, algae, protozoa etc. The two fundamental reasons for the indigenous microorganisms are used for biologically indicator of coastal environment are (i) they are responsible for the regeneration of nutrients (ii) the transfer of primary production from phytoplankton to micro zooplankton and to larger organisms (1). There are many biologically relevant and common general indicators of the quality of the coastal water available to assess the effect of human activities on the functioning of the ecosystems, particularly indicators of chemical contamination (2). Last few decades the population densities in coastal regions are estimated to be nearly three times higher than the global average density (3) which directly increase the level of pollution in the coastal ecosystem.

The majority of bioluminescent organisms reside in the ocean because more than 700 genera of living organisms known to contain luminous species (4). These occupy a diverse range of habitats, from polar to tropical and from pelagic waters to the benthic sea floor (5). It is exhibited by a diverse group of organisms although their number is very less compared to the total number of known species. It has been estimated that luminous organisms may have come from about 30 different evolutionarily distinct origins (6,7). However some animals, including crustaceans, squid, jellyfish and fish, release their light-emitting chemicals into the water, producing clouds or particles of light that serve to distract or blind a predator (8).

Luminescent bacteria are distributed widely in shallow coastal environments and deep pelagic water (9,10). Certain bioluminescent species are established as species-specific symbionts with marine fish and squids and are harboured in highly specialized light organs (11). The most luminous bacteria are classified into three major genera such as *Photobacterium* spp, *Vibrio* spp and *Photorhabdus* spp. Among them the species *Photobacterium* spp and *Vibrio* spp are exist in marine environment and the *Photorhabdus* spp are considered to be a terrestrial species (12).

The *Vibrio* species are a diverse group of bacteria found in abundance in the marine environment and associated with aquatic plants and animals to which they may provide a chemical defence for the host and some of them are showing bioluminescence (13). Research on isolation of bioactive compounds from the 74 species of this group has already shown promise with the isolation and identification of many antibiotic compounds (14,15). This study aimed to isolate and identify the bioluminescent bacterial strains from sediments collected from mangrove areas in Parangipettai.

2. MATERIALS AND METHODS

2.1. Sample collection

The pond Sediment samples were collected at bottom of the pond using the sterile polyvinyl corer (10cm diameter) and these samples were transported to sterile vials and tightly sealed. The collected samples brought to the lab in an ice-box for further analysis.

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2.2. Viable count for enumeration of cell

Sediment samples in each pond were pooled, weighed and pulverized with a mortar and pestle. Dilution plate method was used to isolate bacteria, 1 g sediment sample mixed with 9 ml of sterile distilled water and shaken well. It was assumed that the bacteria were evenly distributed between solid and liquid. Use the pipette to remove 1 ml and delivered into the next dilution tube. Discarded the pipette, and continued for the required number of dilutions (10-, 100-, 1000-fold dilution). Pipetted 0.1 ml of each dilution of sediment samples into center of a plate agar. A separate plate for each dilution was used. Spread each inoculum using sterile bent glass rod over the plate and incubated for 24 to 48 h and then observed the growth. The number of colonies on a plate were counted and calculated.

2.3. Identification of bacteria

The bacterial strains were identified up to genus level based on the morphological and biochemical feature of the bacteria (16,17)

2.4. Morphological characteristics

2.4.1. Gram staining

Thin smear was prepared and gram stained. The slide was observed under 10, 45 and 100X magnification.

2.4.2. Motility determination

The isolated bacterial cultures were stab inoculated into the motility agar in a test tube. Then tubes were incubated at room temperature for 24 to 48 hrs. After incubation, the tubes were observed under the light source.

2.4.3. Biochemical characteristics

The bacterial isolate was subjected to various biochemical tests such as oxidase, catalase triple sugar iron test, gelatinase, arginine decarboxylase, growth on gelatin agar with NaCl, growth on TCBS agar all the tests were done by standard procedure as follows.

2.4.4. Oxidase test

The fresh growth from the plate was scraped with a disposable loop and the colony was touched with the edge of the oxidase disc (Himedia). The disc was examined with blue colour within 10 seconds.

2.4.5. Catalase test

A loop full of 24 hrs fresh bacterial culture was touched with the drop of 3% hydrogen peroxide

on slide. The formation of air bubbles was taken as positive reaction.

2.4.6. Triple sugar iron test

Triple sugar iron agar medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 48 hrs. A formation of acid (Yellow), alkaline (Red) and gas were noted at the end of the incubation period.

2.4.7. Gelatin hydrolysis test

Nutrient gelatin medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 24 hrs. After incubation period tubes were further incubated at 4°C for 1 min and liquefying the medium in test tubes were considered as a positive reaction.

2.4.8. Amino acid decarboxylase test

A decarboxylase broth (Moeller broth) containing 1% L-arginine monohydrochloride, 1% L-lysine dihydrochloride and 1% L-ornithine dihydrochloride amino acids and pH indicator was prepared. After inoculating bacterium, test tubes were incubated for 24 – 48 hrs at room temperature. Development of yellow to dark purple colour was considered as positive reaction.

2.4.9. Growth of bacteria in gelatin agar medium

Gelatin agar medium was prepared with 3% NaCl and without 3% NaCl, sterilized and inoculated with isolates and incubated at room temperature for 24 hrs. After incubation period growth were observed in the medium

2.4.10. Growth of luminescent bacteria in TCBS medium

The TCBS (Thiosulfate citrate bile salt sucrose agar) medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 24 hrs. Appearance of yellow and green colour colonies in this medium indicates the bacterial strain like *Vibrio* spp.

3. RESULTS

The total bacterial density enumerated from sediment is given in table and figure. Only 10 bacterial strains were selected and screened for identification. The isolated bacterial strains were given designated codes from SD.

Table 1: Total bacterial count in sediment

Sample	No of bacteria
Sediment	5 X 10 ⁴ CFU/g

3.1. Identification of bacterial strains

All the isolated bacterial strains were identified as *Vibrio* spp. the results are represented in Table (2).

3.2. Grams characterization

Microscopic observations have confirmed that all the 10 bacteria were pink in colour and were identified as gram negative rod.

3.3. Test for motility

All the 10 bacteria were found to be actively motile in motility agar.

3.4. Catalase test

In Catalase test, all the strain was positive to the formation of bubbles against hydrogen peroxides.

3.5. Oxidase test

In Oxidase test, all strain showed positive and oxidase disc was changed to blue colour.

3.6. Triple sugar iron test

In Triple sugar iron test, nine strains fermented triple sugar and produced acid butt and acid slant (yellow colour), except strains SD2 produced acid butt (yellow colour) and alkaline slant (red colour) and there was no formation of gas.

3.7. Growth of luminescent bacteria on tcbs agar

All bacteria were produced green colour colonies in TCBS Agar which is very selective for *Vibrio* spp. So, it has been confirmed that all the 10 strains were belonging to the genera *Vibrio*.

3.8. Tentative identification of the strains

Reference to the keys provided in the Bergeys Manual of Determinative Bacteriology and the results of biochemical tests that are summarized in the table 5, all the 10 strains were tentatively identified as *Vibrio* spp.

Table 2. Morphological and biochemical characters of isolated bacterial strains

Strain code	Gram stain	Motility	Oxidase	Catalase	TSI	Growth on TCBS agar	Tentative identification
SD1	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD2	Rod /gram negative	Motile	+	+	Alkaline slant	Green color	<i>Vibrio</i> sp
SD3	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD4	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Green color	<i>Vibrio</i> sp
SD5	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD6	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD7	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD8	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD9	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp
SD10	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	<i>Vibrio</i> sp



Fig. 1. Culture of bacteria from sediment

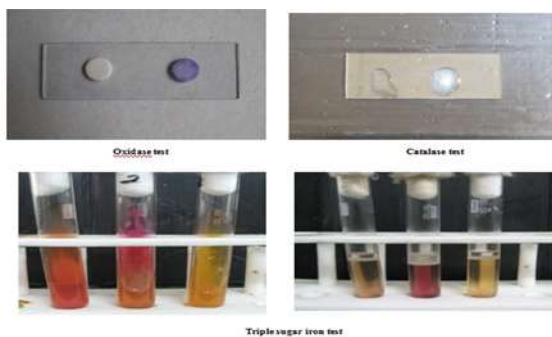


Fig. 2. Morphological and Biochemical Characters of Isolated Bacteria



Fig. 3. Growth of bacteria on TCBS agar

4. DISCUSSION

Luminous bacteria are the most common of all bioluminescent organisms' presents in all environment. The marine luminescent bacteria are presents in either as free living bacteria or in symbiotic association with other marine organisms such as squids, deep water fishes, octopus etc. The occurrence of luminescent bacteria considered as indicative of the physico chemical conditions and indicates the presents increased availability of nutrients. These associated bacterial luminescent

genes are considered as biosensors for marine environmental studies, with special emphasis on the micro toxicity assay (18). In this study the average density of luminous bacteria was 5×10^4 CFU/ml. The present observation of luminous bacterial population was higher when compared with Abraham *et al.* (19) who reported 20 to 1050 cells/ml of luminous bacteria from seawater of Tuticorin bay, 3.0×10^3 CFU/ml in port Harcourt in Nigeria and 1.3×10^4 to 3.0×10^4 in Bonny estuary Nigeria (20), 20 to 90 cells/100 ml in surface seawater of the Seto Inland sea (21).

These results noted that the luminous bacteria found on sediment samples from mangrove environment of Parangipettai probably shows the presence of low level of toxicants. The present observation concurrent with the Ramaiah and Chandramohan (22) reported that luminous bacteria loose their ability to emit light in the presence of a toxicant even at very low concentration. As the presence of luminous bacteria in high numbers in coastal waters indicated healthy and pollution free condition.

Globally, the *Virbrio* sp., are reported to be most common luminescent bacteria. In the present study that all luminous bacterial strains were identified as *Vibrio* spp and according the emission of light the prominent active strain (SD5) was further identified as *Vibrio mediterranei* based on the morphological and biochemical characteristics. This obtained result is backed by the observation of Abraham *et al.* (19,23) who reported different species of *Vibrio* such as *Vibrio harveyi*, *V. orientalis*, *V.splendidus*, *V. fischeri* and *V. mediterranei* in seawater and sediment samples from Tuticorin bay. The results of the present study are in close agreement with the observation of Yang *et al.* (24) who isolated and identified some luminous bacteria such as *Vibrio harveyi*, *Vibrio orientalis*, *V. splendidus*, *Photobacterium phosphorium* and *photobacterium leiognathi*. The present study has revealed the distribution of luminous bacteria in sediments, suggested the healthy environment in Mangrove areas of Parangipettai.

REFERENCES

1. Sherr, E., and B. Sherr, (2000). Marine microbes: An overview, p. 13-46. In D.L. Kirchman (ed.) Microbial ecology of the oceans. Wiley- Liss, New York.
2. Frischer, M.E. and P.O. Verity, (2005). Alternatives to coliform bacteria as indicators of human impact on coastal ecosystems. In Kleppel, O.S., M.R. DeVoe and M. Rawson, (ed.) Implications of land use change to coastal

- ecosystems challenges to effective resource management. Springer-Verlag, Berlin (in press).
3. Small, C., and R.J. Nicholls, (2003). A global analysis of human settlement in coastal zones. *J. Coastal Res.* **19**: 584-599.
 4. Shimomura, O., (2006). Bioluminescence: chemical principles and methods (World Scientific, Toh tuck link, Singapore), pp 500.
 5. Haddock, S.H.D., M.A. Moline and J.F. Case, (2010). Bioluminescence in the sea. *Annu. Rev. Mar. Sci.*, **2**: 443-493
 6. Hastings, J.W., C.J. Potrikas, S.C. Gupta, M. Kurfurst and J.C. Makemson, (1985). Biochemistry and physiology of bioluminescent bacteria. *Adv. Microbiol. Physiol.* **26**: 235-291.
 7. Alldredge, A., T. Cowles, S.S. Macintyre, J. Rines and P. Donaghay, (2002). Occurrence and mechanisms of formation of a dramatic thin layer of marine snow in a shallow Pacific fjord. *Mar. Ecol. Prog. Ser.* **233**: 1-12
 8. Aguilar, J.A., A. Albert, F. Ameli, M. Anghinolfi and G. Anton, (2006). First results of the Instrumentation Line for the deep-sea ANTARES neutrino telescope. *Astropart. Phys.* **26**: 314-324
 9. Reichelt, J.L., and P. Baumann, (1973). Taxonomy of the marine luminous bacteria. *Arch Mikrobiol.* **94**: 283-330.
 10. Ramesh, A., E.G. Loganathan and K. Venkateswaran, (1990). Ecological dynamics of marine luminous bacteria. *J. Basic Microbiol.* **30**: 689-703.
 11. Dunlap, P.V., and K. Kita-Tsukamoto, (2001). Luminous bacteria. The Prokaryotes (Dworkin M, Falkow S, Rosenberg E, Schleifer KH & Stackebrandt E, eds). Academic Press, New York.
 12. Campbell, A.K., (2008). Jean-Marie Bassot (1933–2007): a life of unquenched curiosity—Obituary. *Luminescence*, **23**: 187-190.
 13. Engel, S., P.R. Jensen and W. Fenical, (2002). Chemical Ecology of marine microbial defense. *J. Chem. Ecol.* **28** (10): 1971-1985.
 14. Baranova, N.A., and N.S. Egorov, (1989). Production of antibiotic substances by natural variants of the marine bacterium *Vibrio fischeri*. *Antibiot. Khimioter.* **34**(1): 7-10.
 15. Kobayashi, M., S. Aoki, K. Gato, K. Matsunami, M. Kurosu and I. Kitagawa, (1994). Marine natural products. XXXIV. Trisindoline, a new antibiotic indole trimer, produced by a bacterium of *Vibrio* sp., separated from the marine sponge *Hyrtios altum*. *Chem. Pharm. Bull.* (Tokyo). **42** (12): 2449 – 2451.
 16. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, (1994). Bergey's manual of determinative bacteriology, Ninth edition, (Williams and Wilkins, Baltimore), 260
 17. Abraham, T.J., R. Manley, R. Palaniappan and K. Dhevendaran, (1998). Luminous bacteria as indicator of penaeid shrimp grow out phase abnormalities, in: Proceeding of the national symposium on Frontiers in Applied environmental Microbiology, edited by IS brightSingh, (School of environmental sciences, Cochin University of Science and Technology, Cochin) pp: 205-210.
 18. Halldorson, V.S.N., and N.L. Duran, (2003). Bioluminescent Bacteria: Lux Genes as Environmental Biosensors. *Braz. J. Microbiol.* **34**: 91-96.
 19. Abraham, T.J., S.A. Shanmugam, R. Palaniappan and K. Dhevendran, (2003). Distribution and abundance of luminous bacteria with special reference to shrimp farming activities. *Indian J. Mar. Sci.* **32**(3): 208-213.
 20. Adoki, A.A., and L.O. Odokuma, (2007). Bioluminescent hydrocarbonclastic bacteria of the Niger Delta. *Afr. J. Biotechnol.* **6**(4): 393-399.
 21. Venkateswaran, K., H. Nakano and H. Hashimoto, (1989). Occurrence and distribution of bioluminescent bacteria in the seto inland sea. *Bull. Jap. Soc. Sci. Fish.*, **55**: 467-473.
 22. Ramaiah, N., and D. Chandramohan, (1993). Ecological and laboratory studies on the rate of luminous bacteria and their luminescence in coastal pollution surveillance. *Marine Poll. Bull.*, **26**: 190-201.
 23. Abraham, T.J., R. Palaniappan and K. Dhevendaran, (1999). Simple taxonomic key for identifying marine luminous bacteria. *Indian J. Mar. Sci.* **28**: 35-38
 24. Yang, Y., L.P. Yeh, Y. Cao, L. Baumann, P. Baumann, J.S. Tang and B. Beaman, (1983). Characterization of marine luminous bacteria isolated off the coast of china and description of *Vibrio orientalis* sp. Nov, *Curr. Microbiol.*, **8**: 95-100.