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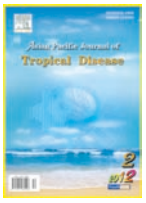

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Association of bioluminescent bacteria from blue swimmer crab *Portunus pelagicus* (Linneaus, 1758)

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ABSTRACT

Objective: To screen the bioluminescent bacteria from *Portunus pelagicus* (*P. pelagicus*) at Thondi coast, Palk Strait, Bay of Bengal, India. **Methods:** Physico-chemical parameter including atmospheric and surfacewater temperature, pH, salinity and dissolved oxygen were analyzed. The population of bioluminescent bacterium was screened in ambient water and blue swimmer crab of *P. pelagicus* (muscle, gill, hemolymph, shell) and colony forming unit (CFU) was calculated. **Result:** Atmospheric and surface water temperatures varied from 26.1 and 27.3 °C to 33.4 and 32.6 °C, respectively; salinity varied from 28.4% to 34.3%, pH varied from 7.6 to 8.6, and dissolved oxygen varied from 4.8 to 6.9 O^2 ml/l. In addition, the maximum CFU value was identified (12.63 x10^4 CFU/ml) during postmonsoon season and the minimum level (1.09 x10^4 CFU/ml) identified during summer season. Further, based on the phenotypic characterizations the isolated strain were identified as *Vibrio harveyi* (*V. harveyi*). **Conclusions:** It is concluded from that the incidence of *V. harveyi* infections was frequently identified with edible crab of *P. pelagicus*, throughout the study periods in different seasons.

1. Introduction

Bioluminescent bacteria can be isolated readily from the marine environment. In nature, diversity of emitting light luminous bacteria is abundant in seawater, on surface of marine animals and in the specialized organ of luminous fish and cephalopods. The edible crabs are commercially important and get high price as there is a rapidly expanding demand for crab meat both in local and international markets. The marine blue swimmer crab, *Portunus pelagicus* (*P. pelagicus*) (Linneaus, 1758) (Family: Portunidae) also known as flower crab is an important nominee and cultivable species for aquaculture in India.

Microbial infections have been a major concern of aquaculture worldwide but to date, the literature does not offers any information on pathogenic microbes with the gut, larval rearing system of *P. pelagicus* and their pathogenic role in the larval culture and survival. With respect to indigenous pathogenic *Vibrio* sp. it appears that crabs from cold waters are far less likely to act as vectors of these pathogens than shellfish from temperate waters[1]. Garcia et al reported on crab and shrimp hatcheries, bacterial infection, especially those carried by luminescent *Vibrio* sp. result in serious disease that affect animal growth and total production[2]. Water near shore is one of the major sources of infection; the midgut content of shrimp and crab broodstocks are to be main source of the luminous vibriosis pathogens[3]. More than a few *Vibrio* sp. and *Pseudomonas* sp. are known to be fatal pathogens in zoeal stages of mud crabs[4]. In the present investigation, we examined the association of pathogens with edible crabs collected in marine ecosystems especially in the Palk Strait region. Hence, there exists a lacuna in understanding the ecology and physiology of luminous bacteria associated with *P. pelagicus*.

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2. Materials and methods

The *P. pelagicus* male crabs were collected from Thondi coast (N 9 ° 44′; E 79 ° 01′) Palk Strait region, India. The samples were collected for one year during January to December 2010 at four different seasons such as postmonsoon to monsoon preferably middle month of every season. Live animals were brought to the laboratory in polythene buckets, surface water samples were taken in sterile 1-liter polypropylene bottles within 1 to 2 h of collection.

2.1. Physico-chemical parameters analysis

The following physico-chemical parameters such as atmospheric and surface water temperatures (mercury centigrade thermometer), pH (Elico pH meter Model-LI-120), Salinity (Refrectometer Model E-2) and dissolved oxygen[5] were investigated at Thondi coastal region.

2.2. Enumeration of bioluminescent bacteria associated P. pelagicus

Samples such as muscle, gill and gut macerated with one-half strength sterile Rila salts solution (pH 8.4). In addition, hemolymph sample also collected using 23-gauge needle by penetrating the intersegmental membrane between the posterior of the carapace and the abdomen after the site was disinfected with alcohol[6]. Collected samples serial diluted with PBS solution up to 10-8 dilutions and spreaded over the luminescent agar medium for the isolation of bioluminescent bacteria. Control plates also maintained without the addition of sample. All the plates incubated at 28 ℃ for 24 h. After attaining the visible growth the bluegreen colonies counted. The collected samples further identified using standard protocols[7-10].

3. Results

Atmospheric temperature ranged from 26.1 to 33.4 ℃ and the maximum temperature identified during summer season and the minimum temperature (26.1℃) identified during the monsoon season. The maximum surface water temperature (32.6℃) recorded during the summer season and minimum temperature recorded during the monsoon season. Further, the maximum and minimum salinity level identified during the summer and monsoon season respectively. The maximum potential hydrogen-ion concentration (pH) recorded during the summer season and minimum recorded during monsoon season. Dissolved oxygen content also varied 4.8 to 6.9 ml/l and the maximum during monsoon and minimum identified summer season (Table 1). In addition, the maximum CFU value identified in water during postmonsoon season and the minimum level identified during summer season (Table 2). Based on the phenotypic characterization the isolated bacterial pathogen was identified as *V. harveyi* (Table 3).

Table 1.
Seasonal variation of physico-chemical parameters were record at Thondi from January to December 2010.

Parameters	Season of collection 2010			
	Postmonsoon	Summer	Premonsoon	Monsoon
Atmospheric temperature (℃)	28.2	33.4	31.2	26.1
Surfacewater temperature (℃)	28.6	32.6	31.8	27.3
Salinity (‰)	32.1	34.3	29.2	28.4
pH water	7.7	8.6	7.6	7.1
Dissolved oxygen (O_2 ml/l)	5.5	4.8	5.6	6.9

Table 2.
Quantitative distribution- of luminous bacteria from different parts of *P. pelagicus* in seawater.

Collection of Season 2010	Bodyweight grams	Counting (CFU g) x10^4			Counting (CFU ml) x10^4	
		Shell	Gill	Muscles	Hemolymph	Water
Postmonsoon	193.67	3.77	5.14	2.07	2.41	12.63
Summer	176.34	1.12	3.16	–	1.09	7.96
Premonsoon	183.26	2.43	4.82	–	–	7.52
Monsoon	270.51	1.88	2.93	1.11	1.22	3.99

Table 3.
Characteristics of luminous strains isolated.

Characteristics		*V. harveyi*
Gram stain		–
Morphology of cell		R
Luminescence		+
Colour on TCBS		Green
Growth In NaCl	0%	–
	3%	+
	8%	+
	10%	+
	12%	–
Growth in Seawater		+
Growth temperature	4℃	–
	28℃	+
	35℃	+
Motiliy		+
Oxidase		+
Catalase		+
Vogas proskauer		–
Amylase production		+
Gelatinase		+
Lipase		+
Chitinase		–
Glucose fermentation		+
Mannose		+
Pyruvate		+
Lactose		–
Acetate		+
Mannitol		+
Propionate		–
Haptanoate		–
Sucrose		–

+: Positive, –: Negative, R: Rod shaped.

4. Discussion

In marine environment, bioluminescent bacteria are exposed to various physico-chemical conditions. The present study was an attempt to identify the distribution of luminous bacteria in seawater and the edible crab of *P. pelagicus* in Thondi coastal area. In general, seawater of the Thondi coastal area found with maximum luminescent bacteria continent than tissue samples of *P. pelagicus*. Atmospheric temperature is most important factor calculating the physiological activities of tropical bioluminescent bacteria. In this study, higher atmospheric temperature was recorded during summer season and the lower surface water temperature noticed in monsoon season. Influence of temperature play an important role in distribution of luminescent bacterial population[11-15].

Salinity is one of the important factors which intensely influence the abundance and distribution of the animals in marine water. Moreover, the maximum level of salinity values recorded during summer season and this may due to higher degree water evaporation in coastal area. The lowest level of salinity found to monsoon season and this may due to heavy rainfall and fresh water inflow. Similar trends in the salinity values were observed in luminescent bacteria distribution[15-17]. The maximum pH level identified during summer season and minimum level of pH identified during monsoon season. The alteration in pH also plays important role in microbial population[18]. The results of the present study suggest that, dissolved oxygen concentration is found higher concentration during monsoon season and found lower during summer season. But, the level of dissolved oxygen in water region is not playing any important role in surface water[19, 20].

The genus *Vibrio* is commonly found in coastal and estuarine waters[21-24] but some species are recognized to be potentially pathogenic to man and marine animals, causing vibriosis, a serious infectious disease. The understanding of the survival strategies of *Vibrio*s is important to control both water quality and the transmission pathways of the disease[23,25,26]. Luminous bacteria serve as valuable tools for biological seawater mass characterization[12]. In the present study the occurrence of *V. harveyi* found maximum in surface water, followed by gill, shell and muscles, but the minimum level of *V. harveyi* was identified with the hemolymph. The result of CFU values improves the knowledge of distribution ratio of *V. harveyi*. Hence, *V. harveyi* is an important secondary bacterial pathogen in mud crabs *Scylla tranquebarica* affected primarily by white spot disease[27]. *V. harveyi* was more pathogenic in comparison to the other luminous bacteria causing mass deaths in crab larvae[28]. The association of various *Vibrio* sp. with *Chionoecetes opilio*, *Cancer magaster*, *C. irroratus* and *Paralithodes camtschatica* were reported for gill, shall, gut and water from Alaskan. Brandin and Pistole, in hemolymph of the Horseshope crab *Limuls polyphemus*. Since *Vibrio* sp. have been isolated from healthy *P. pelagicus* hypothesis on the opportunistic nature of vibriosis associated with crab has become widely accepted[6]. Mud crab for human consumption based on wild mud crab associated bacteria as well as their antibiotic resistant[30].

A present study concluded that the natural *P. pelagicus* of Thondi coastal area presents of bioluminescent bacteria. Through the association of *V. harveyi* isolated from this crab it is not suitable for edible to human consumption. The Indian government has to be instructing the public sector need to cook *P. pelagicus* properly before use, to avoid the microbial contamination.

Conflict of interest statement

We declare that we have no conflict of interest.

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Reference

[1] Fishbein M, Mehlman J, Pitcher J. Isolation of *Vibrio parahaemolyticus* from the processed meat of Chesapeake Bay blue crabs. *Appl Microbiol* 1970; **20**:176-178.

[2] Garcia DK, Faggart MA, Rhoades L, Alcivar Warren AA, WA Wyban J. Genetic diversity of cultured *Penaeus monodon* shrimp using three molecular genetic techniques. *Mol Mar Biol Biotechnol* 1994; **3**: 270-280.

[3] Lavilla-Pitogo CR, Albright LJ, Paner MG, Sunaz NA. Studies on the sources of Luminescent *Vibrio harveyi* in *Penaeus monodon*. In: Diseases in Asian Aquaculture. Shariff IM, Subasinghe RP, Arthun JR. ed. Fish Health Section, *Asian Fisheries Society*, *Manila*, 1992: 157-164.

[4] Jithendran KP, Poornima M, Balasubramanian CP, Kulasekrapandian S. Disease of mud crabs (*Scylla* sp.): An overview. *Indian J Fish* 2010; **57**: 55-63.

[5] Strickland JDH, Parsons TR. A practical handbook of seawater analysis. *Bull Fish Res Bd Canada* 1972; **167**: 1-310.

[6] Faghri MA, Pennington LC, Cronholm LS, Atlas RM. Bacteria associated with crabs from cold waters with emphasis on the occurrence of potential human pathogens. *Appl Environ Microbiol* 1984; **47**(5): 1054-1061.

[7] Nealson KH. Isolation, identification and manipulation of luminous bacteria. *Methods Enzymol* 1978; **57**: 153-165.

[8] Baumann PS, Schubert RHW. The famili II *Vibrio*naceae Veron.

In: Krieg NR. Bergey's Manual of Systematic Bacteriology, Williams and Williams, *Baltimore MD, USA*, 1984; **1**: 515-538.

[9] Alsina M, Blanch AR. A set of keys for biochemical identification of environmental *Vibrio* species. J Appl Bacteriol 1994; 76: 79-85.

[10] Holt JG. Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams & Wilkins, Baltimore; 1994.

[11] Ruby EG, Nealson KH. Seasonal changes in the species composition of luminous bacteria in nearshore seawater. *Limnol Oceanogr* 1978; **23**: 530-533.

[12] Yetinson Y, Shilo M. Seasonal and geographic distribution of luminous bacteria in the eastern Mediterranean Sea and the Gulf of Elat. *Appl Environ Microbiol* 1979; **37**: 1230-1238.

[13] Orndorff SA, Colwell RR. Distribution and identification of luminous bacteria from the Sargasso Sea. *Appl Environ Microbiol* 1980; **39**(5): 983-987.

[14] Ramesh A, Nair GB, Abrham M, Natarajan R, Venugopalan VK. Seasonal distribution of luminous bacteria in the tropical Vellar Estuary. *Microbios* 1987; **52**: 151-159.

[15] Urakawa H, Rivera ING. Ch. Aquatic environment. In: Thompson F.L Austin B, Swings J. The Biology of the *Vibrio*s. ed. Washington, D.C. *ASM Press*. 2006; 175-189.

[16] Reicheld JL, Baumann P. Effect of sodium chloride on growth of heterotrophic marine bacteria. *Arch Microbiol* 1974; **97**: 329-345.

[17] Ramesh A, Venugopalan VK. Ecophysiological studies on luminous bacteria associated with marine Gastropods. *Actes de Colloques* 1986; **3**: 445-450.

[18] Obrien CH, Sizemore RN. Distribution of the luminous bacterium *Beneckea harveyi* in a semitropical estuarine environment. *Appl Environ Microbiol* 1979; **38**(5): 928-933.

[19] Sivakumar N, Jayabalan N. Distribution of luminouscent bacterium *Vibrio harveyi* in Netravathi estuary, Mangalore. *J Mar Biol Assoc India* 1994; **36**: 251-259.

[20] Abraham TJ, Shanmugam SA, Palaniappan R Dhevendaran K. Distribution and abundance of luminous bacteria with special reference to shrimp farming activities. *Indian J Mari Sci* 2003; **32**(3): 208-213.

[21] Caruso G, Zaccone R, Crisafi E. Distribution and numerical taxonomy of *Vibrio*naceae in the waters of the Straits of Messina. *Microbiologica* 1996; **19**: 155-166.

[22] Sainz JC, Maeda-Martinez AN, Ascencio F. Experimemental *vibrio*sis induction with *Vibrio alginolyticus* of larvae of the *Catarina Scallop* (Argopcten ventricosus = circularis) (Sowerby II, 1842). *Microb Ecol* 1998; **35**: 188-192.

[23] Cavallo RA, Stabili L. Presence of *vibrio*s in seawater and *Mytilus galloprovincialis* (Lam.) from the Mar Piccolo of Taranto (Ionian Sea).*Water Res* 2002; **36**: 3719-3726.

[24] Cavallo RA, Stabili L. *Vibrio*s biodiversity in the Northern Ionian Sea (Italian coasts). *Sci Mar* 2004; **68**: 23-29.

[25] Caruso G, Zaccone R, Genovese L, Crisafi E. Microbiological monitoring of Castellammare Gulf (TP) waters for their suitability in marine aquaculture. *Microbiologica* 1998; **21**: 169-182.

[26] Pardio Sedas VT. Influence of environmental factors on the presence of *Vibrio cholerae* in the marine environment: a climate link. *J Infect Dev Ctries* 2007; **1**(3): 224-241.

[27] Poornima M, Singaravel R, Rajen JJS, Sivakumar S, Ramakrishanan S, Alavandi SV, et al. *Vibrio harveyi* in mud crabs (*Scylla tranqubarica*) infected with white spot syndrome virus. *Int J Res Biol Sci* 2012; **2**(1): 1-5.

[28] Parenerngi A, Zafran A, Boer DR, Rusdi I. Identification and pathogenicity of several *vibrio* bacteria in mangrove crab larvae, *Scylla serrata*. J *Coastal Aquacul* 1993; **9**: 125-129.

[29] Brandin ER, Pistole TG. Presence of microorganisms in hemolymph of the Horseshope crab Limuls polyphemus. *Appl Environ Microbiol* 1985; **49**(3): 718-720.

[30] Najiah M, Nadirah M, Sakri I, Shaharom-Harrison F. Bacteria associated with wild Mud crab (*Scylla serrata*) from Setiu wetland, Malaysia with emphasis on antibiotic resistances. *Pakistan J Biol Sci* 2010; **13**(6): 293-297.