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Occurrence of Total Heterotrophic Bacteria from *Lucifer* sp. and its Pathogenicity in Uppanar Estuary (South East Coast of India)

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Abstract: *Lucifer* sp. is a little-known and deteriorate genus of prawns, the only genus in the family Luciferidae. This paper deals with the preliminary investigation of Total Heterotrophic Bacteria (THB) present in the marine planktonic shrimp *Lucifer* sp. and in the water samples from where it was obtained. The THB load was found to be 1.2×10^4 and 2.5×10^4 CFU/mL in *Lucifer* sp. and water sample respectively. The THB were further characterized and identified its diversity bacterial based on the morphological and biochemical aspects. Five different bacterial strains viz, *Staphylococcus aureus*, *Micrococcus* sp., *Serratia marcescans*, *Klebsiella* sp. and *Enterobacter* sp. were identified and its pathogenicity was also referred. The fascinating aspect of the work confirms that similar bacterial species were found to be present in the *Lucifer* sp. as well as in the water sample. Hence, it can be concluded that on environmental link is present between the animal and its surroundings.

Key words: Epiplanktonic shrimps, identification, *Lucifer* sp., pathogenicity, THB

INTRODUCTION

Tropical estuaries are complex productive ecosystems (Correll, 1978), known to be key recruitment areas for many species of exploited populations (Blaber *et al*., 1995; Vidy, 2000; Hajisamae and Chou, 2003). Gelatinous macro zooplankton is an important consumer of zooplankton and icthyoplankton. Therefore, the increase of gelatinous macro zooplankton causes problems in the ecosystem. Birinci-Ozdemir *et al*. (2007) zooplanktons are distributed in any habitats in the sea, from the coasts to the offshore oceans, and from the sea surface to the abyssal depths. Some of them are known to play important roles in marine ecosystem, including those in the food-chain and matter transfer, but there are also many species whose distribution and ecology are mostly unknown. Planktons are very sensitive to the environment; they live in any alteration in the environment leads to the change in the plankton communities in terms of tolerance, abundance, diversity and dominance in the habitat. *Lucifer* is a little known and deteriorates genus of prawns, the only genus in the family Luciferidae. The family is represented by a single genus, *Lucifer*. The length of eye stalks are significant in *Lucifer* species, some species have long eye stalks, where the eye and stalk are about as long as the 'neck'; others have short eye stalks, where they are about half the length of the' neck'. A rapid turnover of generations, each with a short adult life span of 30-40 days and maturing within 19 days at 30ºC, sequential spawning and carrying of eggs until hatching characterize the life strategy of this small shrimp (Lee *et al*., 1992). Heterotrophic bacterial biomass and production in coastal waters have been reported almost from all parts of the world away from the immediate influence of rivers, the heterotrophic microorganism and autotrophic microorganism are the major agents shaping the organic composition of ocean. Temperature and pH are limiting factors for the survival of bacteria in the environment (Whipple and Rohovec, 1994). Hence, the present investigation of their importance and involvement in the biological processes, attempts have been made in the occurrence of THB from the *Lucifer* sp. Zooplanktons are distributed in any habitats in the sea, from the coasts to the offshore oceans, and from the sea surface to the abyssal depths. Some of them are known to play important roles in marine ecosystem, including those in the food-chain and matter transfer, but there are also many species whose distribution and ecology are mostly unknown. Planktons are very sensitive to the environment; they live in any alteration in the environment leads to the change in the plankton communities in terms of tolerance, abundance, diversity and dominance in the habitat. Hence, the present investigation attempts have been made in the occurrence of THB from the *Lucifer* sp. Members of *Vibrio* sp., *Aeromonas* sp., *Escherichia coli*, *Enterococcus* sp., *Campylobacter* sp. and *Arcobacter* sp. has been reported from zooplankton samples (Dixon, 2005). Both the

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abundance and types of autochthonous and allochthonus microbial populations in the near shore estuarine environments are affected by land drainages, domestic sewage outfalls and other discharges. The overall ranges of the monitored groups of bacteria were total coliforms, total streptococci, total vibrios, *Escherichia coli*, *Vibrio cholerae*, *Salmonella* sp., *Streptococcus faecalis* and *Aeromonas* sp. (Nagvenkar and Ramaiah, 2009). There is little information on plankton, although it plays a major role in the structure and the functioning of the trophic networks (Wooldridge and Bailey, 1982; (Wooldridge, 1999; Froneman, 2000, 2003; Kibirige *et al*., 2006).The study also focused on understanding the similarities in prevalence of microbial groups in Lucifer associated and free living pathogenic microbial load.

MATERIALS AND METHODS

Study area: The Uppanar estuary is located at Cuddalore district (Lat 11º43' Long 79º49'). It originates from the north eastern part of the Shervarayan hills and opens into the Bay of Bengal near Cuddalore. Apart from the Uppanar estuary receives municipal, sewage and industrial effluents from SIPCOT (Small Industries Promotion Corporation of Tamil Nadu) Industrial complex. Most of industries are wet process industries and hence consume large quantity of water.

Sample collection: The *Lucifer* samples were collected by using zooplankton nets (mouth diameter 45 cm) made up of bolting silk cloths (Mesh size – 200 μm) for 10 or 15 min tow from the surface water. Surface water samples were collected and using pre sterilized McCartney bottles allowing enough air space in the bottles to facilitate thorough mixing. Precautionary measures were taken to minimize the contamination. The collected samples were aseptically transferred in to sterile polythene bags. *Lucifer* sp and water samples were transferred to the laboratory in an ice box maintained at 4ºC. *Lucifer* sp. were isolated from the collected sample for further studies.

Isolation bacteria from *Lucifer* species: The *Lucifer* species were segregate and homogenized using the motor and pestle and inside laminar flow. 1 mL of sample was suspended in 99 mL sterile (50% aged) sea water and was serially diluted in 9 mL blank up to 10^6 and 0.1 mL from each tube was spreaded on nutrient agar plates and incubated at 37 ºC for 24 h.

Isolation bacteria from water sample: The water sample were collected from Uppanar estuary and brought into the lab as aseptically. 1/mL of sample was suspended in 99 mL sterile 50% aged sea water and was serially diluted in 9 mL blank up to 10^6 and 0.1 mL from each tube was spreaded on nutrient agar plates and incubated at 37 ºC for 24 h.

Identification of bacterial strains: The organism's were collected according to their morphological structure and used for identification.

Gram staining: Transfer a loopful of the liquid culture to the surface of a clean glass slide, and spread over a small area. Allow the film to air dry. Flood the slide with crystal violet solution for up to one minute. Wash with tap water. Flood slide with Gram's Iodine solution, and allow acting for about one minute. Wash off with tap water. Remove excess water from slide and blot, so that alcohol used for decolorization is not diluted. Flood slide with 95% alcohol for 10 sec and wash off with tap water. Drain the slide. Flood slide with safranin solution and allow counterstaining for 30 sec. Wash off with tap water. Drain and blot dry with bibulous paper. All slides of bacteria must be examined under the oil immersion lens. Using gram staining technique, we can able to know gram positive or gram negative bacteria. According to this further identification was done up to species level following Bergey's manual of Determinative bacteriology (Buchanan and Gibbons, 1974) and the identified from strain was stored in slant at 4ºC.

RESULTS AND DISCUSSION

Isolation of bacteria from *Lucifer* species and water sample: The aim of the study is to isolate the total heterotrophic bacteria present in both are water sample and *Lucifer* species. The present investigation was shows the distribution of the total heterotrophic bacteria and pathogenic bacteria in the water samples and *Lucifer* species collected from the Uppanar estuary. Here the bacterial density is higher in water sample when compared to *Lucifer* species Wollast (1991) reported that the coastal and shelf sediments play a significant role in the demineralization of organic matter which supports the growth of microbes. Anonymous (1997) also reported the higher bacterial population density in the sediments than water in generally due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column than the sediments the Total heterotrophic bacterial population various from seasons to season. The high bacterial population during monsoon may be due to the rain water flow which brings huge quantities of nutrients (Martin, 1981; Sathiyamurthy *et al*., 1992). In the present investigation was the total heterotrophic bacteria of water sample is 2.5×10^4 CFU/mL and in *Lucifer* species the total count is about 1.2×10^4 CFU/mL (Fig. 1, 2, 3, Table 1).

The early reports supports to the result obtained as and in the sediments showed it's lower of $7x10^3$ CFU/g in (summer) and higher of $46.5x10^3$ CFU/g in (Monsoon). In areas where there is not a lot of sunlight, bacteria thrive and maintain a good population (Swaranakumar *et al*., 2008). In the presence of sunlight the bacteria become

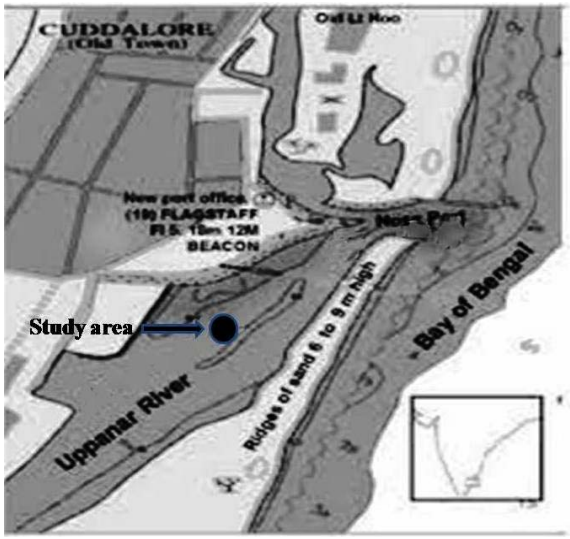

Fig. 1: Showing study area map

Table 1: Total bacterial density in water sample and *Lucifer* species

Sample	Number of colonies (CFU/mL)
Water sample	2.5×10^4 CFU/mL
Lucifer species	1.2×10^4 CFU/mL

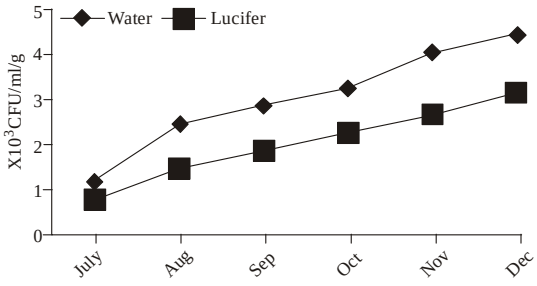

Fig. 2: THB in water sample and *Lucifer* sp.

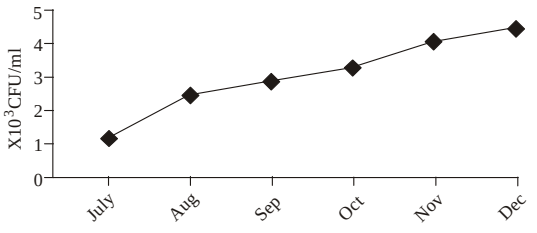

Fig. 3: THB in water sample

inactive and eventually die and Visual light may be the cause rather than the ultra violet from the effect and decrease in the bacteria population (Fujioka *et al*., 1981).

Identification of bacteria from Lucifer and water sample: In the present investigation was five morphologically different strains were isolated and identified (Table 2).

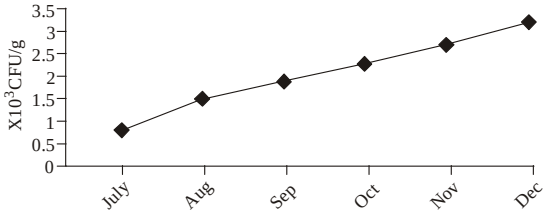

Fig. 4: THB in *Lucifer* sp.

Biochemical methods for bacterial identification: The five bacterial strains were identified based on the biochemical methods (Fig. 5) as follows.

In the present investigation was all the five strains were pathogen and identified as *Staphylococcus aureus, Serratia marcescans,* Micrococcus sp., *Enterobacter* sp. and *Klebsiella* sp. But Urakawa *et al*. (2000) and Thompson *et al*. (2004) recorded pathogenic bacteria such as *V. cholera, V. parahaemolyticus* and *E. coli*. According to them the population density of *Vibrio* sp. in the marine environment is usually more because *Vibrios* can occur in a wide range of the aquatic environments including estuaries, marine and coastal waters and sediments.

Description of pathogen: All the five strains which were identified are pathogen and it will cause harmful disease for humans.

***Staphylococcus aureus*:** *Staphylococcus aureus* is known as common cause of bacterial foodborne disease in the worldwide. Including the symptoms of vomiting and diarrhea that occur shortly after ingestion of *S. aureus*-contaminated food. The symptoms were arising from the ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are

Table 2: Identification of bacteria from *Lucifer* sp and water samples

Bacterial name	*Staphylococcus aureus*	*Serratia marcescans*	*Micrococcus* sp.	*Enterobacter* sp.	*Klebsiella* sp.
Gram reaction	+	-	+	-	-
Motility	-	+	-		-
voges-proskauer	-	+	-	±	-
Indole	-	-	-	±	-
Catalase	+	+	+	+	+
Oxidase	-	-	+	-	-
Mannitol	+	+	-		+
Dextrose	+		-	-	+
TSI	+	+		+	+
Citrate utilization	-	+	-	+	+
Nitrate reduction	+	-	-		+
Morphology	Cocci	rod	cocci	rod	rod

Isolation of bacteria from *Lucifer* species and water sample

(a) Citrate utilization test

(b) Carbohydrate fermentation test

(c) Sugar fermentation test

(e) Methyl red test

(d) Indole test

Fig. 5: Identification of Bacteria using for biochemical test

superantigens and, as such as, have adverse effects on the immune system. The enterotoxin genes are accessory genetic elements in *S. aureus*, meaning that not all strains of this organism are enterotoxin-producing. The enterotoxin genes are found on prophage, plasmids, and pathogenicity islands in different strains of *S. aureus*. Expression of the enterotoxin genes is often under the control of global virulence gene regulatory systems. *Staphylococcus aureus* is an important pathogen associated with diseases in a variety of hosts including

humans. It produces several toxins and virulence factors that contribute to its pathogenic potential such as staphylococcal enterotoxins. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic Shock Syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. . *S. aureus* worldwide is an additional problems and resistance to antimicrobial compounds reduces their effectiveness and increases morbidity, mortality and health care costs worldwide.

***Serratia marcescans*:** *S. marcescens* occurs in the natural environment, including the soil, water, and surfaces of plant parts, and occurs as an opportunistic human pathogen. A human pathogen, *S. marcescens* is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections, (Hejazi and Falkiner, 1997; Auwaerter, 2007) and is responsible for 1.4% of nosocomial bacteremia cases in the United States (Ania, 2008). It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. *S. marcescens* may also be found in environments such as dirt, supposedly "sterile" places, and the subgingival biofilm of teeth. Due to this, and the fact that *S. marcescens* produces a reddish-orange tripyrrole pigment called prodigiosin, *S. marcescens* may cause extrinsic staining of the teeth.

***Micrococcus* sp.:** *M. luteus* has been isolated from the human skin, animal and dairy products, and beer. It can be found in many other places in the environment, as well as, like water, dust, and soil. *M. luteus* on human skin breaks down compounds in sweat into compounds with bad odor. *M. luteus* can grow well in the environments with little water or high salt concentrations. They grow optimally at 37ºC and can be easily grown on inorganic nitrogen agar or Simmon's citrate agar. Although some, like *Micrococcus antarcticus*, are cold-adapted, and have been found living in Antarctica and in the marine environments. *Micrococcus* sp as the cause of infections is easy to overlook because infections caused by this bacterium are rare as well as the bacterium is a natural part of the skin's bacterial flora. Most *Micrococcus* sp infections are discovered through process of elimination (all other bacterial, fungal, etc. tests showing up negative) along with the presence of abundant *Micrococcus* tetrads in the lesions or cysts (Smith *et al*., 1999). Though today immunocompromised patients the risk of infection has grown. They have been several deaths in the immunocompromised children (caused by leukemia) from pulmonary hemorrhages because of *Micrococcus* sp. Recently, these organisms were recognized as an opportunistic pathogen and have been implicated in recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration, and cavitating pneumonia in immunosuppressed patients.

***Enterobacter* sp.:** *Enterobacter* is a genus of common Gram-negative, facultatively-anaerobic, rod-shaped bacteria of the family Enterobacteriaceae strains of these bacteria are pathogenic and caused opportunistic infections in immunocompromised (usually hospitalized) hosts in those on the mechanical ventilation. The urinary and respiratory tract are the most common sites of infection. It has been also the fecal coli form, along with *Escherichia*.

***Klebsiella* sp.:** *Klebsiella* sp. are ubiquitous in nature (Bagley, 1985). Klebsiella probably have two common habitats, one being the environment, where they are found in the surface water, sewage, and soil and on plants and the other being the mucosal surface of mammals such as humans, horses, or swine, which a rare colonize. *Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, ankylosing spondylitis, and soft tissue infections (Podschun and Ullmann, 1998). *Klebsiella* species are ubiquitous in nature (Bagley, 1985). Frequent cause of nosocomial urinary and pulmonary infections; wound infections; secondary infection in lungs of patients with chronic pulmonary disease; enteric pathogenicity (enterotoxin); ozena (atrophy of nasal mucosa) and rhinoscleroma. *Klebsiella* ranks second to *E. coli* for urinary tract infections in older persons. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. Feces are the most significant source of patient infection, followed by contact with contaminated instruments.

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