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RESEARCH ARTICLE

BACTERIAL DENSITY IN FISH *Etroplus suratensis* AND ITS SURROUNDINGS

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ABSTRACT

Seasonal variations of bacteria in surrounding environment and *Etroplus suratensis* were studied. Maximum bacterial counts was recorded during (Feb-May, 2009) in water and sediment samples (2.78 x 10^6 CFU.g-1). Bacterial counts associated with fishes was recorded in healthy and diseased fishes (6.15 x 10^4 CFU. g-1) in station II (Buckingam Channel) and *Bacillus sp* and *Micrococcus sp* were predominant in gills and skin in healthy fish and *Vibrio sp*. were predominant in gills and skin in diseased fish. This comprehensive study could provide an information to findout the etiology of fish health to improve the quality and quantity of the fish for human consumption.

INTRODUCTION

Etroplus suratensis is the only chichlids indigenous to Asia. They primarily inhabit estuaries in India. The distribution of these cichlids within a particular estuary is restricted by the sea on one side and by the flowing water of Feeder Rivers on the other (Jayamathi and Samarakoon, 1983). Bacteria present in the environment exhibit a diverse relationship with the living organisms of in the same environment. Usually when talking about microbes we think of their pathogenic impact. It is true that in the marine environment there are pathogenic microbes which affect not only marine organisms but human beings as well (Garland Science, 2011). A variety of species of bacteria are responsible for the cause of diseases among fishes. So fish diseases are common in the natural environment. Bacterial load of the environment and the adverse environmental conditions are responsible for the outbreak of variety of diseases among fishes living in the natural waters. In Parangipettai coastal waters various diseases like, fin rot, tail rot and ulcerative lesions were observed among numbers of various fish species (Lakshmanaperumalsamy, *et al.,* 1983; Loganathan, 1985). Bacteria of the genera *Vibrio*, *Pseudomonas, Aeromonas* and *Corynebacterium* were found to be responsible for fish diseases (Sindermann, 1979; Singh *et al.* 1981, Lakshmanaperumalsamy, *et al.,* 1983; Loganathan, 1983) and *Vibrio sp*. pathogen for coral *Pocillopora damicornis* disease (Ben-Haim, and Rosenberg, 2002). Various genera of bacteria were present in water and sediment are associated with even normal healthy fishes (Mary, 1977; Palaniappan, 1982).

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Generally, the range of bacterial genera in an aquatic habitat of the fish varies with factors such as the salinity of the habitat and the bacterial load in the water. In many investigations, identification of isolates to the genus level only makes it difficult to determine the precise relationships of aquatic and fish microfloras. Bacteria recovered from the skin and gills may be transient rather than resident on the fish surfaces. Microfloras of fish intestines appear to vary with the complexity of the fish digestive system (Marian M. Cahill , 1990) so sea water is undoubtedly a source of bacteria found in fish, especially the main source of disease causing bacteria. Maya *et al.* (1995) reported that, seasonal variations of bacteria in gills, alimentary canal and reproductive organs of *Etroplus suratensis* and *E. maculatus* were studied. Maximum bacterial population was recorded during premonsoon (Jan.-April) and minimum during post monsoon (Sept.-Dec.) seasons. *Micrococcus spp* were predominant in gills, alimentary canal and reproductive organs of both fish. Selected bacterial cultures were characterized for their physiological activities. Bacterial load in the alimentary canal of both the fishes exhibited positive correlation with weight of that organ. Therefore, detailed study of the bacterial flora of the environment from where the fish with bacterial diseases are collected becomes inevitable. Especially a study of the quantitative and qualitative nature of bacteria present in the water and sediment samples would be more meaningful for understanding the process of bacterial infection in fishes living in the natural environment and as well in aquaculture system. So the present study has indicated to find out the quantitative bacterial density in healthy and diseased fishes corresponding to its surroundings.

MATERIALS AND METHODS

Collection of the sample

Surface water samples were collected aseptically from Vellar Estuary (Station 1) and Buckingham channel (Station 2) for a period of 7 months. Sediment samples were collected using a Peterson grab. Healthy fishes from wild fishes from wild is also collected for the enumeration of bacterial density as per the standard procedure. Triplicate samples were brought to the laboratory, diluted serially and plated. Replicate samples were plated over Zobell's marine Agar (2216e) medium and incubated at room temperature (28±2^{o}C). The dry weight of the sediment and the tissue samples were determined after drying at constant temperature in an incubator. The number of bacterial colonies developed on the plates were counted and expressed as number of CFU. g^{-1}/ml^{-1} in sediment and water samples respectively.

morphology, the biochemical characteristic ,were studied in all the strains collected from water, sediment and from the fish and then identified them up to generic level following the scheme of Schewan(1962).

RESULTS

Bacterial density in Surroundings

The total heterotrophic bacterial population in water samples collected from station 1 and 2 during the study period is given in Table 1. The total colony forming units (CFU) in station I ranged from 9.5 x 10^4 ml^{-1} to 7.4 x 10^4 ml^{-1}. The maximum number of CFU of 9.5 x 10^4 ml^{-1} was recorded during the month of February and the lowest CFU of 8.6 x 10^4ml^{-1} was recorded May . The heterotrophic bacterial population in sediment was relatively higher than in water in both the stations (Table 2).

Table 1 Bacterial density in water and sediments of two different stations

Sampling Months	Vellar estuary Station 1		Buckingam Channel Station 2	
	Water ($x10^4$ $CFUml^{-1}$)	Sediment ($x10^6CFUgm^{-1}$)	Water ($x10^6CFU\ ml^{-1}$)	Sediment ($x10^7$ $CFUgm^{-1}$)
February	9.5±0.59	3.65±0.36	9.5±0.59	2.10±0.47
March	8.2±0.3	2.9±0.45	8.7±0.32	1.30±0.25
April	8.6±0.33	3.1±0.25	9.2±0.55	1.98±0.09
May	7.4±0.4	2.8±0.25	8.6±0.32	1.17±0.25
June	7.8±0.7	2.9±0.25	8.9±0.33	1.65±0.34
July	8.1±0.3	2.85±0.25	8.88±0.41	1.55±1.24
August	8.0±0.3	2.78±0.25	9±0.58	1.52±0.09

Table 2. Bacterial density in healthy fish *Etroplus suratensis*

Station	Tissue (Mean CFU x 10^4gm^{-1})	Gill (Mean CFU x 10^4gm^{-1})	Gut (Mean CFU x 10^4gm^{-1})
Vellar estuary (S1)	5.044±0.23	5.075±0.28	5.135±0.31
Buckingam Channel (SII)	6.15±0.086	6.82±1.21	7.25±0.241

Table 3. Bacterial density in diseases fish *Etroplus suratensis*

Station	Tissue (MeanCFUx10^6gm^{-1})	Gill (MeanCFUx10^7gm^{-1})	Gut (MeanCFUx10^8gm^{-1})
Vellar estuary (S1)	1.987±0.064	1.027±0.024	1.878±0.065
Buckingam Channel (S2)	1.878±0.05	1.124±0.024	1.923±0.06

Quantitative estimation of bacteria in healthy and diseased fish of *Etroplus suratensis*

E.suratensis occurs in the entire stretch of Vellar estuary and Buckingham channel. The healthy and infected animals of *E.suratensis* were collected using a vellan screen or cast net. During every collection fish were subjected for diseases diagnosis. The normal fishes were brought to the laboratory in clean plastic buckets containing estuarine water. The affected fishes were screened for bacterial pathogens. Small pieces of gill, skin with underlying muscles (approximately $1cm^{3)}$ just below the base of the dorsal fin and gut of fish were excised from both normal and infected fish. In the case of fish showing pathological condition such as finrot, ulcer, haemorragic skin lesions etc., The abnormal regions were excised and transferred aseptically to a sterilized physiological saline solution. This was homogenized and diluted serially. From this, one ml was transferred to the plates and plated on the Zobell's marine Agar (2216e medium). The colony

In station 1 maximum bacterial density (3.65x $10^6/gm^{-1}$) was recorded at the same month. The bacterial density was found low in August (2.78 x 10^6 CFU gm^{-1}) at station I and in May (1.17x 10^7CFU gm^{-1}) at station 2.

Bacterial density in fish

The average total length and weight of the fish along with mean bacterial load of the skin muscle, gill and gut of the fish is given in Table 3. The quantitative distribution of bacterial flora in fish collected from different regions reveals that the bacterial density (CFU) of the skin with muscle of the fish collected from both the stations ranged from 5.044 x 10^4 CFU gm^{-1} to 6.15 x 10^4 CFU gm^{-1} and in the gill the counts ranged between 5.075 x 10^4 CFU gm^{-1} and 6.82 x 10^4gm^{-1} . The mean bacterial density in the gut of the fish fluctuated from 5.135 x 10^4CFUgm^{-1} to 7.25 x $10^4CFUgm^{-1.}$ Quantitative analysis of bacterial population was performed in different organs of diseased fish with ulcerative lesion collected from

both the stations and the mean values are given in Table 3. The bacterial load of different organs of fish with ulcerative lesion is remarkably higher when compared to the bacterial load of healthy fish. The mean bacterial load of skin with muscle of fish collected from the two stations ranged between 1.878 x 10^6CFU gm $^{-1}$ and 1.987 x 10^6CFUgm^{-1}. The mean bacterial load in gill samples ranged from 1.027 x 107CFUgm^{-1} and 1.124 x 10^7CFUgm^{-1} and in the gut ranged from 1.878 x 10^8CFUgm^{-1} and 1.923 x 10^8CFUgm^{-1} respectively.

Generic diversity

The generic composition of bacterial flora isolated from water and sediment samples was found out using various biochemical characteristics. Water and sediment samples comprised both gram positive. *Bacillus sp, Micrococcus sp, Flavobacterium sp, Cytophaga sp* and *Coryne bacterium* and the gram negative forms by *Vibrio sp, Psedudomonas sp* and *Aeromonas sp.* The bacterial genera observed in the skin, gill and gut of the healthy *E.suratensis* were *Bacillus sp, Micrococcus sp* and *Vibrio sp*. obviously the bacteria of genus *Bacillus sp* was absent in diseased fish. Likewise, species like *Vibrio anguillarum* and other *Vibrio sp*. were not represented in the bacterial population collected from skin associated with muscle and gills of healthy fish. So it is quite evident that, the generic composition at various regions of healthy fish is different from that of diseased fish.

DISCUSSION

Bacterial flora, its type and density present in the water and sediments are often responsible for the cause of infection of fish and resulting in the most commonly occurring diseases like fin and tail rot, columnaris disease, gill disease and septicaemia (Gillmour, 1977; Loganathan, 1985). The observed pattern of bacterial fluctuation in water and sediments in the present observations reflect the available organic load of the water and sediments. The stations where the studies were performed normally contain high organic matter (Balasubrmanaian, 1981; Rajendren, 1984). Hence, higher bacterial population was observed in these stations 1. Further, the bacterial load of water and sediments in the present observation reflect the variations in environmental parameters especially the available organic load in water and sediments. The sediment samples in station 2 always registered higher bacterial density when compared to that of the samples of station 1. This might be due to the discharge of sewage water and along with dissolved and particulate organic matter would have resulted leading to the maximum bacterial density in water and sediment. According to Rajendren (1984), the bacterial load of the water fluctuated parallel with that of other living organisms and the organic load. Higher magnitude of bacterial population observed in the water and sediments may also be responsible for the cause of the disease in *E.suratensis*. Normally, when the fish is robust and healthy their muscle, liver and blood will be sterile. Rarely, the bacterial occurrence in the blood was noticed (Loganthan, 1985). But skin, gills and gut of the fish which are in constant contact with external milieu, where the bacterial load is abundant, might contain high bacterial flora. The microflora of the external environment definitely influence the microflora of the skin (Liston, 1956), eventhough the colonization of microflora in the skin of healthy fish is inhibited by the presence of mucoproteins or peptides (Sieburth, 1976), whereas it is not that extent in diseased fish. The environmental parameters of both physical and chemical might cause physiological stress on fish and sometime result in production of excess mucus in the skin and in gills also affect the tissue integrity thus resulting in the heavy colonization of bacteria in these organs. Higher bacterial load and poor environmental condition at station 2 might be the reason for the higher incidence of fish diseases in this region (Sieburth, 1976).

The higher bacterial population observed in the gills of diseased fish of *E.suratensis* than in healthy fish. The maximum bacterial counts were recorded in the gills of *E.surtensis* with ulcerative lesion was 1.124 x 10^7CFU gm. Similarly higher bacterial population (1.94 x 10^7 /gm; 1.10^7/gm) were recorded in the gills of *Lates calcarifer* and *Ambassis commersoni*_respectively infected with fin rot and ulcer diseases (Loganathan, 1985). The skin of *E. suratensis* harbored slightly lesser number of bacteria than in gills and this in agreement with that reported by Loganathan (1985). But the bacterial density of the gills and the skin of healthy fish were more or less in the same level. These clearly indicate that the diseased fish with the excess secretion of mucus from the gills might have favoured better growth of bacteria. Loganathan (1985) has opined that, the high content of mucoid slime produced in the gills might favor the selection of polysaccharide splitting bacteria. Bacterial flora of the gut content of healthy fish was very low when compared to that of diseased fish. Bacterial density of the gut of the fish is generally influenced by the bacterial density of the environment in which it live and the type of feed it takes. Interestingly the bacterial density of skin, gill, and gut content of normal healthy fish was similar, whereas, in diseased fish the bacterial population of the gut content of the fish was higher when compared with skin and gill. In healthy fish, the bacteria from surroundings entering into the gut along with food may be digested by the animal but not in the case of diseased fish. This might be the reason for higher bacterial density observed in the gut content of diseased fish when compared to healthy fish. Since the skin and gill parts of the fish are always in contact with the water. The pathogen present will always be in contact with these parts. So it is important to know the bacterial genera occurring in water and sediment and comparing them with that in fish skin and gills. Almost all the genera that occur in water and sediments are encountered in the skin, gills, and gut of the animals. Liston (1957) pointed out that, the generic population of skin and gills are influenced by the nature of bacterial population present in water and sediment, because they are always in constant contact with these organs. The generic composition of diseased fish is different from that of healthy fish. In diseased fish, *Vibrio anguillarum* type A and B and other *Vibrio sp*. were the dominant forms. Apart from these, species representing *Micrococcus* was present but not species belonging to the genera *Bacillus* and *Cytophaga*. *Micrococcus* was observed in both types of fishes. Such variations in generic distribution of bacteria in healthy and infected fish of *L. calcarifer* were reported from Parangipettai waters by Loganathan (1985). The normal surface flora of healthy fish did not appear to cause any disease by itself (Striezko, 1972), unless there was an incidence between the hosts and the pathogens or other disease causing agents and the

environment. *Vibrio* generally occurs in large numbers in water and in particulate matter (Loganathan, 1985; Sathyamurthy, 1991). But, more occurrence of *Vibrio* in the environment and in the skin, gills and gut of the fish could not be accounted for the incidence of the disease vibriosis on its own. However, these bacterial flora vary rapidly when these organs injured or under stress thus making it easy for the entry of pathogens into the fish tissues. After entry of *Vibrio* into the tissue, the elimination of other microflora may depend on the survival of *Vibrio* in the microecological niche. The elimination of bacterial species belonging to other genera might be due to the inhibitory effect of slime or that they fail to compete with *Vibrio*, for a microecological niche. Possibly, *Vibrio* took the advantage of the environmental stress and invade the fish tissues through various possible routes causing the infection and disease, in which *Vibrio* predominated in the finrot and ulcerative diseases. Vibriosis disease observed in *E. suratensis* during summer months (April to June) appeared to be due to increase in temperature. Which favours the outbreak of vibriosis. The above observations supports the views of earlier investigators (Hastein, 1972; Muroga *et. al.* 1984 a,b, Loganathan; 1985). Further studies on the mode of entry of pathogen into the fish, and its component with autochthonous and adherent bacterial community and its ability to multiply inside the fish to threshold level to cause disease processes would be much more useful for complete understanding of vibriosis.

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