



ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL PATHOGEN IN COMMERCALLY IMPORTANT SHRIMP AND FISH COLLECTED FROM GHERS IN SATKHIRA DISTRICT, BANGLADESH

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Abstract: Investigations were carried out for isolation, enumeration and characterization of bacterial pathogens from shrimp *Penaeus monodon* and *vetki* *Lates calcarifer* from different *ghers* at Asasuni thana of Satkhira district. Shrimp and *vetki* were randomly sampled with a cast net from each *gher* individually during the period of September to December, 2005. After sampling, the live specimens of 20 shrimp and 12 fish were brought to the laboratory and immediately dissected. Muscle, hepatopancreas and gill were removed aseptically, weighed and homogenized with 0.85% NaCl solution. The homogenate was diluted using consecutive decimal dilution and inoculated on standard agar plates for enumeration of total bacterial load. A total load of 10^{6-7} CFU g^{-1} , 10^{5-6} CFU g^{-1} and 10^{7-10} CFU/ g^{-1} were found in hepatopancreas, muscle and gill of shrimp and 10^{5-6} CFU g^{-1} , 10^{6-9} CFU g^{-1} and 10^{7-10} CFU ml^{-1} in fish muscle, gill and slime, respectively. The total bacterial load in water of the *ghers* was estimated to be 10^{7-10} CFU ml^{-1} . A total of 32 and 20 representative isolates were selected from shrimp and fish respectively to identify them up to the genus through morphological, physiological and biochemical properties. Four genera namely, *Vibrio*, *Micrococcus*, *Aeromonas* and *Pseudomonas* were identified from the samples. *Vibrio* (56%) was predominantly isolated followed by *Micrococcus* (19%), *Aeromonas* (16%) and *Pseudomonas* (9%) in *P. monodon* whereas *Pseudomonas* (45%) dominated in *L. calcarifer* followed by *Aeromonas* (30%), *Micrococcus* (15%) and *Vibrio* (10%). High bacterial load in shrimp, fish and water of the *ghers*, it can be assumed that shrimp culture of Satkhira district is susceptible to bacterial contamination specially *vibrio* which is one of the devastating pathogens causing vibriosis in our country.

Key words: Bacterial content, opportunistic pathogen, *Vibrio*, *Penaeus monodon*, *Lates calcarifer*

Introduction

Disease has become one of the limiting factors for the development of fisheries sector especially in culture fisheries of Bangladesh. Shrimp plays an important role in our economy which has been cultured broadly in the South Western part of Bangladesh, especially in the districts of Khulna, Satkhira and Bagerhat. Production of fish and shrimp using traditional operation practice is established in this region, where farmers pay little or no attention to water quality management that often creates an unhealthy, disease producing environment for the aquatic animals. Diseases do not occur as single causal event but are the end results of interactions of the etiologic agents, the host (fish/shrimp) and the environment. Aquatic animals are continuously affected by environmental fluctuations and by inadequate management procedures such as stocking handlings, feeding, and so on which affects physiological and biochemical homeostasis of aquatic animal and expose fish to various sorts of infectious diseases in presence of various known and unknown etiologies.

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Ideally host and pathogen remain in equilibrium condition in the aquatic environment. Because of management problem when this equilibrium is damaged the aquatic animals become stressed. As a result the pathogenic microorganisms may establish on the fish/shrimp, especially younger ones (juvenile, fry or fingerlings) in culture system to produce disease. Bacteria are often found to be associated with many fish and shrimp disease condition as primary causative agents or secondary invaders. Among all other bacteria, *Aeromonas*, *Vibrio*, *Pseudomonas* etc. are major fish pathogens which are widely distributed in aquatic environment. These bacteria infect a wide range fish from freshwater to seawater. Various information regarding good culture practice, feed, management techniques are available (Karim, 1993; Hossain, 1994). However, information regarding disease, pathogen of fish/shrimp is limited in our country especially covering Khulna, Satkhira region. So this study was attempted to assess common microbial pathogen especially bacterial flora available on cultured shrimp and fish from shrimp gher. The objectives of the present investigations were to isolate, enumerate and characterize some bacterial pathogens from commercially important shrimp *Penaeus monodon* and vetki fish *Lates calcarifer*.

Materials and Methods

Selection of the study area: The study area had been selected at the southern part of Bangladesh which is well known shrimp resort in Bangladesh. Samples were collected from three different gher locally named as Barainer, Koikhali and Uttar beel at Asasuni thana in Satkhira district.

Samples and sampling: Juvenile live shrimp *P. monodon* and fish *L. calcarifer* weighing of 16 g and 152 g respectively were randomly collected by netting from the selected gher during the period from September to December, 2005. After harvesting the samples were then immediately taken to the laboratory using oxygenated polythene bags containing water from the same gher. In each sampling, five shrimp and three fish were examined from each gher. For the isolation of suspected bacterial pathogen, the organ samples of gill, muscle and hepatopancreas from *P. monodon* and gill, muscle and slime from *L. calcarifer* were removed aseptically, weighed and homogenized properly with 0.85% NaCl solution by tissue homogenizer. The homogenate were then diluted using consecutive decimal dilution and inoculated on to the medium.

Enumeration of total bacterial load: Bacterial content of the samples were determined by standard plate count method. An aliquot of 100 μ l of each decimal dilution was spread separately on duplicate plates having the nutrient agar medium and incubated for 24-48 h. When the colonies became clearly visible within the range of 30-300, then total bacterial load were counted and expressed in CFU g^{-1} or ml^{-1} of sample.

Characterization and identification of the isolates: In order to identify the bacteria, some representative isolates were selected from *P. monodon* and *L. calcarifer*. Morphological and biochemical characteristics of each isolate were studied according to the methods described in Cowan and Steel's Manual for the Identification of Medical bacteria edited by Barrow and Fetham (1993). Isolates were identified up to the genus level using the taxonomic key developed by Tonguthai *et al.* (1999). The mean percentage of identified bacterial genera isolated from the gill, muscle, slime and hepatopancreas of the shrimp and fish was also calculated.

Results

A total bacterial load of 10^{6-7} CFU g^{-1} , 10^{5-6} CFU g^{-1} and 10^{7-10} CFU g^{-1} were found in hepatopancreas, muscle and gill respective of shrimp and 10^{5-6} CFU g^{-1} , 10^{6-9} CFU g^{-1} and 10^{7-10} CFU ml^{-1} respectively in fish muscle, gill and slime. The average bacterial load in the organs of

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gill in both *P. monodon* and *L. calcarifer* were higher than those from muscle. The bacterial load in gher water was found ranging from 10^{7-10} CFU ml⁻¹ (Table 1). Monthly variation was also observed in our study from September to December in different gher which was higher in September than that of December.

Table 1. Total bacterial content of various organs of shrimp and fish collected from three different gher in Satkhira district.

Name of gher	Avg. weight		Month	Average No. of bacteria (log CFU ml ⁻¹ or g ⁻¹)						
	Shrimp (n=20)	Fish (n=12)		water	Organs from shrimp			Organs from fish		
					Gill	Muscle	Hp	Gill	Muscle	Slime
Baraine r beel	17.46g	134g	Sept.	9.7	8.3	5.1	7.9	7.5	6.2	8.5
			Oct.	9.3	8.1	6.5	7.6	8.4	5.1	9.3
			Nov.	8.6	7.9	5.3	6.9	7.9	5.8	7.9
			Dec.	7.2	7.7	5.5	6.4	7.1	5.7	8.3
Koikha li beel	16.10g	175g	Sept.	10.2	10.3	5.3	6.6	9.7	5.9	10.4
			Oct.	8.4	8.7	5.8	6.3	7.2	5.8	9.5
			Nov.	7.7	7.3	5.2	7.2	7.5	6.6	8.7
			Dec.	7.8	7.1	5.1	6.1	7.6	5.6	8.8
Uttar beel	15.86g	147g	Sept.	9.7	7.7	5.3	6.7	6.3	6.1	8.9
			Oct.	8.4	7.4	5.2	6.3	7.8	5.9	8.7
			Nov.	8.3	8.6	5.7	6.4	7.6	5.3	7.6
			Dec.	7.8	7.2	5.6	6.6	7.4	5.4	7.7

Hp: Hepatopancreas

According to morphological characters, a total of 32 and 20 isolates were randomly selected from different organs of shrimp, fish and the water of the gher. Various biochemical characterization tests were performed to identify up to genus level and the results are given in Table 2. Among the selected colonies from shrimp and fish, four types of genera *Vibrio*, *Micrococcus*, *Aeromonas* and *Pseudomonas* were identified from both shrimp and fish. Among them *Vibrio* (56%) was predominantly isolated followed by *Micrococcus* (19%), *Aeromonas* (16%) and *Pseudomonas* (9%) in *P. monodon* whereas *Pseudomonas* (45%) was dominant flora in *L. calcarifer* followed by *Aeromonas* (30%), *Micrococcus* (15%) and *Vibrio* (10%) which was shown in Table 3.

Table 2. Comparison of biochemical characteristics of the recovered isolates from shrimp and fish collected from three different gher at Asasuni thana of Satkhira district.

Characteristics	<i>Vibrio</i>	<i>Pseudomonas</i>	<i>Aeromonas</i>	<i>Micrococcus</i>
Growth at 37°C	+	+	+	+
Motility	+	+	+	-
Gram staining	-	-	-	+
Growth with 0% NaCl	-	+	+	+
Growth with 1% NaCl	+	-	+	-
Growth with 3% NaCl	+	-	-	-
Oxidase test	+	+	+	+
Catalase test	+	+	+	+
O-F test	F	O	F	O
Resistance to O/129 150µg	-	NR	+	NR

NR: Not required; O: Oxidative; F: Fermentative

Table 3. Pattern of the recovered isolates from shrimp and fish of three different gher at Asasuni thana of Satkhira district.

Name of the recovered isolates (%)	
<i>Peneus monodon</i> (n= 32)	<i>Lates calcarifer</i> (n=20)
<i>Vibrio</i> (56%)	<i>Vibrio</i> (10%)
<i>Micrococcus</i> (19%)	<i>Micrococcus</i> (15%)
<i>Aeromonas</i> (16%)	<i>Aeromonas</i> (30%)
<i>Pseudomonas</i> (9%)	<i>Pseudomonas</i> (45%)

Discussion

The results revealed that quantity of bacterial flora in both *P. monodon* and *L. calcarifer* vary from organ to organ. Higher quantity of bacteria ranging from 10^7 to 10^{10} CFU g⁻¹ or ml was observed in the gill and slime of both shrimp and fish, and *gher* water as well. Significant monthly variations in bacterial content from September to December were also observed during the study period. Result showed (Table 1) that the higher content in September and comparatively lower in December which could be due to the lower environmental temperature during that time. This finding is similar to the findings of Austin (1982), Kimura and Yashimizu (1983) and Sugita *et al.* (1985). Uddin *et al.* (1990) suggested that bacterial load might be increased with the increase of water temperature as primary production is usually higher in warmer season. In our result, it was observed that in September, bacterial content in the *gher* water, and gill, slime of fish and shrimp in Koikhali beel was considerably higher (10^{10} CFU g⁻¹ or ml⁻¹) than the other months (Table 1). Presence of high organic substances might be the reason for that. The isolated genus, *Vibrio*, *Aeromonas*, *Pseudomonas* and *Micrococcus* are common opportunistic pathogens in aquatic environments of both seawater and freshwater (Austin and Austin, 1987). Many authors reported that most septicaemic forms of diseases in fish are the result of aeromonad and pseudomonad infection which is expressed by ulceration in the skin and muscle. *L. calcarifer* is one of the most popular and commercially valuable fishes around the Khulna, Satkhira region and cultured commonly in the *gher* system. In our study period, *Pseudomonas* was dominantly isolated from the *L. calcarifer* followed by *Aeromonas*. The overall results from the study suggest that *P. monodon* and *L. calcarifer* could be infected with the isolated bacteria in a stressful condition, which may result in serious economic loss.

Conclusion

Considering high bacterial load on various organs of shrimp, fish and the water of *ghers*, and the genus detected in our investigation, it can be concluded that shrimp culture of Satkhira district are susceptible to bacterial contamination, specially *Vibrio*, which is one of the devastating pathogen causing vibriosis in shrimp in our country.

Though it was not possible in our study to pinpoint which genus caused various clinical diseases faced by the farmer, however this baseline data could serve as a useful indicator to determine the potential hazards to *P. monodon* and *L. calcarifer* in *gher* poly-culture system which has a great economic consequence to our country.

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