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Correlation of Bacteria Between Estuarine Sediments and Fish Skin

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Abstract

Bacterial samples from surface sediments and cutaneous deposits on fishes living in the Rajakkamangalam estuary (Lat. 8º 20' N & Long. 77º 30' E), Tamilnadu, South India, were analysed to study their relationship. The bacterial load in the sediment and fish skin ranged from $2.1 \pm 2.4 \times 10^5$ to $1.7 \pm 1.0 \times 10^6$ cfu/g and $1.2 \pm 2.4 \times 10^4$ to $1.0 \pm 2.5 \times 10^5$ cfu/cm^2 respectively. A total of 35 bacterial strains were identified from the sediment. *Aeromonas* sp. was 40.0 %, significantly higher ($p<0.005$) in the population followed by *Pseudomonas* sp. (28.57 %, $p<0.001$). Among the 24 bacterial strains identified from fish skin, the predominant colonies were *Aeromonas* sp. (58.33 %, $p<0.005$) and *Pseudomonas* sp. (41.67 %, $p<0.001$). The results indicated a positive correlation of bacteria (*Aeromonas* sp. and *Pseudomonas* sp.) present in sediments and fish skin.

Key words: Estuary, Sediment, Fish skin, Bacteria.

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1. Introduction

The estuarine sediments act as a rich medium for the growth of microorganisms [1]. The interaction of microorganisms with sediments may enhance their survival by reducing exposure to stressors, such as sunlight and predation, or by increasing the availability of nutrients [2]. In this way sediments may act as reservoirs for pathogenic micro-organisms. Hence there may be an increased risk of infection to fishes due to the re-suspension of potentially pathogenic micro-organisms from the surface sediment. The present study was carried out with an objective to evaluate the correlation of bacteria between sediments and fish skin.

2. Materials and methods

The study was undertaken at Rajakkamangalam estuary (Lat. 8^{o} 20' N & Long. 77^{o} 30' E), Tamilnadu, South India. Surface sediments (0-5 cm depth) were collected monthly once in sterile polythene bag with the help of fishermen from December 2018 to March 2019 from four places, named as S1, S2, S3 and S4. The first three samples (S1 to S3) were collected from a distance of approximately 1000, 500 and 250 meter from the barmouth and the S4 samples were collected exactly at the barmouth of the estuary. Sediment samples (S1, S2, S3 and S4) were mixed thoroughly to make a composite sample. One gm well-mixed wet sediment was triturated in 9.0 ml sterile normal saline. Thereafter ten-fold dilutions of the triturated sample were made in sterile normal saline, plated on nutrient agar plates using the pour plate method and incubated at 37^{o}C for 24 h [3]. The predominant colonies were isolated and named as SB 1 to SB 35.

In addition to sediment samples, fishes (Juvenile Tilapia, *Oreochromis mossambicus*) of 5.0 – 10.0 cm length were also collected with the help of fisherman from the same area in each month (n=5). The live fishes were brought to the laboratory in polythene bags and kept in a plastic tub. The samples were processed for the bacterial isolations within two hours of collection. Cellophane squares with 1.0 x 1.0 cm square were made and sterilized. The surface of the fishes was wiped with alcohol and placed these cellophane templates on the skin and swabbed the area with 1.0 x 1.0 cm square using sterilized cotton swab. Placed the entire swab in nutrient broth (5.0 ml) for overnight. Ten-fold dilutions was made from this broth, plated on nutrient agar by pour plate method and incubated at 37 ^{o}C [3]. The predominant colonies were isolated and named as FB 1 to FB 24. The strain determination was made by means of morphological and biochemical characteristics, according to the procedures in Bergey's manual [4]. All the statistical analysis was conducted using Microsoft Statistica Software Version 2.01.

3. Results and Discussion

The bacterial load of sediment from December 2018 to March 2019 are given in the **Table 1.**

Table 1. Bacterial load of sediment and Fish skin during the study period.

Month	**Sediment x 10^5 cfu/g**	**Fish skin x 10^4 cfu/g**
December 2018	5.8 ± 2.7	1.3 ± 2.8
January 2019	15 ± 2.3	10 ± 2.5
February 2019	2.1 ± 2.4	1.2 ± 2.4
March 2019	17 ± 1.0	10 ± 2.3

It is found that the bacterial load in fish skin and sediment was low during the month of December and February. During these months the bar mouth was opened and the sea water entered in this area. This sudden influx of sea water might have been the reason for the reduction in bacterial load. A total of 35 bacterial isolates from sediments and 24 from fish skin were partially characterized during the entire study period. The details of biochemical tests undertaken for genus identification of these isolates are listed in the **Table 2.**

The obtained data referred that the predominant genera of bacteria from sediments were *Aeromonas* sp. (40.0 %; $p<0.005$), *Pseudomonas* sp. (28.57 %; $p<0.001$) *Bacillus* sp. (20.0 %) and *Proteus* sp. (8.57 %) and that of fish skin were *Aeromonas* sp. (62.5 %; $p<0.005$) and *Pseudomonas* sp. (41.67 %; $p<0.001$). The percentage wise occurrence of bacterial genera on each month was given in the **Table 3.**

Table 2. Biochemical test results of the bacterial isolates

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
SB-3,4, 7,12,13,14,15, 20,21,22,26, 27,30,31	-	Rod	+	+	+	+	+	-	-	+	+	+	±	+	+	*Aeromonas sp.* (40.0 %)
SB-1,6,9,10,11, 18,19,28,29, 32	-	Rod	+	-	-	-	+	+	-	+	±	-	+	+	-	*Pseudomonas sp.* (28.57 %)
SB-2,16,17,24,25, 33,35	+	Rod	+	-	±	-	-	+	-	+	±	+	+	+	-	*Bacillus sp.* (20.0 %)
SB-8,23,34	-	Rod	+	+	-	+	±	+	+	+	-	-	-	+	+	*Proteus sp.* (8.57 %)
FB-1,2,3,6,7,8,9,13,14,15,18,19, 20,21	-	Rod	+	+	+	+	+	-	-	+	+	+	±	+	+	*Aeromonas sp.* (58.33 %)
FB-4,5,10,11,12, 16,17,22,23, 24	-	Rod	+	-	-	-	+	+	-	+	±	-	+	+	-	*Pseudomonas sp.* (41.67 %)

Note **A**- isolate name; **B**- Gram staining; **C**- shape; **D**- motility; **E**- methyl red; **F**-Voges proskauer; **G**-indole; **H**- citrate utilization; **I**- nitrate reduction; **J**- urea hydrolysis; **K**- catalase; **L**- oxidase; **M**- starch hydrolysis; **N**- lipid hydrolysis; **O**- gelatin liquification; **P**- hydrogen sulphide production; **Q**- isolates.

Note: + = positive; ± = variable reaction; - = negative

Table 3. Percentage composition of genera of bacterial isolates identified

Month	Source	*Aeromonas* sp. (%)	*Pseudomonas* sp. (%)	*Bacillus* sp. (%)	Proteus sp. (%)
December 2018	Sediment	42.86	28.57	14.23	-
	Fish skin	60.0	40.0	-	-
January 2019	Sediment	40.0	30.0	20.0	10.0
	Fish skin	57.14	42.86	-	-
February 2019	Sediment	37.5	25.0	25.0	12.5
	Fish skin	60.0	40.0	-	-
March 2019	Sediment	40.0	30.0	20.0	10.0
	Fish skin	57.14	42.86	-	-

From December 2018 to March 2019 the predominant colonies in the sediment were *Aeromonas* sp. (42.86, 40.0, 37.5 & 40.0 %) and *Pseudomonas* sp. (28.57, 30.0, 25.0 & 30.0 %). Similar trend of predominance of *Aeromonas* sp. (60.0, 57.14, 60.0 & 57.14 %) and *Pseudomonas* sp. (40.0, 42.86, 40.0 & 42.86 %) were observed on the surface of fishes. However, the fishes caught were apparently healthy without showing any pathological symptoms, it is noticed that the predominant bacteria in the sediment such as *Aeromonas* sp. and *Pseudomonas* sp. were also present as major bacteria in the skin of fishes showing a positive correlation of bacteria between sediment and fish skin (**Figure 1**).

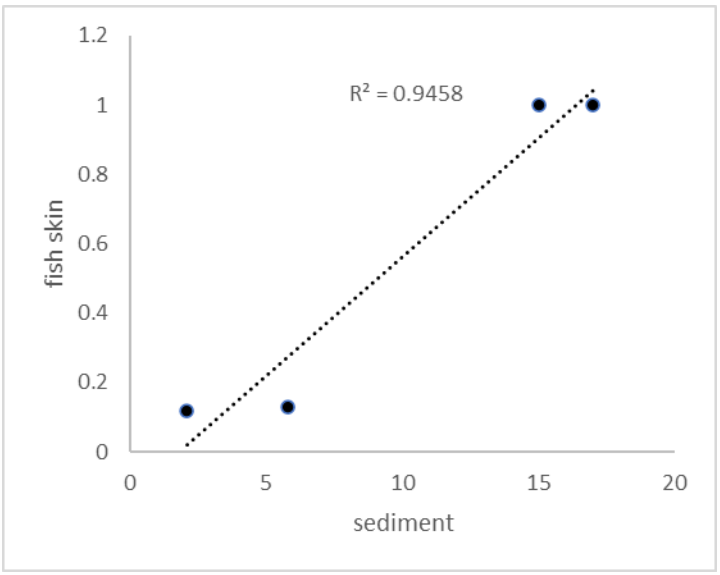

Figure 1. Correlation of bacterial load between sediment and fish skin

This can be a potential threat to fish population in this region because these bacteria were reported as secondary invaders/opportunistic pathogens of wide host range and can cause disease when the fish is in stress [5-7]. Motile aeromonads are considered to be the main cause of bacterial haemorrhagic septicemia in fresh water fish and have been reported in association with various ulcerative syndromes and red spot disease [8-9]. These infections can cause high mortalities in fish hatcheries and in natural fresh water fish population [10-11]. Similarly, *Pseudomonas* is also considered as facultative pathogenic genera though mortality is observed in cases involving skin injury to the fish [12]. Therefore, it is important to identify the motile aeromonads and pseudomonads in order to determine the true etiology of the fish disease outbreaks.

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