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RELATIVE ABUNDANCE AND DISTRIBUTION OF BACTERIA IN THE GUT OF *MACROBRACHIUM VOLLENHOVENII* FROM BADAGRY CREEK AND EPE LAGOON, LAGOS STATE NIGERIA

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Abstract

Quantitative analyses of bacterial flora associated with the intestine of *Macrobrachium vollenhovenii*, sediment and water from Epe lagoon and Badagry Creek were carried out. Total viable counts (TVC) of bacteria in the intestine of the prawn ranged from $5.7 \pm 0.3 \times 10^7$ to $8.1 \pm 0.3 \times 10^7$ (cfu g^{-1}) and $2.8 \pm 0.6 \times 10^7$ to $5.3 \pm 0.4 \times 10^7$ (cfu g^{-1}) at Epe lagoon and Badagry Creek respectively. There were significant variation ($p<0.05$) in the total viable counts of bacteria isolated at both aquatic systems. Altogether, 11 gram negative bacteria genera were identified and found to be common to the lagoon environment. *Escherichia coli, Aeromonas hydrophila, Salmonella typhi* and *Pseudomonas aeruginosa* were the most abundant species in the samples from Epe Lagoon while *Escherichia coli*, *Aeromonas hydrophila, Proteus vulgaris* and *Pseudomonas aeruginosa* were predominant in Badagry Creek. It was noteworthy that *Citrobacter sp.* was not found in Epe lagoon while *Salmonella typhi* and *Vibrio cholerae* was absent in Badagry Creek. This study provides amongst others baseline information on bacteria of wild stock of *Macrobrachium vollenhovenii* thus providing needed thrust when farming the species.

Key words: Bacteria; *Macrobrachium vollenhovenii*; Lagos state.

Introduction

Macrobrachium vollenhovenii, African river prawn was listed as belonging to prawn with aquaculture and commercial potential globally according to Jayachandran (2001). However, in Nigeria the aquaculture potential have not been exploited, therefore, the inland water systems remain the source of the prawn both for consumption and foundation stocks in case of recruitment into aquaculture (Anetekhai *et al.*, 2007). Previous work of the prawn were focused on distribution (Marioghae, 1982), ecology (Anetekhai, 1986) and metal accumulation (Anetekhai, 1986; Anetekhai *et al.*, 2007). Isolation of bacterial flora in the gastrointestinal tracts of aquatic animals living in different geographical locations has been carried out (Fernandes *et al.*, 1997) but limited information exists about the bacterial flora of fresh water prawn in the country.

Bacteria diseases are responsible for heavy mortality in both wild and cultured prawn after viral diseases. The actual role of these microorganisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other diseases process (Richards and Roberts, 1978). Opportunistic pathogens are organisms that generally do not usually

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cause disease unless the individual's body defenses are impaired (Nester *et al.,* 1998).

Many bacteria live abundantly on various surfaces of aquatic animals and the aquatic biomes and may not necessary cause diseases particularly when pollutants do not find their way into the aquatic systems. Bodies of water have always been convenient places for people to dispose of wastes and together with wastes from aquatic denizens, the water looses its ability to dilute and wash away most of the wastes that were dumped into it (Anetekhai, *et al.,* 2007).

It has been shown by research that the microfauna of aquatic animals is highly variable and is a reflection of the habitat of the individual animal, especially the food they eat (Nieto *et al.,* 1984), and a majority of these bacterial flora are carried in the gastrointestinal tract (Buras *et al.,* 1987).

Unfortunately, knowledge of bacterial infections and diseases of prawns denizen in Africa are comprised almost entirely of presumptive diagnosis of pathological or clinical conditions from studies carried out elsewhere, outside Africa (Fernandes *et al.,* 1997; Buras *et al.,* 1987) but are non-specific and common to non-African fish. Shell fishes are known to be prone to food borne disease organism leading to food borne illness in human, majority which are rarely reported because of absence of regulatory framework. Therefore, this work provides a baseline data on the composition and distribution of bacteria in the intestine of wild stock of *M. vollenhovenii* from two important natural biomes of the prawn and provides amongst others baseline information on bacteria of wild stock thus providing needed thrust when farming the species.

Materials and methods

(i) Samples collection:

M. vollenhovenii collected live were transported in ice chest to the laboratory and measured with the aid of top- loading Mettler balance (Model PE 1000) to the nearest 0.001g. Specimen with weight range of 28-56g and 16-24g from Epe Lagoon and Badagry Creek respectively were used for the study. Temperature was measured in-situ with the aid of thermometer and in the laboratory a pH meter (model PW 19418) was used in determination of the pH. Standard buffer solution (pH 7.0 – 6.87) containing 0.025M potassium-dihydrogen phosphate (KH_2PO_4) and 0.025M anhydrous -disodium hydrogen phosphate (Na_2HPO_4) was used as reference.

Sediments at the bank were collected by means of a sterilized plate while those at middle were collected using grab lined with a sterilized lynon aided.

(ii) Bacteriological sampling and analysis

Four samples from each lagoon were studied every month for microbial investigation of intestine of prawn covering May to August.

(iii) Sterilization of equipment

All the equipment and glass wares for this work were new and those which have to be used again were washed, rinsed and sterilized at 160^{o}C for 2 hours in the autoclave.

(iv) Sample preparation

The four randomly chosen specimens were sacrificed and the number of incidental organism was reduced by washing their skin with 70% ethanol before opening the ventral surface with sterile scissors to expose the body cavity. From each sample, approximately 1g of intestine

was taken aseptically, weighed and homogenized in a mortar. Then 0.2g of the homogenate was transferred to a test tube containing 9ml of saline water.

One millimeter of the solution was serially diluted to 10^{-6}. Volumes (0.1ml) of the dilutions were cultured on two bacteriological media of Macconkey agar (for isolation of enterbacteiaceal) and plate count agar (for numeration of total aerobic bacteria).

(v) Identification of microbial isolates

Gram's Staining and motility test were made following the method of Harrigan and McCance (1976). Biochemical tests were done according to tests and descriptions of Collins and Lyne (1984). Further identification of bacterial isolates into species was done according to tests and description in Bergey's manual of Systemic bacteriology (Krieg and Holt, 1984).

(vi) Statistical analysis

Data were analysed using SPSS (2006)® Version 15 statistical package with test of significance at alpha level of 0.05. Independent t test and correlation were conducted as applicable.

Results

The highest mean temperature of 30.5 ± 2.5^{o}C, (range = 28.0-31.0^{o}C) was recorded at Badagry Creek in May while the lowest mean temperature of 27 ± 2.0^{o}C (range= 26-30^{o}C) was recorded at Epe lagoon in the same month. On the other hand, the highest pH mean value of 6.9 ± 0.15 and lowest mean value of 6.0 ± 0.05was recorded in Epe Lagoon in August and May respectively (Table I).

Correlation coefficient between weight of samples and bacteria load in the intestine was low and was not significantly different in the two sites (r= 2.4, P>0.05) as shown in Table II. Prawns with mean weight of 46.3± 5.7 had the highest total bacteria counts of 8.1 ± 0.1 × 10^{7} (cfug^{-1}) in Epe Lagoon whereas the prawn with mean weight 19.8 ± 3.2 g had total bacteria counts of 3.0 ± 0.7 × 10^{7} (cfug^{-1}).

In Epe, *Escherichia coli* and *Salmonella typhi* were present throughout the period of study. The highest distribution of *E. coli* were found in August (n= 31,) representing 41% of the isolates followed by *Aeromonas hydrophila* in the same month. *E. coli* was predominant throughout the months of study. However, *Citrobacter sp.* and *Pasteurella sp.* were never found in any of the samples of the guts of the prawn at this site (Table II). The morphologies of the bacterial isolates are presented in Table VI.

Relative distribution of isolates from Badagry Creek showed that *Aeromonas hydrophila* was higher in the guts of the prawn in May, June and July accounting for 36.23% (n=25), 35.60% (n=21) and 26.98% (n=17) respectively (Table IV). *Salmonella typhi, Vibro cholerae and Enterobacter aerogenes* were not isolated in the guts of the animal.

Total bacteria counts in the banks of both sampling stations were significantly higher (p<0.05) compared to the values obtained in the column of middle part of the two water bodies (Table V). However, monthly total bacteria counts at banks and middle in both stations do not differ significantly (p>0.05).

Table I. Mean ± Std values of Temperature and pH at Epe Lagoon and Badagry creek

Months	Epe Lagoon		Badagry Creek	
	Temperature (°C)	pH	Temperature (°C)	pH
May	27.0 ± 2.0 (26-30)	6.0 ± 0.1 (6.0-6.1)	30.5± 2.5 (28-31)	6.3 ± 0.1 (6.2-6.5)
June	28.5 ± 2.5 (26-31)	6.1 ± 0.05 (6.0-6.1)	30.5± 1.5 (28-30)	6.4 ± 0.0 (6.2-6.5)
July	29.0 ± 1.0 (29-30)	6.6 ± 0.2 (6.0-6.2)	30.0±1.0 (29-31)	6.2 ± 0.5 (6.2-6.7)
August	29.0 ± 2.0 (28-30)	6.9 ± 0.2 (6.0-7.0)	29.5± 3.5 (29-31)	6.2 ± 0.3 (6.2-6.5)

Values are not significantly different between sites ($p<0.05$)

Table II. Mean ± Std weight and total bacteria counts ($cfug^{-1}$) in the guts of *Macrobrachium vollenhovenii* from Epe Lagoon and Badagry creek

Epe Lagoon		Badagry Creek	
Weight of Prawn (g)	Total Bacterial Counts ($cfug^{-1}$)	Weight of Prawn (g)	Total Bacterial Counts ($cfug^{-1}$)
49.3 ± 6.7	$5.7 \pm 0.3 \times 10^7$	18.3 ± 2.7	$3.3 \pm 0.8 \times 10^7$
31.3 ± 5.7	$6.0 \pm 0.4 \times 10^7$	21.0 ± 5.7	$2.8 \pm 0.6 \times 10^7$
40.0 ± 6.0	$6.8 \pm 1.2 \times 10^7$	19.8 ± 3.2	$3.0 \pm 0.7 \times 10^7$
46.3 ± 5.7	$8.1 \pm 0.3 \times 10^7$	19.2 ± 2.1	$5.3 \pm 0.4 \times 10^7$

Values are not significantly different between sites ($p<0.05$)

Table III. Percentage distribution of isolates in the guts of *Macrobrachium vollenhovenii* from Badagry creek

ISOLATES	May		June		July		August	
	N	%	N	%	N	%	N	%
Escherichia coli	18	20.9	22	26.50	26	35.62	31	41.33
Aeromonas hydrophila	24	27.9	20	24.09	14	19.17	16	21.33
Salmonella typhi	11	12.79	5	6.02	11	15.07	7	9.33
Klebsiella pneumonia	4	4.65	7	8.43	4	5.48	2	2.67
Proteus vulgaris	0	0	0	0	0	0	9	12.0
Pseudomonas aeruginosa	9	10.47	21	25.30	16	21.92	0	0
Serratia liquefaciens	6	6.97	6	7.22	2	2.74	5	6.67
Vibro cholerae	12	13.95	0	0	0	0	3	4.0
Enterobacter aerogenes	2	2.32	2	2.40	0	0	2	2.67

N= Number of individual isolate

Table IV. Percentage distribution of isolates in the guts of *Macrobrachium vollenhovenii* from Epe Lagoon

Bacteria	May		June		July		August	
	N	%	N	%	N	%	N	%
Escherichia coli	9	13.04	11	18.64	16	25.40	19	24.56
Aeromonas hydrophila	25	36.23	21	35.60	17	26.98	13	15.79
Klebsiella pneumonia	4	5.80	6	10.17	0	0	2	14.04
Proteus vulgaris	8	11.59	7	11.86	11	17.46	6	3.51
Pseudomonas aeruginosa	0	0	6	10.17	12	19.05	11	17.54
Serratia liquefaciens	11	15.94	8	13.56	0	0	7	10.53
Pasteurella sp.	10	14.49	0	0	4	6.35	2	10.53
Citrobacter sp.	2	2.90	0	0	3	4.76	0	3.51

N= Number of individual isolate

Table V. Total bacteria counts in the sediments of Epe Lagoon and Badagry creek in the period of study

Months	Epe Lagoon		Badagry Creek	
	Bank	Middle	Bank	Middle
May	$1.6 + 0.5 \times 10^8$	$4.4 + 0.1 \times 10^7$	$5.9 + 0.3 \times 10^7$	$4.8 + 0.1 \times 10^7$
June	$2.09 + 0.02 \times 10^8$	$4.9 + 0.1 \times 10^7$	$5.7 + 0.1 \times 10^7$	$4.2 + 0.2 \times 10^7$
July	$2.05 + 0.02 \times 10^8$	$5.0 + 0.1 \times 10^7$	$5.7 + 0.1 \times 10^7$	$4.2 + 0.2 \times 10^7$
August	$20.6 + 0.02 \times 10^8$	$3.9 + 0.1 \times 10^7$	$5.8 + 0.1 \times 10^7$	$4.7 + 0.2 \times 10^7$

Monthly value variations were not significant $p>0.05$
Values of bank and middle section varied significantly in each station $p<0.05$

Table VI: Morphological and biochemical characteristics of bacterial isolates from the gut of *Macrobrachium vollenhovenii* from Epe Lagoon and Badagry creek

Lab Code for Isolates	Gram Stain	Shape	Motility	Growth at 37°C	Citrate	Oxidase	Urease	Indole	H_2S Production	Methyl Red	Voges Proskauer	Gelatin Hydrolysis	Oxygen Relationship	Lactose	Sucrose	Fructose	Mannitol	Sorbitol	Maltose	Arabinose	Glucose	Xylose	Possible Organisms
TP1	-	Rod	+	+	-	-	-	+	-	+	-	-	fa	+	+	+	+	+	+	+	A/G	+	*Escherichia coli*
TP2	-	Rod	+	+	+	-	-	-	-	-	+	+	a	-	+	+	+	+	+	+	+	+	*Serratia liquefaciens*
TP3	-	Rod	+	+	-	-	+	+	+	+	-	+	a	-	+	+	+	-	+	+	+	+	*Proteus vulgaris*
TP4	-	Rod	+	+	+	+	-	+	-	+	+	+	fa	-	+	+	+	+	+	-	-	-	*Vibrio cholerae*
TP5	-	Rod	+	+	-	+	-	+	-	+	-	-	fa	-	+	+	+	+	+	+	+	-	*Aeromonas hydrophila*
TP6	-	Rod	+	+	+	+	-	-	-	-	+	-	fa	+	+	+	+	+	+	+	A/G	+	*Enterobacter aerogenes*
TP7	-	Cocco Bacilli	-	+	-	+	+	-	-	+	-	-	fa	-	-	+	+	-	+	+	+	+	*Pasteurella sp.*
TP8	-	Rod	-	+	+	+	+	-	-	-	+	-	fa	+	+	+	+	+	+	+	A/G	+	*Klebsiella pneumonia*
TP9	-	Rod	+	+	+	+	-	-	-	-	-	+	a	-	-	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
TP10	-	Rod	+	+	-	-	-	-	+	+	-	-	fa	-	-	+	+	+	+	-	A	+	*Salmonella typhi*
TP11	-	Rod	+	+	+	-	+	-	+	+	-	-	fa	+	-	+	+	+	+	+	A/G	+	*Citrobacter sp.*

Where += Positive for the test ; - =Negative for the test; fa= Facultative anaerobe; a= Aerobe; A/G= Acid and Gas Production; A=Acid Production

Discussion

Both Epe Lagoon and Badagry Creek are important natural resource providing the optimum conditions that supports the well being of the prawn. Range of temperature and pH in this study, 26-39^{o}C and 6.0-7.0 were within limit reported for the production of prawns and shrimps (Gürel, 2007). These values are known to be suitable for bacterial growth as well (Nester *et al.*, 1998).

Evidence indicates that the gastro-intestinal bacteria of fish is highly variable and is a reflection of their aqueous environment, especially the food of the individual fish (Nieto *et al.*, 1984). The poor correlation between weights and total bacteria load in this study showed that ingestion of isolates by prawn may be related to the relative abundant of bacteria in the sands as well as water column in which they are found. Compared with the weight of the prawn samples employed in this study, there were wide variations in the bacterial load isolated, meaning that the weight of the samples does not determine the population of the bacteria found in their intestine.

The data of the bacteria load obtained in this study were similar for both locations except that some bacteria were present in one location but absent in the other. The bacteria isolated from the various samples were 11 gram negative rods altogether, of different genera. At Epe, *Escherichia coli*, *Salmonella typhi* and *Aeromonas hydrophila* were the most dominant organisms while *Escherichia coli, Aeromonas hydrophila, Proteus vulgaris* and *pseudomonas aeruginosa* were more prevalent in Badagry Creek.

It is noteworthy that *Citrobacter sp.* was not found in Epe throughout the study but found in Badagry in small composition while *Salmonella typhi* and *Vibrio cholerae* of which the quantity of the former was high in Epe were never detected in Badagry. Occurrence of both *Salmonella typhi* and *Vibrio cholerae* is an evidence of higher human interaction at the banks of Epe Lagoon as seen in the course of the study. Pollution through washing, faeces and urine take place frequently at Oluwo a landing site for fishers and traders at Epe Lagoon. The presence of coliform bacteria simply indicates previous contamination of the lagoon with raw sewage.

Although the purpose of this study was not to diagnose any disease of the Prawn, it is very likely that the animals may suffer from bacterial diseases caused by these isolates, especially if the animals undergo stress (Snieszko, 1974).

It should be however noted that the sediments at the bank of the lagoon accommodated more bacteria, the coliforms included, than the sediments at the middle of the lagoon. This simply proved that the activities of human such as excretion of faeces, urine and sputum may contribute largely to microbial pollution of the bank and consequently leading to high bacterial isolates especially the pathogenic ones.

Plate counts of psychrotrophs were relatively constant across the months and not significantly different ($P > 0.05$) in both the bank and middle part of the creek and lagoon. This pattern may be attributed to the long adaptation period for psychrotrophs from the original mesophile microbiota of *M. rosenbergii*, a tropical water prawn, to be able to grow in refrigeration temperatures (Leitão and Rios, 2000).

Counts match the acceptable range for fresh marine fish (below log 7.0 CFU/g) (ICMSF, 1986). Initial counts were below those reported by Angel *et al.* (1981) and

Leitão and Rios (2000) who found log 6.1 and 5.2 CFU/g, respectively, in whole fresh *M. rosenbergii*.

This present study indicates that bacteria such as *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae* which are usually agents of food poisoning are prevalent in the lagoons in the two important aquatic systems and consequently in the prawn inside it. These bacteria can thus be passed to human through consumption of these food products or other activities such as swimming in the water body. However, since their presence in the gut of the animal were below the standards mentioned earlier, the prawns are safe for human consumption. It is hoped that the results from this study will form the basis for future research and development particularly those related to probiotics.

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