BACTERIAL FLORA OF THE GUT OF THE AFRICAN SNAKEHEAD, CHANNA OBSCURA (PISCES: CHANNIDAE)

Kori-Siakpere, Ovie and Evbakhare, Choice Iyore
Departments of Zoology and Microbiology
Edo State University, P. M. B. 14,
Ekpoma, Nigeria.

Keywords:- Bacterial flora, stomach, pyloric caeca, intestine, temperature, Channa obsura.

ABSTRACT

A qualitative and quantitative investigation of the bacterial flora of the gut of the African snakehead, *Channa obscura* was undertaken. The types of bacteria isolated from the different parts of the gut of *C. obscura* include Pseudomonas, Streptococcus, Citrobacter and Proteus. The coliform *(Escherichia coli, Enterobacter)* and some other Enterobacteriaceae such as Salmonella were also present. The stomach and intestine were found to have a preponderance of *Pseudomonas* and *Vibrio* species. *Klebsiella* sp and *Bacillus* sp (only in the pyloric caeca) were also isolated. On the whole, the correlation coefficients of the two incubation temperatures showed a high statistical significance. Thus the bacterial load of the gut of *C. obscura* has been shown as a function of temperature.

INTRODUCTION

The flora of living fish depends on the types and abundance of micro-organisms present in the water in which they live (Horsley 1973; Trust, 1975). Generally, microbes including moulds and yeast are considered responsible for the degradation of fish, leading to the formation of products of undesirable nature, and innocuous physical characteristics.

The presence in fish, of substantial amounts of low molecular weight compounds such as peptides and amino acids, of glycogen, lipids and a variety of metal ions, together with an abundant supply of water provides an ideal environment for the growth of micro-organisms (Okolo, 1977). Consequently, when fish dies, micro-organisms which are associated with it, or acquired by *post-mortem* external contamination are able to grow rapidly, being limited only by such factors as temperature, redox potential, P^H, chemicals smoking, radiation and antibiotics (Frazier and Westhoff, 1978).

In Nigeria, where poor sanitary practices abound, freshly caught fish during its journey, from the local fisherman to the consumers, frequently pass through the market retailer, who collects the fish wholesale direct from the fisherman's boat. At the retail market, fish are usually displayed on wooden board (which may be heavily bacteria-laden) where they are handled by prospective buyers. Consequently fish ultimately become heavily contaminated with bacteria including potential pathogens (Fodeke, 1979).

The reappears to be little information on the microflora of Nigerian freshwater, brackishwater and marine fishes. However, the flora of fresh and spoiling fish have been extensively investigated throughout the world (Liston, 1956; Shewan, 1962).

The present investigation involves the qualitative and quantitative studies of the bacterial flora of the gut of the African snakehead, Channa obscura. The choice of this species was based on its commercial importance, its availability all year round and its well-known biology.

MATERIAL AND METHODS

Experimental fish

Live specimens of *Channa obscura* weighing 152.20 - 178.05g used in this investigation were obtained from a local fish market at Illushi, Edo State of Nigeria. They were brought to the laboratory under aseptic condition and maintained in 250 litres (capacity) sterile glass aquaria. All fish were considered healthy on the basis of their healthy-looking appearance and the absence of obvious signs of disease. Both sexes were used without discrimination.

Sample Preparation

Fish were caught individually from the aquaria, weighed and disinfected with 70% ethanol. They were then opened up and the gut removed aseptically using sterile instruments. The gut was initially flushed with normal saline to remove the contents and divided into three regions namely stomach, pyloric caeca and intestine. Approximately one gram (1.0g) of each tissue sample was weighed and properly blended with 99ml of peptone water in a sterile all-glass electronic blender. The homogenates were then transferred into a 250ml flasks and allowed to stand for 30 minutes. Serial ten-fold dilutions were then made in physiological saline (0.85 NaCl).

Qualitative Examination

This involved the identification and confirmation of the organism present in the homogenates of the different regions of the gut. About five (5) colonies were picked from the specific plates and tests carried out for the presence of the presumptive organisms. Colonies were also picked randomly from the Nutrient agar (NA) and Mac Conkey agar (MCA) plates, subcultured into nutrients agar and subjected to routine test for identifications.

Test carried out are:

(i) Growth on nutrients agar

(ii) Gram staining

(iii) Carbohydrate utilization with basal medium containing substrates at 1% (w/v) concentration

(iv) Other tests include catalase, indole, mobility, oxidase, coagulase and

(v) Examination for morphological features.

Quantitative Examination

For the quantitative examination of the different regions of the gut, replicates of the diluted samples (0.1ml) were spread on Nutrients agar (NA), MacConkey agar (MCA) and Thiosulphate Citrate Bile Salt Sucrose agar (TCBS), to determine the total viable count, coliform count and vibrio count. All agar plates were incubated aerobically at 25° and 37°C for 24 hours. All colonies on NA were counted to give the total viable count, and the colonies on MCA were counted for the coliform count. All sucrose - fermenting and non-fermenting colonies on TCBS agar presumed to be Vibrio sp. on the basis of oxidase reaction were counted for the Vibrio count.

After counting was done, the arithmetic mean of the colony counts at the chosen dilution were used to calculate the microbial concentration (load) in the original sample. For convenience of presentation, the value of the microbial load were recorded as logarithm to the

base ten (log) of the number of bacteria counted.

Data Analysis

The correlation between the count for the different regions of the gut at 25°C and 37°C were examined using Pearson's coefficient of correlation. Similarly, for the two incubation temperatures the correlation between the different counts for all the portions of the gut combined was also determined. The correlation coefficient (r) was then analysed for statistical significance using the student's t-test.

RESULTS

Qualitative Investigation

The organisms found to be present on the different portions of the gut of *C. obscura* are presented in Table 1. These include *Pseudomonas, Streptococcus, Citrobacter* and *Proteus.* The coliforms *(Escherichia coli, Enterobacter)* and some other Enterobactriaceae such as *Salmonella* were also isolated. Others include *Vibrio* sp, *Klebsiella* sp and *Bacillus* sp.

TABLE 1: TYPES OF BACTERIA ISOLATED FROM THE PARTS OF GUT OF *C. OBSCURA*

Bacteria	Parts of the Gut		
	Stomach	Pvloric Caeca	Intestine
Salmonella sp	+	+	+
Enterobacter sp	+	-	+
Escherichia coli	+	+	+
Vibro sp	+	+	+
Pseudomonas sp	+	-	+
Proteus sp	+	+	+
Bacillus sp	-	+	-
Streptococcus	+	-	+
Citrobacter	+	+	+
Klebsiella aerogenes	+	+	+

Key : + = present; - = absent

The stomach and intestine were found to have a preponderance of *Pseudomonas* and *Vibro* species. *Streptococcus* and *Coliforms* have also been isolated. These were mostly gram negative species.

Quantitative Investigation

The results of bacterial count carried out on the gut of *C. obscura* are presented in Tables 2, 3 and 4. As earlier stated, the values are presented as the logarithms to the base ten of the number of bacteria counted for the two incubation temperatures of 25°C and 37°C.

TABLE 2: MEAN VIABLE COUNTS AT 25°C AND 37°C

Sample	Incubation Temperature 25°C	37°C	Correlation Coeff. (r)	P>
Stomach	4.74 ± 0.95 (4.57 - 7.23)	5.97 ± 0.84 (5.00 - 7.30)	0.99	0.001
Pyloric Caeca	5.91 ± 0.95 (4.65 - 7.23)	6.02 ± 0.98 (4.80 - 7.54)	0.99	0.001
Intestine	6.14 ± 0.98 (4.94 - 7.41)	5.75 ± 0.77 (5.01 - 6.98)	0.46	NS
Combined	5.93 ± 0.97	5.91 ± 0.87	0.78	0.001

Counts reported as Mean Log No CFU/g ± S.D. Ranges in parentheses

TABLE 3: MEAN COLIFORM COUNT AT 25°C AND 37°C

Sample	Incubation 25°C	Temperature 37°C	Correlation Coeff. (r)	P>
Stomach	5.73 ± 1.03 (4.50 - 7.36)	6.04 ± 0.99 (4.99 - 7.23)	0.36	NS
Pyloric Caeca	5.77 ± 0.99 (4.60 - 7.29)	5.90 ± 1.06 (4.19 - 7.24)	0.94	0.01
Intestine	5.73 ± 1.08 (4.62 - 7.30)	6.06 ± 1.01 (4.81 - 7.52)	0.99	0.001
Combined	5.74 ± 1.04 (4.50 - 7.36)	6.00 ± 1.01 (4.19 - 7.52)	0.77	0.001

Counts reported as Mean Log No CFU/g ± S.D. Ranges in parentheses

TABLE 4: MEAN VIBRIO COUNT AT 25°C AND 37°C

Sample	Incubation 25°C	Temperature 37°C	Correlation Coeff. (r)	P>
Stomach	5.66 ± 0.86 (4.65 - 6.59)	5.55 ± 0.84 (4.53 - 6.48)	0.99	0.001
Pyloric Caeca	5.52 ± 0.96 (4.25 - 6.70)	5.59 ± 0.85 (4.49 - 6.61)	0.97	0.01
Intestine	5.81 ± 0.9 (4.83 - 6.77)	5.63 ± 0.90 (4.50 - 6.61)	0.99	0.001
Combined	5.66 ± 0.91 (4.25 - 76.77)	5.59 ± 0.86 (4.49 - 6.61)	0.98	0.001

Counts reported as Mean Log No CFU/g ± S.D. Ranges in parentheses

The total viable count obtained from the stomach at 25°C ranged from 4.57 to 7.23 and 5.00 to 7.30 at 37°C. In the pyloric caeca, the counts recorded were 4.65 to 7.24 at 25°C and 4.80 to 7.54 at 37°C. And the intestine had counts of 4.49 to 7.41 at 25°C and 5.01 to 6.98 at 37°C. When the total viable count from the three portions of the guts were combined, a mean value of 5.93 at 25°C and 5.91 at 37°C were recorded. The mean total viable counts were higher at 37°C in the stomach and pyloric caeca, and in the intestine at 25°C in which the different counts for the two incubation temperature did not differ significantly.

The coliform counts for the stomach at 25°C ranged from 4.50 to 7.36 and 4.94 to 7.23 at 37°C. In the pyloric caeca, the counts were 4.60 to 7.29 at 25°C and 4.19 to 7.24 at 37°C. Intestine had count of 4.62 to 7.30 at 25° and 4.81 to 7.52 at 37°C. For the three portions combined, a mean value of 5.74 at 25° and 6.00 at 37°C were recorded. The correlation coefficient for the counts at the two temperatures was statistically significant, though in all cases the counts were higher at 37°C.

The vibrio counts ranged from 4.65 to 6.59 at 25°C and 4.53 to 6.48 at 37°C in the stomach. In the pyloric caeca, the counts were 4.25 to 6.70 at 25°C and 4.49 to 6.61 at 37°C. Intestine had counts of 4.83 to 8.77 at 25°C and 4.50 to 6.61 at 37°C. Combined, the three portions had a mean value of 5.66 at 25°C and 5.63 at 37°C. The mean Vibrio counts were higher at 25°C in the stomach and intestine whereas the pyloric caeca exhibits a higher count at 37°C. Though the correlation coefficient for the two incubation temperatures for all the portions of the gut were statistically significant, the mean values were within a small range of 5.55 to 5.81.

The following relationships were found between the bacterial counts at 25°C and 37°C.

Mean Total *Viable* Count (MTVC)

$y = 1.716 + 0.708x$, $r = 0.79$, $P < 0.001$

Mean Coliform Count (MCC)

$y = 1.684 + 0.751x$, $r = 0.77$, $P < 0.001$

Mean Vibrio Count (MVC)

$y = 0.365 + 0.922x$, $r - 0.98$, $P < 0.001$

Where y = counts at 37°C and

x = counts at 25°C.

Similarly, the following relationships were found to exist between the different counts at the different incubation temperatures.

At 25°C

MCC = 0.762 + 0.840 MTVC, r = 0.79, P < 0.001

MVC = 0.586 + 0.859 MTVC, r = 0.92, P < 0.001

MVC = 1.943 + 0.648 MCC, r = 0.73, P< 0.01

At 37°C

MCC = 0.775 + 0.883 MTVC, r = 0.77, P < 0.001

MVC = 1.034 + 0.770 MTVC, r = 0.78, P < 0.001

MVC = 0.796 + 0.799 MCC, r = 0.93, P < 0.001

DISCUSSION

The bacterial load of aquatic organisms have been reported to be a reflection of the bacterial contamination of their habitat. The growth of these microorganisms is encouraged by organic matter and micronutrient of the water (Anson and Ware, 1975). The checklist of bacteria isolated from the gut of *Channa obscura* include those belonging to the genera *Bacillus, Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Pseudomonas, Salmonella, Streptococcus* and *Vibro*. These microorganisms reported in the present study have been isolated from different bodies of water in Edo and Delta (Bendel) States (Otunola, *et al* 1983; Nkwodimmah 1985; Isaton, 1987).

Although the flesh of newly caught healthy-looking fish may appear sterile, the skin, gills and intestine of fish which have been recently feeding may carry considerable bacterial load. Though the bacterial flora appears to be a function of the environment, the intestinal flora differ quite considerably from that of the slime and gills (Shewan, 1866).

Quantitatively, in feeding fish the bacterial number are much greater in the gut than in either gill or slime. However, quantitatively, the flora is conditioned not so much by food or external environment as by the special ecological conditions existing in the stomach and intestine such as P^H enzymes and bile salts (Sera and Kimata, 1972).

Zelibe (1991) reported bacterial count within the gut of *Tilapia zilli* consistently higher than those on the skin and gills. The bacterial load of the gut have been attributed to the nutritional status of the fish. Intestinal coliforms have the capability of degrading urio acid in ruminants (Armstrong and Wiseman, 1962). The ability of *T. zilli* to utilise the bulk of non-protein nitrogen (urio acid) in feed composed of industrial and agricultural by-products including poultry litter has been traced to the gut microflora (Zelibe, 1986).

The presence of certain strains such as *E. coli* and *Salmonella*, which are normal intestinal flora and indicators of faecal contamination is attributable to the human waste (faecal) disposal habits of the riverine populace, which results in the contamination of the water body from which the fish were caught. In addition, the fish during the process of osmotic and ionic regulation drinks water from its surrounding and thus form one of the major source of contaminants in the gut.

In the riverine areas, where harvesting of clams, shrimps and fish is the major occupation, exposure to infected water habitats and the processing or harvested food items are important risk factors (Utsalo *et al.*, 1988). Okodugha (1986) attributed the high microbial quality of the water used in dressing meat. Similarly, Fodeke (1979) drew attention to the poor sanitary practices where freshly caught fish during its frequent journey from the local fisherman to the consumer, frequently pass through the market retailer who collect the fish wholesale direct from the fisherman's boat. At the retail market, fish are usually displayed on wooden boards (which may be heavily bacterial-laden) where they are handled by prospective buyers; consequently fish ultimately become heavily contaminated with bacteria, including potential pathogens.

Johnson (1987) examined five different Nigerian species of freshly caught fish to establish the microflora of the skin and reported no difference in bacterial load due to species, but seasonal variation in the microflora was observed.

From a practical standpoint, fish dishes prepared locally are usually well-cooked before serving and this may not be important in direct infection transmission. However, a number of diseases have been reported to be caused by microorganisms isolated from the gut to *C. obscura* in the present study. These include the involvement of *Escherichia coli* in acute gastroenteritis and urinary tract infections Jawetz *et al*, 1984). *Salmonella* sp have been found to be responsible for most enteric fever (typhoid and paratyphoid) and gastroentertis (Lundbeck *et al.*, 1955; Frazier and Westhoff, 1979) *Aeromonas* sp in gastroenteritis, septicemia and wound infection (Agbonlahor, 1983). Others include *Klebsiella* sp, *Pseudomonas* sp and *Proteus* sp which involved in Otitismedia, urinary tract infection, septicemia, and occasionally meningitis and diarrhoea (Cruickshank *et al.*, 1975).

Correlation coefficient analysis showed high positive correlation between the individual count, and within the two incubation temperatures. The high correlation coefficient values between the microbial load of the gut of *C. obsura* and incubation temperatures suggests a dependency of the microbial load on temperature. Georgala (1958) reported that peak bacterial load coincided with the maximum water temperatures.

REFERENCES

Agbonlahor, D. E. (1983). The role of *Aeromonas* in acute diarrhoea disease in Nigeria. *Cent. Afr. J. Med.* 29 (7): 142 - 148.

Anson A. E. and Ware E. W. (1974). Survey of the distribution of bacterial population of Bristol channel. *J. Appl. Bacteriol.* 37: 657 - 661.

Armstrong P. R. Jr. and Wiseman R. F. (1962). Uric acid degradation by intestinal bacteria of the rat *Bacterial Proc.* p. 159

Cruckshank, R., Duguid, J. P. Marmion B. P. and Swain, R.H.A. (1975). *Medical Microbiology* vol. 2, 12th ed. Churchhill Livingstone Edinburgh London.

Fodeke V. A. (1979). Studies on heavy metal and microbial contamination of *Tilapia* species in Lagos Lagoon. M. Sc. Dissertation. University of Lagos.

Frazier W. C. and Westhoff D. C. (1978).
Food Microbiology. Tata McGraw Hill Publishing Co. Ltd. New Delhi p. 440 - 451.

Georgala G. E. 1958). The bacterial flora of the skin of North Sea Cod. J. *Gen. Microbiol.*, 18: 84 - 91.

Hosley, R. W. (1973). The bacterial flora of the Atlantic Salmon, *Salmo salar* in relation to its environment. *J. Appl Microbiol.*, 36: 377-386.

Isaton G. (1987). The bacteriological and physicochemical examination of two fish ponds in relation to the bacterial flora of the fish (*Clarias* sp). M. Sc. Disserta.ion. University of Benin.

Jawetz, E., Meinick, J. L. and Adelberg E. A. (1984).
Review of Medical Microbiology, 16th Ed. Lange Medical Publications California pp. 235 - 251.

Johnson, A. M. (1987). The microbiological studies of the Nigerian marine fish species a preliminary investigation into the microflora of the skin. *J. Food Agric.*, 1: 47 - 49.

Liston J. (1958). Quantitative variation in the bacterial flora of fresh flat fish. J. Gen. Microbiol 52: 305 - 314.

Lundbeck, H., Plazikowski U, and Silvertoipe, L. (1953).
The Swedish Salmonella Outbreak of 1953. *J. Appl. Bacteriol.* 18: 535 - 55.

Nkwodimmah, C. N. (1958).
The bacteriological and physicochemical quality of Ojirami Dam. M. Sc. Thesis University of Benin.

Okudugha, S. A. (1986). Microbiological safety and stability of Intermediate moisture beef. M. Sc. Thesis University of Nigeria Nsukka.

Okolo G. O. (1977). Microbiological studies of fish spoilage. Ph. D. Thesis University of Lagos.

Otunola, E. T. Ogunsanya C. O. and Ekundayo, J. A. (1983).
Microbial quality of *Gryphaea gasar* Adamson from the coastal waters of Nigeria and Ghana. Microbios Letters 24: 91 - 98.

Sera H. and Kimata, M. (1972).
Bacterial flora in the digestive tract of marine fish. *Bull. Jpn. Soc. Sci. Fish.* 38: 50 -55.

Shewan J. M. (1962). The bacteriology of fish and spoiling fish and some related chemical changes. In *Recent Advances* in *Food Science* vol 1 (commodities) Eds. Hawthorn J. and J. Mulleitch Butterworths, London p. 167 - 193.

Shewan J. M. (1966). Bacteriology and biochemcial changes opccurring durin microbial spoilage of sih. Supplement to Medlemsbland for Den Norska Veterinaer - forening No 11: 13 - 30.

Trust T. J. (1975). Bacteria associated with the gills of Salmonid fishes water. *J. Appl. Bact.* 38: 228 - 233.

Utsalo, S. J., Mboto, C. I., Gemade E. I. I. and Nwabgwa M. A. (1988).
Halophilic *Vibrio* spp. associated with hard clams (*Mercenaria* spp) from the Calabar river estuary. *Trans. Roy. Soc. Trop Med. & Hyg.*, 82: 327 -329.

Zelibe S. A. A (1986). The feeding cycles and response of *Tilapia zilli* (Gervais) to varied feed formulations. Ph. D. Thesis University of Ibadan.

Zelibe S. A. A. (1991). Isolation of some organisms of public health significance from a population of *Tilapia zilli* (Gervais) in Awba reservoir Ibadan. *Nig. Ann. Nat. Sci.* 1: 45 - 49.