Journal of Science and Multidisciplinary Research
Volume 6, No. 1, 2014
ISSN: 2277-0135

COMPARATIVE STUDY OF BACTERIAL ISOLATES ASSOCIATED WITH THE SKIN OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS BIDORSALIS* IN KAINJI LAKE AREA

Kolndadacha, O.D[1]. Adikwu, A. I.[2], Okaeme A.N.[1], Orgem, C.M.[3], Atiribom, R.Y[1]. and Mshelia M.B[1].
[1]National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria.
[2]Department of Biological Science, Benue State University, Benue State, Nigeria.
[3]Department of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.
E-mail: kolnda@yahoo.com

Abstract: One hundred and twenty (120) apparently healthy *Heterobranchus bidorsalis* and *Clarias anguillaris* from National Institute for Freshwater Fisheries Research (NIFFR) as cultured and Monai and Wara villages as wild environment were obtained for the studies. The total bacterial load varied from $2.2x10^5$ to $1.08x10^8$ and $2.27x10^5$ to $6.3x10^8$ CFU/g of the skin of *C. anguillaris* and *H. bidorsalis* in the culture respectively, while the load varied from 1.77 x 10^5 to $1.17x10^8$ and 2.27 x 10^5 to $9.0x10^7$ CFU/g in the wild respectively. Eleven bacterial genera/species were identified which include: *Bacillus species, B. firmus, Pseudomonas* species*, P. aeruginosa, Escherichia coli, Klebsiella aerogenes, K. ozaenae, Staphylococcus aureus, Streptococcus* species, *S. faecalis, and Aeromonas* species. The aims of this investigation is to compare the type and the load of bacteria isolates on the skin of catfish in culture and wild environment

Keywords: Disease, *Heterobranchus bidorsalis*, *Clarias anguillaris,* Fish Skin.

INTRODUCTION

Disease is a primary constraint to the growth of many aquaculture species and is now responsible for severely impeding both economic and socio-economic development in many countries of the world (Subasinghe *et al.* 2001). Diseases decrease production, causes, morbidity and mortality and pose a serious setback for continued growth of fishing industry. Infectious diseases take precedence over all other contributing causes for production losses. Paying attention to health problems with both pro-active and reactive programmes have thus become a primary requirement for sustaining aquaculture production and product trade (Subasinghe and Bernoth, 2000).

MATERIALS AND METHODS

Fish Sample

A total of one hundred and twenty (120) apparently healthy *Heterobranchus bidorsalis* and *Clarias anguillaris* species from Monai and Wara, Niger and Kebbi States respectively, representing wild and culture environment were collected for this study. Culture samples were obtained from the fish skin. Each of the skin part was obtained by excavation with scalpel of a dissecting set in an aseptic manner for bacterial culture samples.

Culture Samples

One gram of each skin measured with a Mettla sensitive weighing balance model was crushed into 9mls of sterilized distilled water for serial dilution in the order of 1/10, $1/10^2$, $1/10^3$, $1/10^4$ and $1/10^5$. Then, 0.1m of each dilution was inoculated on culture media (BHIA) and incubated for 18-24 hours at 37°C. The total colony forming units were counted on each plate of varied dilution using Quebec dark field colony counter. Only plates with 30-300 colonies were used to calculate colony forming unit (CFU). The mean colony counts were determined per plate

of each dilution. The colony count per milliliter of a particular dilution was calculated by dividing the volume of the liquid transferred to each plate.

The count per milliliter of the original sample was obtained by multiplying with the reciprocal of the dilution which gave the colony forming unit (CFU) represented by the formula: Colony forming unit.

$$(CFU) = cc \times df / iv$$

Where;

cc	=	Colony Count
df	=	Dilution Factor
iv	=	Inoculum's Volume

Identification of Bacteria Isolates

A random collection of colonies were carried out and streaked on fresh media with sterilized wire loop and incubated at 37°C for 18-24 hours. After incubation, a colony was subjected to Gram stain procedure according to Cowan and Steel (1993) to identify the group of the organisms. These colonies were then subjected to characterization to identify the isolates to species level, based on the criteria documented by Mette *et al.* (2004).

Duncan Multiple range test was used to identify the significant difference in the mean log of CFU of the bacterial load using Instat Graphpad statistical package.

RESULTS

Bacterial Count

The total plate counts were counted under a Quebec dark field colony counter with a guide plate ruled in square centimeter. After incubation the plates with 30-300 colonies were chosen for counting and the total plate count bacteria is expressed as a number of colonies forming unit (CFU) per gram of the sample of fish skin. The result of total plate counts of bacteria from *Clarias anguillaris* and *Heterobranchus bidorsalis* in both wild and culture environment were compared.

The total bacterial load varied from $2.2x10^5$ to $1.08x10^8$ and $2.27x10^5$ to $6.3x10^8$ CFU/g of the skin of *C. anguillaris* and *H. bidorsalis* in the culture respectively, while the load varied from 1.77×10^5 to $1.17x10^8$ and 2.27×10^5 to $9.0x10^7$ CFU/g in the wild respectively. In the culture it was found that 23.33% of cultured *C. anguillaris* and 40% of culture *H. bidorsalis* had high number ($>10^7$), 13.33% and 16.67% of culture *C. anguillaris* and *H. bidorsalis* respectively had extremely high number of count ($>10^8$). While only 13.33% and 6.67% of culture *C. anguillaris* and *H. bidorsalis* respectively had acceptable level of total plate count ($<10^5$) (FAO, 2001). In the wild, 20% *C. anguillaris* and 13.33% *H. bidorsalis* had high count (10^7), 6.67% *C. anguillaris* had extremely high count ($>10^8$), but non in *H. bidorsalis* while 16.67% *C. anguillaris* and 33.33% *H. bidorsalis* had acceptable count ($<10^5$). Generally the total plate count revealed the same ranges of $1x10^5$ to $6x10^8$ except for the wild *H. bidorsalis* which had maximum of $< 9.0x 10^7$. Majority of count fall in the average of 10^6. Very few numbers of fish had extremely high bacterial count.

Bacterial Identification

A total of 129 pure colonies were subjected to biochemical tests for identification. Eleven bacterial genera/species were identified which include: *Bacillus species, B. firmus, Pseudomonas* species, *P. aeruginosa, Escherichia coli, Klebsiella aerogenes, K. ozaenae, Staphylococcus aureus, Streptococcus* species, *S. faecalis, and Aeromonas* species. Five (45.45%) of the bacteria identified were Gram-positive while six (54.55%) were Gram-negative. Table 2 shows the result of the biochemical tests.

Bacillus spp, B. firmus, P. aeruginosa, Pseudomonas sp., E. coli, and *K. aerogenes* were identified from cultured *C. anguillaris* in which *Bacillus* species was found to be most predominant (43.33%) (Fig: 2). While in cultured *H. bidorsalis, P. aeruginosa, Streptococcus spp, E. coli, Bacillus spp, Aeromonas sp, K. aerogenes, Staphylococcus aureus* and *Streptococcus faecalis* were identified in which *P. aeruginosa* was found to be most predominant (35.29%). Wild *Clarias anguillaris* were found to harbor *Bacillus spp, S. aureus, P. aeruginosa, K. ozaenae, B. firmus* and *E. coli* in which *Bacillus spp* were most predominant (58.54%). Bacterial flora of some freshwater fishes in tropical water showed *Aeromonas* species to be the most predominant microorganism isolated from the skin, gills and intestine of fish. In wild *Heterobranchus bidorsalis* also, *Bacillus* was the predominant (60%), other species include *S. aureus* and *P. aeruginosa*. The frequency of occurrence of the bacteria in both wild and culture environment is presented in Table 1.

Table 1: The Frequency of Occurrence of Bacteria in Wild and Cultured Facility

Bacteria Isolates	Culture		Wild		Occurrence	
	C. anguillaris	*H. Bidorsalis*	*C. anguillaris*	*H. bidorsalis*	Total	%
Bacillus. spp	13	1	24	9	46	45.5
B. firmus	7	-	6	-	13	12.87
P. aeruginosa	4	6	1	1	12	11.88
Pseudomonas spp	1	-	-	-	1	0.99
E. coli	1	4	1	-	6	5.9
K. aerogenes	4	1	-	-	5	4.95
S. aureus	-	1	8	5	14	13.86
K. ozaenae	-	-	1	-	1	0.99
Streptococcus spp	-	1	-	-	1	0.99
Aeromonas spp	-	2	-	-	2	1.98
S. faecalis	-	1	-	-	1	0.99
Total species	30	17	41	15	101	
% Occurrence	29.70	16.83	40.59	14.85		100

Table 2: The Distribution of Bacterial Genera in *C. anguillaris* and *H. bidorsalis* in Both Wild and Cultured Environment

Bacteria Isolates	Culture		Wild	
	C. anguillaria	*H. bidorsalis*	*C. anguillarias*	*H. bidorsalis*
Bacillus. Spp	+	+	+	+
B. firmus	+	-	+	-
P. aeruginosa	+	+	+	+
Pseudomonas ssp	+	-	-	-
E. coli	+	+	+	-
Klebsiella. Aerogenes	+	+	-	-
Staphylococcus aureus	-	+	+	+
Klebsiella ozaenae	-	-	+	-
Streptococcus spp	-	+	-	-
Aeromonas spp	-	+	-	-
Streptococcus fecalis	-	+	-	-
No. of genera	6	8	6	3
% Occurrence	54.55	72.72	54.55	27.27

Cultured *Clarias anguillaris* haboured higher number of bacteria (29.70%) compared to that of culture *H. biodorsalis* (16.83%) (Table 2). Similarly wild *Clarias anguillaris* haboured higher number of bacterial occurrence (40.59%) compared to that of wild *H. bidorsalis* (14.85%). There was no significant different (P>0.05) in the occurrence of bacteria between *C. anguillaris* and *H. bidorsalis* in the culture or between the wild. The occurrence of the bacterial isolates in descending order was as follows; *Bacillus* species (45.5%), *Staphylococcus aureus* (13.86), *Bacillus firmus* (12.87%), *P. aeruginosa* (11.88%). *K. ozaenae, Streptococcus* spp and *Streptococcus faecalis* were 0.99% each (Table 2). The distribution of bacterial genera in the *C. anguillaris* and *H. bidorsalis* is presented in Table 4. The distribution of bacteria isolates recorded shows that, there are fewer bacterial genera/species in wild *H. bidorsalis c*ompared to the culture counterpart. Six (54.55) bacteria genera were found in cultured and wild *Clarias anguillaris* while three (27.27%) and eight (72.72%) in wild and culture *H. bidorsalis* respectively (Table 2).

The analysis of variance according to Duncan's Multiple Comparison test revealed only significant difference (P<0.05) between cultured and wild *H. bidorsalis*. *Bacillus sp* and *Pseudomonas aeruginosa* were identified in the catfish studied in both culture and wild environment. Most of the bacterial microflora associated with the skin of the *C. anguillaris* and *H. bidorsalis* were Gram negative rods and Gram positive-cocci.

DISCUSSION

The lower and higher limits of bacterial load in this study is higher than that of Hatha *et al* (2005) who got a maximum of 10^7 CFU/g representing 15% in their study. But the result from wild *H. bidorsalis* conforms to their studies. The study of Sugita *et al.* (1985) reported higher bacteria count compared to this study. They reported that the TVC of intestinal content of fish ranged from 10^3 to $10^9/g^{-1}$. Such variation in TVC could be associated with diet, species of host animal and their physiological content (Sugita *et al.*, 1985).

Bacterial flora of some freshwater fishes in the tropics showed that *Aeromonas sp* was the most predominant microorganism isolated from the skin, gills, and intestine of the fish contrary to this study which recorded *Bacillus spp* more dominant in cultured and wild *Clarias anguillaris* and *Heterobranchus bidorsalis* except in culture *H. bidorsalis* whereas *Pseudomonas*

aeruginosa was predominant. The predominance of *Bacillus* sp in this study corroborates with the result of Hatha *et al.* (2005) who reported the predominance of *Micrococcus* and *Bacillus* among the nine (9) bacterial genera isolated in their study. Most bacteria isolated in this study are those that are usually associated with bacterial disease such as ulcer, fin rot, acute septicemia, and gill diseases resulting to mortality and loss in productivity during disease outbreak (Okaeme 2006). The 11 bacteria genera/species harbored by the two fish species in wild and culture environment in this work are among the common ones isolated by several workers (Okaeme, 2006; Ibiwoye *et al.*, 2001). The insignificant difference ($P > 0.05$) recorded in the species, between and within the groups means that bacterial species have no specificity to either fish species or the environment. There is only significant difference ($P < 0.05$) recorded in the mean log of CFU/g^{-1} between cultured and wild *H. bidorsalis,* which was probably because the cultured pond is routinely fertilized with animal dung from different animals resulting in introduction of some contaminants which might be attributed to higher number of bacterial load and genera, especially the *Enterobactericeae*. It is recorded that the mixed isolates are always present in ponds with poor water quality as a result of accumulation of fish faeces, decomposition of uneaten fish feed, dead fish, over fertilization and high toxic metabolite in fish receptacle. Ogbondeminu and Okaeme (1986) had reported that the variation in the distribution of bacteria isolates in the different culture system may be a regulatory mechanism, which includes natural inactivation process, absorption to sediment, and uptake by fish might be in existence.

This study therefore revealed that bacterial load and species of bacteria isolates haboured by wild and cultured catfish is similar but may in combination be influenced by the management practices resulting to the alteration of quality of the aquatic environment which was attributed to significant difference ($P<0.05$) found in the mean log of CFU/g^{-1} between the cultured and wild *H. bidorsalis*. The eleven bacterial genera/species isolated on the skin may reflect those from the environment in which the fish lives with direct contact with the microorganisms. In some sense, microbes in the aquatic environment have the choice of living in association with potential host (intestinal tract, gills or skins) or the environment.

REFERENCE

Cowan, and Steel (1993). *Manual for Identification of Medical Bacteria.* Third Edition. Edited by G.I. Barow and R.K.A. Fatham, Cambridge. Pp 1- 300.

FAO (2001). Evaluation of Health and Nutritional Properties of Powdered Milk and Live Lactic Acid Bacteria. Cordoba, Argentina: *Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.* Pp. 1-34.

Hatha, M.A.A., Christi, K.S., Singhi, R. and Kumar, S. (2005). Bacteriology of Freshwater Bivalve Clam Batissa Violaech (Kai) Sold in the Suva Market. *The South Pacific Journal of Natural Science.* 23:48-50.

Ibiwoye, T.I.I., Okaeme, A.N and Agbontale, J.J. (2001). Distribution and Occurrence of Bacterial Fish Disease in Different Culture Facilities in Kainji Lake Area. *A Paper Presented at the 16th Annual Conference and Sliver Jubilee of FISON at Maiduguri 4th to 9th Nov. 2001* pp 221-226.

Mette H., Bergh G; Riaza A; Nielsen J., Melchiorsen J., Duncan H. Ahrens, Birkbeck H. and Gram L. (2004). Selection and Identification of A*utochthonous* Potential Probiotic

Bacterial from Turbot Larvae (*Scophltalnus maximus*) Rearing Units. *Systematic and Applied Microbiology 27:360-371.*

Okaeme A.N. (2006). Infectious diseases. In: *Fish Disease Prevention and Control, A Simple Field Guide.* Pp 1-31.

Ogbondeminu, F.S. and Okaeme, A.N. (1986). Bacterial Flora Associated with Organic Manure-Aquaculture System in Kainji Lake Basin Area. *Nigeria. Int. J. Zoon. 13:* 54-58.

Subasinghe R. and Bernoth E. (2000). Disease control and health management. *Aqualcuture in the 3rd millennium, Bankok. Declaration and strategy Bankok, Thailand. Pp1563-1615.*

Subasinghe R.P. Bondad-Reantoso M.G. and McGladdery S.E. (2001). Aquaculture Development, Health and Wealth. Aquaculture in the Third Millennium. Pp 1-36

Sugita, H.,Hirose, Y., Matsuo, N. and Deguchi, Y., (1998). Production of the Antibacterial Substance by *Bacillus* sp Strain NM12, An Intestinal Bacterium of Japanese Costal Fish, *Aquaculture 165:*269-280.

Reference to this paper should be made as follows: Kolndadacha, O.D. *et al.*, (2014), Comparative Study of Bacterial Isolates Associated with the Skin of *Clarias gariepinus* and *Heterobranchus bidorsalis* in Kainji Lake Area. *J. of Sciences and Multidisciplinary Research,* Vol. 6, No. 1, Pp. 64 – 69.