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Bacteria of cultured mudfish *Clarias anguillaris* (Pisces: Clariidae) in a tropical hatchery

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Resumen: Se examinó la relación entre microflora bacteriana y dieta del pez *Clarias anguillaris* en la cuenca del Lago Cainyi, Nigeria, Africa. La microflora se examinó en agua, alimento. huevos e intestinos y el pez se mantuvo en condiciones de cultivo intensivo. *Pseudomonas* y *Aeromonas* fueron los géneros dominantes en agua, huevos y juveniles. *Pseudomonas* predominó en el intestino de peces de diez días de edad y *Aeromonas* en intestinos de 30-60 días. La microflora de los individuos en crecimiento parece ser una función de la encontrada en huevos, agua y alimento. La dieta artifical usada para los adultos es rica en bacterias de tipo Gram positivo. No hubo correlación notable entre la microflora del alimento artificial y la de intestinos de 30 y 60 días.

Key words: Fish culture, bacterial microflora, fish food, diet, mudfish.

Most studies of the microflora associated with aquaculture have dealt with microbial ecology, fish nutrition, fish desease and public health.

Modern aquacultural practices are new in Nigeria (Africa). Therefore published information on bacterial microflora associated with cultured fish is scarce. In intensive rearing systems mass production of fish eggs and larvae can be affected by low egg quality and mortality of fry due to microbial infections and poor water conditions. The microflora of fish eggs and fry may be important for the establishment of the intestinal flora of adult fish and for the health of cultured fish.

The present study reports on the bacterial flora associated with eggs, food, associated water and intestines of mudfish *(Claria anguillaris)* cultured in a hatchery located within the Kainji Lake Basin, Nigeria.

The investigation was carried out in a hatchery unit previously described by Madu *et al.* (1988). Fertilized eggs were collected from vinyl nests placed at the tank bottom two days after hormone-induced breeders spawned. The eggs were washed with autoclaved 0.1 % peptone water, weighed to 0.1 % and homogenized in 9.9 ml of sterile phosphate buffer saline solution, pH 7.4. Serial 1 ml of each homogenate was done with autoclaved 0.1 % peptone water, and 0.1 ml aliquots of appropriate dilutions were used for bacteriological analyses. Samples of fish fry were collected at 10, 30 and 60 days after egg hatching, washed with 70 % ethanol before teasing out the intestinal contens with a sterile surgical scarpel; the pooled sample was weighed to 0.1 g, homogenized and the suspension treated as previosly described for the eggs. A mixed culture of zooplankton comprised principally of copepods and rotifers was grown in a concrete outdoor tank treated with animal manure. A sample of the zooplankton was collected with a 63 µm mesh net into a sterile petri-dish, and 0.2 g of the sample weighed into 9.8 ml of 0.1 % peptone water, homogenized and the resultant suspension diluted appropriately. Aliquots (0.1 ml) of the suspension were used for bacteriological analyses. Samples of

TABLE 1

Isolation frequencies (per cent) for bacterial isolates from samples of eggs, intestinal tracts at different ages (days), water and diets of *C. anguillaris*

	Fishes at different ages (days)								Diet		
	Eggs n = 80	(Water)[a] n = 62	10 days n = 105	(Water) n = 82	30 days n = 110	(Water) n = 72	60 days n = 95	(Water) n = 86	*Artemia*[b] nauplii n = 38	Zoopl-ankton n = 35	Artificial n = 77
Pseudomonas	37.5	(25.0)	15.0	(20.0)	25.5	(18.5)	23.2	(16.3)	28.5	34.0	15.6
Aeromonas	27.5	(26.0)	23.0	(25.0)	32.5	(30.0)	31.6	(36.1)	34.0	16.0	12.9
Enterobacter	0	(7.5)	1.0	(2.0)	5.0	(21.0)	9.5	(10.5)	0	8.5	12.9
Citrobacter	0	(12.5)	2.0	(2.0)	4.0	(2.0)	0	(2.3)	0	8.5	7.8
Esscherichia	0	(0)	0	(0)	0	(1.0)	0	(2.3)	0	2.8	2.5
Klebsiella	0	(0)	0	(2.0)	0	(10.5)	3.2	(4.6)	0	2.8	7.8
Protens	0	(2.0)	0	(0)	0	(2.5)	0	(1.2)	0	0	0
Acinetobacter	12.5	(10.5)	16.0	(10.0)	18.0	(0)	16.8	(10.5)	0	5.7	0
Flavobacterium	10.5	(12.5)	14.0	(10.0)	10.0	(0)	9.4	(2.3)	0	5.7	0
Bacillus	0	(0)	0	(2.5)	0	(2.5)	0	(2.3)	15.0	0	5.2
Gram positive cocci	6	(0)	0	(0)	1.0	(5.0)	0	(4.6)	6.5	8.5	24.6
Not identified	6	(4)	19	(26.5)	4.0	(7.0)	6.3	(6.9)	11.0	7.5	10.7

a. Associated water

b. 5% *Vibrio* sp. isolated

c. Viability test on routine sub-culture

Artemia salina nauplii grown in 3 % saline solution were treated as previously described for zooplankton. Artificial food (mainly fish meal with a 30 % crude protein content) was administered daily ad libitium to the growing fish from three weeks after hatching. One gram of this food was weighed into 9.0 ml of sterile 0.1 % peptone water and homogenized. The homogenate was serially diluted, and appropriate dilutions were used for bacteriological analyses.

The bacterial microflora of the aerated culture tank water samples was also determined. The bacteriological qualitative profile of the tank water, fish eggs, fry intestine and diets were assessed with the following media: Trypticse soy agar, macConkey agar, 5 % sheep blood agar, thiosulphate citrate bili sucrose agar, brain heart infusion and triple sugar iron agars. The inoculated plates were incubated at 28 ± 1 °C for 48-72 hours. A random selection of representative colonies from the various samples were streaked into fresh agar plates to ensure purity. Then, pure cultures were identified according to the procedures of Cowan (1974), Mac Faddin (1980), and Kreig and Host (1984).

The results are summarized in Table 1.

Pseudomonas, Aeromonas, Acinetobacter, Flavobacterium and Gram positive cocci were isolated from the eggs. In 10-day old fish *Pseudomonas* was predominant, while both *Pseudomonas* and *Aeromonas* dominated in the associated culture tank water. No gram positive organism was isolated from culture water during the period between egg fertilization and 10 days of fish development. Predominant bacterial groups isolated from the eggs were represented in the 10-day old fish intestine. After 30 days of hatching *Klebsiella* and *Escherichia* were not isolated from the intestinal tract of fish fed with the artificial diet, while *Aeromonas* and *Pseudomonas* were predominant. The culture water of the 30-day old fish contained these bacterial groups and some members of the Enterobacteriaceae which include *Enterobacter, Citrobacter* and *Klebsiella*. A similar trend appears to occur in fish and water an the 60-day stage. There was a persistence of the predominant bacterial groups in the intestine from 10 to 60 days with minor variations in the species composition. The percent isolation frequencies of *Aeromonas* in both 30 day and 60 day old fish were not significantly different (Student's t test, $P>0.05$). This may indicate the stability of the bacterial strain in the intestine of the growing fish within this period. No members of the Enterobacteriaceae were isolated from the *Artemia* diet but were isolated from the zooplankton and the artificial food.

The most predominant identifiable bacterial groups from *C. anguillaris* eggs were *Pseudomonas, Aeromonas, Flavobacterium* and *Acinotobacter.* This finding is similar to the observations of Sugita *et al.* (1988) on goldfish (*Casassius auratus*) eggs and to those by Yoshimizu *et al.* (1980) on eggs of *Oncorychus masou* and *O. keta.* On the surfaces of both cod and halibut eggs, *Pseudomonas, Alteromonas, Aeromonas* and *Flavobacterium* were predominant (Hansen and Olafsen 1989). It is likely that these groups of bacteria are indigenous to the surface of freshwater and marine fish eggs.

The prevalence of *Pseudomonas, Aeromonas, Acinetobacter* and *Flavobacterium* and few isolates of *Citrobacter* and *Enterobacter* in the fish intestine 10-day after hatching may be due to input of bacterial cells from the natural diets fed to the growing fish. From the results of the present study, it appears that the intestinal microflora of cultured fish could originate from their food and culture environment as well as from the egg. The microflora established thanks to favourable ecological conditions in the post larval intestine. It could further be hypothesized that the normal intestinal microflora of adult *Clarias* may be established in the fish within a 60-day post-hatching period. The occurrence of some members of Enterobacteriaceae in the culture indicates contamination. Therefore, the hatchery operators should be particularly careful regarding sanitation of water and food provided to the fish at early stages of development.

The successful maintenance of fishes employing the gnotobiotic technique has been reported (Battalora *et al.* 1985). This method may give a better understanding of the microflora of cultured fish in the tropics.

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