

No Antibacterial Properties Found in Buccal and Skin Mucus of the Mouth-brooding Cichlid *Oreochromis mossambicus*

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Abstract - The skin and buccal mucus of maternal mouth-brooding cichlid fish *Oreochromis mossambicus* at different stages of their breeding cycle were investigated for possible antibacterial activity against five recognized fish pathogenic bacteria: *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Edwardsiella tarda* and *Flavobacterium* sp. Neither skin nor buccal mucus of prespawning, egg-carrying or fry-carrying female *O. mossambicus* showed positive antibacterial activity against any of the bacteria. The results are discussed with regard to the protective mechanism in mouth brooding.

It is obvious that mouth brooding in tilapia provides physical protection from environmental hazards to the eggs and fry. It is still not clear if the brooding parents possess any other specialized anatomical or biochemical adaptation in the buccal cavity which favors survival of the eggs and fry.

Subasinghe and Sommerville (1985) showed that the survival of eggs of *Oreochromis mossambicus*, a maternal mouth brooder, outside the buccal cavity could be improved by disinfecting them against microbial pathogens in the aquatic environment. If there is similar protection in the buccal cavity, it might be manifested through the qualities of the buccal mucus, which changes according to the

breeding cycle of the brooding parent. The present study was designed to investigate possible antimicrobial qualities of the skin and buccal mucus of *O. mossambicus* females at different stages of their breeding cycle.

Genetically pure *O. mossambicus*, as identified by McAndrew and Mujumdar (1983), were used throughout the study. The female fish used had a mean weight of 164.6 g, range 146.4-184.3 g; and a mean standard length of 17.4 cm, range 17.0-18.2 cm.

To collect mucus from the skin and the buccal epithelium, twelve fish were selected in three different categories: prespawning, egg carrying and fry carrying. The prespawning females were selected on the basis of their courtship behavior and the appearance of the genital papillae. They were considered to be not more than 24 hours prior to spawning. All the egg-carrying females were within 72-96 hours post-spawning and the fry-carrying females were between 9 and 10 days post-spawning.

The methods used by Harris (1972) and Fletcher and White (1973) for the collection of mucus were modified and used during the present study.

The buccal and skin mucus saline washings were centrifuged separately at 8,000 g_n for 30 minutes in a refrigerated centrifuge at 4°C. The supernatants were dialyzed against distilled water for 24 hours at 4°C. The dialyzed material was then stored in sterile plastic stoppered ampules at -70°C. The skin and buccal mucus extracts from fish belonging to the same category were pooled and then freeze-dried in an Edwards Pirani II freeze drier.

Five Gram-negative bacterial species were used in the antibacterial assay. *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Flavobacterium* sp. used were original isolates from the aquarium water where the fish were maintained. *Yersinia ruckeri* (isolate NCMB 1316) and *Edwardsiella tarda* (isolate NCMB 2034) were obtained from the reference collection at the Institute of Aquaculture, University of Stirling. All these species have been recognized as fish pathogens (Richards and Roberts 1978; Frerichs 1984).

The pure cultures were transferred to TSA slopes prepared in sterile 25-ml glass universal bottles. The slopes were incubated at room temperature (18-20°C) for six days before the assay.

For the antibacterial assay, antibiotic medium No. 1 (Oxoid Ltd.) was used as it is recommended for microbiological assay.

Bacterial suspensions were prepared in sterile glass bottles (25 ml) by suspending a loopful of bacteria from a culture slope into 10 ml of sterile phosphate-buffered saline (PBS). Their concentration was individually estimated from optical density using a spectrophotometer (WPA S 105) with PBS as the blank. The suspensions were prepared to give a close range of optical densities ensuring somewhat uniform concentrations. The suspensions were then placed in the same water bath as the agar medium at 47°C.

Five ml of bacterial suspension were added to 100 ml of agar medium and mixed by gentle shaking in the bath to prevent solidification. Aliquots of 12 ml were placed in 7-cm diameter sterile plastic petri-dishes, adapted from the "pour plate method".

After approximately two hours, once the culture plates had solidified, four wells were cut in each culture plate using a sterile gel punch. The freeze-dried mucus extracts were resuspended in 5-ml portions of sterile PBS. Aliquots of 0.2-0.3 ml were then placed in the wells. Separate plates were used for skin and buccal mucus extracts; water from the fish tanks was used as a control. After inoculation of plates, an antibiotic assay disc containing 30 µg of oxytetracycline was placed in the center of each culture plate as a positive control. The plates were then incubated at 26°C for 48 hours.

The tissue lining over the posterior dorsum of the mouth of the females, i.e., posterior palate, showed an extremely mucoid, spongy, thick and whitish appearance. This was more prominent in females carrying eggs or fry than in females prior to spawning. No other differences were observed in the general appearance of the buccal cavity between prespawning, egg-carrying and fry-carrying females.

During the antibacterial assay, a definite, clear, bacteria-free zone was observed around the positive control antibiotic assay discs placed in all five types of bacterial culture plates, indicating the effect of the antibiotic on the bacteria. The assay was repeated twice, but no antibacterial activity was noted around the wells containing either buccal or skin mucus.

Shaw and Aronson (1954) investigated the oral incubation of *Sarotherodon macrocephalus*, a paternal mouth brooder and reported that a pair of unusual pharyngeal glands was found on the dorsal surface of the palate. Areas of similar appearance were identified in breeding *O. spilurus*, *O. macrochir* and *O. niloticus* (Ntheke 1984). During the present study similar areas were observed in the posterior palate of breeding *O. mossambicus*. Incidental observations made on

the buccal cavities of substrate spawners revealed no such glands, which may coincide with the breeding method and cycle of the fish.

Noakes (1972) found a positive correlation between the mucus cell concentration in the epidermis of parent *Cichlosoma citrinellum* (Linnaeus) and the degree of physical contact between the parent fishes and young. Thus, the eggs and/or fry may act as a trigger mechanism (Pickering and Macey 1977) for the production of mucus in the buccal cavity during mouth brooding.

Varute and Jirge (1971) on their histochemical investigation of mucosubstances in the oral mucosa of *O. mossambicus*, showed the presence of sulfate carbohydrate sulfomucines, sialomucin along with some neutral mucosubstances, and their cyclic variations in concentration during the breeding cycle. They also reported that these sulfomucins, sialomucins and glycogens reached a maximum during mouth brooding. This seems to be a biochemical adaptation of the oral mucosa for oral gestation. However, the role of these mucosubstances in such oral gestation still remains unclear.

Pickering (1974) suggested that continuous replacement of mucus in fish prevents colonization by parasites, fungi and bacteria. Hjelmeland et al. (1983) reported that *Vibrio anguillarum* was not inhibited when exposed to protease extracted from rainbow trout skin mucus in a standard nutrient medium. However, the bacteria lost their viability faster than normal. This suggests the possibility that the proteases, lysozymes, sialomucins, sulfomucins, etc., in the buccal mucus may reduce the viability of bacteria when in contact with eggs and fry, delaying the access of bacteria to them. This explanation is supported by the observation that the surfaces of dead eggs in the buccal cavity did not show any sign of bacterial activity until a few days after death (Subasinghe 1986).

These explanations, however, do not disregard the possibility that the buccal mucus of mouth-brooding cichlids possesses antibacterial properties not revealed by the present methods.

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