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DISTRIBUTION OF *PSEUDOMONAS AERUGINOSA* IN SWAMPS AND IT'S INFECTION TO *OREOCHROMIS NILOTICUS*

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Abstract

The bacterial load, *Pseudomonas aeruginosa* and their artificial infection to *Oreochromis niloticus* were studied in water of four swamps in the Rajshahi University campus during November, 2001 to October, 2002. The load of *P. aeruginosa* was found to vary from 6.80×10^5 to 5.08×10^6 ml^{-1}. In artificial infection to *Oreochromis niloticus*, the mortality rates was recorded at 90, 50, 30, 20 and 0% at 1.92×10^8, 1.92×10^7, 1.92×10^6 1.92×10^5 and 1.92×10^4 CFU fish ml^{-1}, respectively. The average bacterial load in the swamps (1.92×10^6CFU ml^{-1}) was slightly higher than that prevailed in the control pond (1.90×10^6CFU ml^{-1}). Swamps can be utilized at the present bacterial load for the fish culture.

Key words: Swamp, pathogenicity, *Pseudomonas*, *Oreochromis niloticus.*

Introduction

Swamps are potential water bodies for fish culture in Bangladesh (Rahman *et al*. 1998). Beadle and Lind (1960) defined swamps as sufficiently shallow, slow- moving water, which permits the establishment of aquatic vegetation. Ecologically, the swamps through their different phases of dereliction are considered to be the final stage of the evolution from ponds and lakes (Hora and Pillay, 1962). Department of Fisheries (DoF, 2001) of Bangladesh reported that the total area of ponds and ditches in Bangladesh is 2, 15,000 ha. The numbers of ponds vary between 13, 42, 000 to 17, 69, 000 and among the ponds the percentage of swamp is 0.19 to 0.34. Rahman *et al.* (1998) reported that swamps can be utilized for production of various fish species of different size requiring short time and small investment.

*O. niloti***cus* is an omnivorous, quick-breeding fish in ponds. This is a popular exotic fish of Bangladesh.

In Bangladesh, information bacterial diseases in fish are very scare. Wakabayashi and Egusa (1972) reported that *Pseudomonas* is a common bacterial pathogen of fishes. *Pseudomonas* spp. are found in soil, fresh water, sediments and sea water, and are known as plant and root colonizers. They are gram-negative, non-acid fast, nonsporing rods with single polar flagellum, measuring about $2\times0.4\mu$. The characteristic symptom of the disease produced by the bacteria is a remarkable septicemia hemorrhage in the skin of the mouth region, opercula and ventral side of the body (Wakabayashi and Egusa, 1972). The aim of the study was to investigate the load of *P. aeruginosa* in swamps and its infection to *O. niloticus*.

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Materials and Methods

Load of *L. aeruginosa* in swamp:

For investigation of the load of *P. aeruginosa*, a total of four swamps and a typical control pond were selected. Water samples were collected twice a week and counting of bacteria was performed according to Rahman *et al*. (1998) with slight modification. Water was inoculated into nutrient agar medium through 10 fold dilution in sterile physiological saline and then plates were incubated at 25°C for 48 h. After incubation the bacteria were counted directly by plate count method.

Collection of diseased fish:

A total of four swamps were selected for collection of severely affected fish for isolation of bacteria. The diseased fish were brought to the laboratory immediately after collection for bacteriological study. Besides, the affected fishes were counted monthly.

Isolation of *P. aeruginosa* from diseased fish:

Bacteria were collected from kidney of diseased fish (*O. niloticus*) and inoculated into agar plate. P. aeruginosa was identified according to Bergey's manual. A pure colony of *P. aeruginosa* was detected and reisolated into agar medium, and then cultured at 25^0 C for 24 h and used for artificial infection.

Artificial infection:

Pure colony of *P. aeruginosa* was identified and then bacteria were cultured in nutrient agar at 25^0C for 24 h. Bacterial cells were harvested by centrifugation at 3,000xg for 20 min. and washed twice in a physiological saline (0.85% Nacl solution). Experimental concentrations of bacteria for inoculation through injection were then prepared.

Artificial infection:

O. niloticus were selected for artificial infection. The fish weighing 5 to 10 gm were kept in 100 tank with well aerated flowing water until used. The fishes were fed with commercial pelleted foods. Different concentrations of the bacteria were made in the physiological saline by 10 fold dilution, then injected intraperitonially into 5 groups (5x10) of fishes. The concentrations of the bacteria were 1.92×10^4, 1.92×10^5, 1.92×10^6, 1.92×10^7 and 1.92×10^8 CFU/fish/ml. Injected fishes were reared for 15 days in water at 20-25^0C and mortality was recorded. Infection was confirmed by reisolation of bacteria from the kidney of dead fish using agar medium.

Results

Load of *P. aeruginosa*: The maximum number of *P. aeruginosa* was recorded to be 5.08×10^6 CFU ml^{-1} in September, 2002 and the minimum number was 6.80×10^5 CFU ml^{-1} in December, 2001. In the control pond, the highest number was 4.02×10^6 CFU ml^{-1} in September, 2002 and the lowest was 7.60×10^5 CFU ml^{-1} in January, 2002. The yearly average number of the bacterium were 1.92×10^6 CFU ml^{-1} and 1.90×10^6 CFU ml^{-1} in the swamps and in the control pond, respectively (Table 1).

Table 1. Monthly variation in total number of *Pseudomonas aeruginosa* in different swamps and the control pond.

Month	Load of *Pseudomonas aeruginosa* (CFU ml^{-1})				
	Swamp-1	Swamp-2	Swamp-3	Swamp-4	Control pond
Nov '01	1.36×10^6	1.18×10^6	1.54×10^6	1.26×10^6	1.28×10^6
Dec	9.60×10^5	6.80×10^5	1.12×10^6	7.40×10^5	8.80×10^5
Jan '02	8.40×10^5	7.60×10^5	1.00×10^6	8.40×10^5	7.60×10^5
Feb	1.14×10^6	9.60×10^5	1.32×10^6	1.02×10^6	1.06×10^6
Mar	1.44×10^6	-	1.62×10^6	1.26×10^6	1.36×10^6
Apr	1.76×10^6	-	1.90×10^6	-	1.68×10^6
May	2.08×10^6	1.90×10^6	2.24×10^6	1.96×10^6	2.04×10^6
Jun	2.34×10^6	2.12×10^6	2.56×10^6	2.16×10^6	2.28×10^6
Jul	2.64×10^6	2.40×10^6	2.90×10^6	2.50×10^6	2.56×10^6
Aug	2.76×10^6	2.52×10^6	3.20×10^6	2.56×10^6	2.68×10^6
Sep	4.10×10^6	5.08×10^6	3.04×10^6	3.14×10^6	4.02×10^6
Oct	2.32×10^6	2.16×10^6	2.10×10^6	2.24×10^6	2.24×10^6

* Yearly average load of *P. aeruginosa* in swamps was 1.92×10^6 CFU ml^{-1}
** Yearly average load of *P. aeruginosa* in the control pond was 1.90×10^6 CFU ml^{-1}

Diseased fish: Fishes infected with *P. aeruginosa* were collected from the swamps from November, 2001 to October, 2002. The maximum diseased fish were found in May and June, 2002 and the minimum in March, September and October, 2002.

Artificial infection: The mortality of *O. niloticus* varied from 0% at 1.92×10^4 to 90% at 1.92×10^8 CFU/fish/ml (Table 2).

Table 2. Artificial infection with *Pseudomonas aeruginosa* to *Tilapia nilotica.*

Dose No.	Injection dose (CFU ml^{-1})	Injected fish	Dead fish	Mortality (%)
1	1.92×10^8	10	9	90
2	1.92×10^7	10	5	50
3	1.92×10^6	10	3	30
4	1.92×10^5	10	2	20
5	1.92×10^4	10	0	0

Discussion

Diseases have become the major problem in fish production both in culture system and wild condition in Bangladesh (Rahman and Chowdhury, 1996). In Bangladesh, the major carp species have been found suffering from ulcer type diseases of different expressions, including epizootic ulcerative syndrome (EUS), bacterial hemorrhagic septicemia, tail and fin rot, bacterial gill rot, dropsy, columnaris disease, fungal disease and parasitic disease (Chowdhury and Baqui, 1997). A wide range of bacterial flora is abundant in water that is associated with fish especially with the bottom living fishes. Effective water management in fish pond is one of the important factors contributing to the success of fish culture. Study of aquatic bacteria associated with fish is very limited in Bangladesh (Chowdhury *et al*., 2001). Romanenko (1971) reported that the total bacterial number in reservoir water was 1.43-0.18$\times10^6$ CFU/ml. Chowdhury *et al.* (2001) reported that the range of bacterial load was 1.01$\times10^6$ CFU/ml to 5.06$\times10^8$ CFU/ml of water in this country. The highest bacterial load was recorded in August and September. In the present investigation the range of bacterial (*P. aeruginosa*) load was recorded at 6.80$\times10^5$ to 5.08$\times10^6$ CFU ml^{-1}.

Fish pathogenic bacteria have developed many strategies for infection to host fish species. Adhesion to the epithelial tissue of host, resistance against body surface mucus and serums of host are the important mechanisms during initial stages of infection (Thune *et al*., 1993). Several researchers (Wakabayashi and Egusa, 1972, Finlay and Falkaw, 1989, Hacker and Goebel, 1987) studied the pathogenicity of bacteria to fish. The average load of *P. aeruginosa* was recorded as 1.92 $\times$ 10^6 in swamps in the present study in the Rajshahi University Campus. The same concentration when injected produced 30% mortality in *O. niloticus*. An average load of 1.92 $\times$ 10^6 CFU ml^{-1} was recorded in control pond where fish culture was well developed. This indicates that swamps can be profitably utilized for fish culture.

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