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Viable count of bacteria in swamp water and its effect on Tilapia fish

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Abstract: The viable number of bacteria of four swamp water and their effect on *Tilapia* were determined through artificial infection. During the study period (November, 2015 to October, 2016) viable number of *Aeromonas sp.* were counted ranging from 1.9 x $l0^6$ to 3.8 x $l0^6$ CFU/ml in swamp sample-1, 2.1 × $l0^6$ to 3.9 × $l0^6$ CFU/ml in swamp sample-2, 3.1 × $l0^6$ to 3.8 × $l0^6$ CFU/ml in swamp sample-3 and 3 × $l0^6$ to 4.l × $l0^6$ CFU/ml in swamp sample-4. The highest effect of *Aeromonas* sp. on *Tilapia* was experimentally observed at 3.3 × $l0^8$ CFU/fish (80% fish mortality) and the lowest effect was observed at 3.3 × $l0^6$ CFU/fish (20% fish mortality). In this study artificial infection was performed using the isolated bacteria in *Tilapia* showed that the LD_{50} was $10^{6.6}$ CFU/ fish.

Key words: Viable bacteria, Infection, Swamp, Fish

Introduction

Swamps are one of those types of lentic water bodies which form links between terrestrial and aquatic ecosystems. Dehadrai and Tripathi (1976) have characterized these water bodies as waterlogged, shallow water areas with a loose peaty bottom, rich in decaying organic matter retaining water either periodically or shrinking or drying in summer months. There are numerous water bodies, including swamps, present in Bangladesh (Rahman *et al*., 1998). But, all of these water bodies are not used for scientific fish culture. Fish production in Bangladesh is comparatively moderate as compared to other countries of the world. To meet the increasing protein demand and to solve the unemployment problem in Bangladesh, swamps should be used for fish culture scientifically. This may also be helpful to destroy the habitat of many infectious disease vectors such as mosquitoes. Effective water management is one of the important factors contributing to the success of fish culture. Rahman *et al*. (1998) reported that swamps are suitable for fish culture in Bangladesh. However, the viable bacterial count of swamp water remains undetermined in Bangladesh.

A wide range of bacterial flora may be abundant in water and associated with fish diseases. Environment plays a crucial role in disrupting the balance between the host and the pathogen. Bacteria in aquatic fresh water systems, have been employed as an index of abundance of the microbial community.

There was a relationship between the bacterial flora of Salmon and its environment and observation reveal that the bacteria of skin were similar to the bacteria in water (Horseley, 1973). Frazier &Westhoff (1978) stated that the bacterial flora of living fish depends upon the microbial content of the water in which they live.

Rahman *et al.* (2001) reported that aeromonads were very harmful pathogens for freshwater fishes. It is recognized that *Aeromonas salmonicida,* a gram-negative non motile bacterium causing furunculosis, is one of the most serious infectious diseases of fresh water fishes (Austin *et al*., 1989). The major economic impact of this disease in recent years has been on salmon cultivation, principally in Europe, North America and Japan (Roberts, 1993) and some devastating epizootics of furunculosis have been also

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recorded in wild fish populations (Chapman *et al*., 1999). It has been observed that *A. salmonicida* shows high growth rate at 25°C. *A. salmonicida* populations may be high in polluted warm water bodies of Bangladesh. Study of aquatic bacteria associated with fish is very limited in Bangladesh. Tilapia is one of the economically important fishes and abundant in swamps. The diseased Tilapia has been found but the pathogens remain unidentified. Considering the above reasons, the present research was undertaken to investigate the viable bacterial number of *A. salmonicida* in swamp water and its effect on Tilapia fish.

Materials and Methods

Sampling

Four swamps were selected for the present investigation situated in the Rajshahi University Campus. Water samples of each swamp were collected randomly in sterilized reagent bottles from the surface and bottom on monthly basis during November, 2015 to October, 2016.

Viable count of bacteria in swamp water

To investigate the viable number of bacteria in swamp water, 3.5% furunculosis agar medium was prepared by using distilled water and it was sterilized. The agar plate was prepared and kept at room temperature for several hours. The sample water (0.5 ml) was diluted in physiological saline (0.85% NaCl solution) through 10 fold dilution and then inoculated the solution (0.5 ml) on to agar plate as duplicate. With a sterilized glass rod, the content was spread as quickly as possible and the medium was allowed to set. The petridishes were inverted and incubated at 25°C for 48 hours. The plates having brown diffusible colonies were counted by direct counting method.

Virulence of isolates to Tilapia

In this study one strain was selected from the isolated bacteria to determine the virulence to *Tilapia.* The fishes weighing 10g- 15g were obtained from a fish farm near Rajshahi University. They were kept in 50 liter tanks with well-aerated water and were fed commercial pellets for 2 weeks to acclimatize in laboratory conditions. Different concentrations of the bacterial suspension of the selected strain were made in physiological saline by 10 fold dilution and then injected intraperitonially into 6 groups of *Tilapia*, each consisting 25 fishes. Control fishes were injected sterile distilled water. Injected fishes were reared for 15 days at room temperature and the mortality was recorded. The infection by seleted strain was confirmed by re-isolating bacteria from the kidney of dead fish using furunculosis agar plate.

Isolation of pathogen from the dead fish

To isolate this pathogenic bacterium from the dead fish, the body surface of fish was disinfected with 70% ethyl alcohol and the abdomen was opened by aseptic dissection. The pathogens were isolated carefully from the kidney and lesions of dead fish. The samples were homogenized for preparation of suspension in physiological saline. The suspension was inoculated on to furunculosis agar plate and incubated at room temperature for 48 hours and then brown pigmented colonies were observed on agar plates. Bacteria appearance on farunculosis agar indicated *A. salmonicida*.

Results

Viable number of bacteria in swamp water

During the study period the viable number of bacteria in the four studied swamps varied from $1.9 \times l0^6$ CFU ml-$^{-1}$ (November, 2015 in swamp-1) to $4.1 \times l0^6$ CFU ml^{-1} (September, 2016 in swamp-4) are presented in Table 1. In swamp-1 the maximum viable number of bacteria were observed as $3.8 \times l\ 0^6$ CFU ml^{-1} in October, 2016 and the minimum was observed as $1.9 \times l0^6$ CFU ml^{-1} in January, 2016. The maximum viable number of bacteria in swamp-2 was recorded as $3.9 \times l0^6$ CFU ml^{-1} in October, 2016 and the minimum was $2.1 \times l0^6$ CFU ml^{-1} in January, 2016. In swamp-3 the maximum viable number of bacteria were recorded as $3.8 \times l0^6$ CFU nil^{-1} in October, 2016 and the minimum as $3.1 \times l0^6$CFU ml^{-1} in May, 2016.

Table 1. Monthly variation in total viable number of bacteria in swamp water samples during November, 2015 to October, 2016 in Bangladesh.

Month	Bacterial viable number in swamp water (CFU/ml)			
	Swamp-1	Swamp-2	Swamp-3	Swamp-4
November, 015	3.3×10^6	3.2×10^6	3.3×10^6	3.4×10^6
December, 015	2.5×10^6	2.5×10^6	ND	ND
January, 016	1.9×10^6	2.1×10^6	ND	ND
February, 16	2.5×10^6	2.6×10^6	ND	ND
March 16	3.2×10^6	3.2×10^6	3.3×10^6	ND
April, 16	3.5×10^6	3.5×10^6	3.6×10^6	ND
May, 16	2.9×10^6	3.1×10^6	3.3×10^6	3.3×10^6
June, 16	3.0×10^6	3.1×10^6	3.3×10^6	3.4×10^6
July, 16	3.5×10^6	3.2×10^6	2.5×10^6	3.5×10^6
August, 16	3.7×10^6	3.4×10^6	3.0×10^6	3.8×10^6
September, 16	3.8×10^6	3.0×10^6	3.2×10^6	4.1×10^6
October, 16	3.0×10^6	3.3×10^6	3.3×10^6	3.9×10^6

ND, not determined.

The minimum bacterial viable number of bacteria in swamp-4 was observed as $3 \times l0^6$ CFU ml^{-1} in May, 2016 and the maximum was found as $4.1 \times l0^6$CFU ml^{-1} in September, 2016. The results also suggested that a handsome number of bacteria were distributed in swamp water. It is also reasonable to state that the strain used for artificial challenge to *Tilapia* is *Aeromonas* sp.

Effect of bacteria on Tilapia

The LD_{50} of the bacteria determined as $10^{6.6}$ CFU/fish (Table 2). The highest mortality (80%) by bacteria on Tilapia was observed at $3.3 \times l\ 0^8$ CFU/fish ml^{-1} the lowest (20%) was observed at $3.3 \times l0^6$ CFU/fish ml^{-1} respectively. No mortality was observed at $3.3 \times l0^5$ and $3.3 \times l\ 0^4$ CFU/fish ml^{-1} (Fig.-l). This data revealed that the isolated bacteria showed low virulence to *Tilapia*.

Table 2 Virulence of isolated bacteria to Tilapia

Bacterial dose	Injected fish	Dead fish	Mortality %	LD_{50}
Control with DW	25	0	0	
3.3×10^3	25	0	0	
3.3×10^4	25	0	0	
3.3×10^6	25	5	20	$10^{6.6}$ CFU/fish
3.3×10^7	25	15	60	
3.3×10^8	25	20	80	

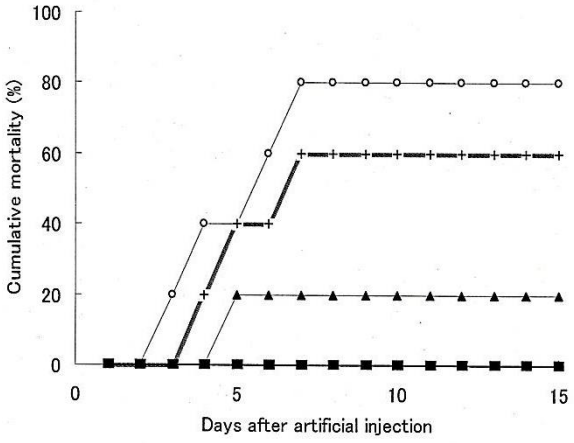

Figure 1. Cumulative mortality of tilapia fish by artificial infection of bacteria with different doses. Fish were injected intraperitoneally with 3.3×10^8 CFU/fish (o), 3×10^7CFU/fish (+), 3.3×10^6 CFU/fish **(▲),** 3.3×10^5 CFU/fish (×), 3.3×10^4 CFU/fish (♦) and control fish group were injected by sterile distilled water (■).

Discussion

Microbial studies on aquatic environment and fishes are very limited in Bangladesh. No works have been carried out on the viable count of bateria in swamp water and its impact on Tilapia. So, it was not possible to compare the present results with other findings. In Bangladesh, Banu *et al*. (2001) investigated the bacterial load in pond water. They observed that the mean bacterial load in surface water varied from 1.39×10^5 (July) to 3.11×10^7 CFU ml^{-1} (September) while that of bottom water ranged from 10×10^6 (May) to 5.90×10^7 CFU ml^{-1} (October). Romanenko (1971) reported that the bacterial number in reservoir water was $1.43\text{-}0.18\times 10^6$ ml^{-1} of water. Tewary and Mishra (1985) reported that fresh water bacteria varied from 1 to 3×10^{-1} CFU ml^{-1} in the lake water. Araki and Kitamikadi (1978) pointed out that the population density of bacteria ranged from 0.0 to 1.8×10^5 cells ml^{-1} of water in some river and pond water of Japan.

Rahman *et al*. (2001) observed the virulence of viable but non culturable state (VBNC) of *Aeromonas hydrophila* to carp *Carassius auratus* and recorded the LD_50 values of $10^{6.2}$ CFU/fish, $10^{8.45}$ CFU/fish and $>10^{9\ 11}$ cell/fish in VBNS cells. A perusal of the data shows that the natural average bacterial load of 3.3×10^6CFU ml^{-1} or below do not produce any significant mortality in *Tilapia.* Much more comprehensive studies are needed in this area in Bangladesh.

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