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PRELIMINARY STUDY ON *IN VITRO* CONTROL OF BACTERIA ISOLATED FROM *MACROBRACHIUM ROSENBERGII* (DE MAN) HATCHERY

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Abstract: Bacterial species were isolated from the dead larvae and hatchery water of Backyard Freshwater Prawn (*Macrobrachium resenbergii*) hatchery at Noakhali, Bangladesh. The selected isolates were identified as *Aeromonas sp., Bacillus badius, B. brevis, B. cereus, B. megaterium, Legionella sp., Micrococcus luteus, Moraxella Phenylpyruvica, Pseudomonas aeruginosa, Staphylococcus gallinarum and Streptococcus sobrinus*. Formalin and antibiotics were used for *invitro* control of these bacteria. Three hundred ppm formalin effectively controlled the growth of all the selected species. Susceptibility test against the antibiotics showed that Gentamycin, Kanamycin, Streptomycin and Tetracycline should be the drug of choice to control bacterial flora of the hatchery.

Key words: Bacteria; Prawn larvae; Hatchery; Formalin, Antibiotics

Introduction

Commercial cultivation of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) highly depends on adequate supply of seed. *Macrobrachium* seed is produced by some private and public hatcheries established in the coastal areas of Bangladesh. Production of seed is also based on some backyard hatcheries systems in Bangladesh. However, such a backyard freshwater prawn hatchery established at Noakhali, Bangladesh faces acute mass larval mortality. Bacteria associated with prawn larval moralities were observed by different workers (Brock, 1983; New and Singholka, 1985; Anderson *et al.,* 1990). Lavilla-Pitago *et al*. (1990) observed the *Vibrio harveyi* and *V. splendidus* were the causes of larval moralities of *Penaeus monodon* (Fabricius) in hatcheries in Panay island, Philippines. In case of *Penaeus indicus*, Hameed (1993) reported that total number of bacteria increased from egg to post-larvae and in turn survival rate decreased. From the nutritional point of view, Khan *et al.* (1996) observed that bacteria possess all the common enzymes by which it competes with small larvae. Innumerable bacterial population could be the cause of larval mortality of the hatchery. Therefore, the present study was initiated to find out the suitable inhibitor of prawn hatchery's bacteria that could be used in hatchery.

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Materials and Methods

Dead larvae of *M. rosenbergii* and water of larval rearing tanks were collected from the hatchery. Bacteria associated with the water samples and macerated larvae were isolated by pour-plate method using nutrient agar media. The selected isolates were identified according to Bergey's Manual (Krieg and Holt, 1984). Susceptibility of the isolates against formalin (BDH Chemicals Ltd., Poole England, Formaldehyde solution 37 to 41% HCHO) was determined by tube dilution technique. Nutrient broth consisting different concentrations of formalin viz. 0, 10, 20, 30, 50, 100 and 200 ppm were used. In case of antibiotics, commercially available antibiotic-impregnated discs were used.

Results and Discussion

The selected bacterial isolates were identified considering the morphological, biochemical (Table 1) and ecological characters.

Table 1. Biochemical characters of the selected bacterial species.

Name of Bacteria	Case iase	Amy lase	Catalas e	Gelati nase	Citrate	Indole	Nitrate reducta se	Tyrosi- ne degrad- ation	Acetyl methyl carbin ol
Aeromonas sp.	-	-	+	-	-	-	+	-	+
B. badius	+	+	+	+	-	-	+	-	+
B. brevis	+	+	+	+	-	-	-	-	-
B. cereus	+	+	+	+	+	-	-	+	+
B. megaterium	+	+	+	-	-	-	-	-	-
Legionella sp.	-	-	+	+	-	-	-	-	-
M. luteus	+	-	+	+	-	-	-	+	-
M. phenylpyruvica	+	+	+	+	+	-	-	-	-
P. aeruginosa	+	-	+	+	+	-	+	+	+
S. Gallinarum	-	-	+	-	+	-	+	+	+
S. Sobrinus	-	-	+	-	+	-	+	+	+

"+" sign indicates positive result and "– " sign indicates negative result.

The selected bacteria were *Aeromonas sp, Bacillus badius, B. brevis, B. cereus, B. megaterim, Legionella sp., Micrococuus luteus, Moraxella phenylpyruvica, Pseudomonas aeruginosa, Staphylococcus gallinarum* and *Streptococcus sobrinus*. The most frequent bacterial genera in larvae and larval culture water of *Penaeus indicus* were *Aeromonas, Alcaligenes, Flavobacterium, Pseudomonas* and *Vibrio* (Hameed, 1993). New and Singholka (1985) reported that in the hatchery condition, filamentous bacteria reproduce rapidly and make net-like structure that clog the gills and interfere with larval respiration. Subsequently the larvae become frail, lethargic, develop anorexia and finally die. It was observed that all the identified *Bacillus* sp. and *S. sobrinus* were filamentous. The isolate of *B. cereus* consisted prominent long chain filament.

Minimum Inhibition Concentration (MIC) for formalin was found to be 100 ppm for *B. badius, B. brevis, Legionella* sp., *M. luteus, M. phenylpyruvica* and *S. gallinarum* and 200 ppm for *Aeromonas* sp., *B. cereus, B. megaterium* and *S. sobrinus* and 300 ppm for *P. aeruginosa* (Table 2). It was observed that formalin at a concentration of 10 ppm did not differ from the 0 ppm control treatment in its ability to reduce the growth of bacterial species. Twenty ppm of formalin was the

lowest level of concentration that reduced the growth of *B. badius, B. brevis, B. megaterium, Legionella sp., M. luteus, Mor. Phenylpyruvica* and *S. gallinarum* while 30 ppm for other species. The higher the level of formalin used, the more pronounced was the reduction of bacterial growth. Formalin was used to control mass mortality in the hatchery and showed effective result. New and Singhoka (1985) recommended that 250 ppm formalin may be used to prevent infectious diseases in hatchery. Anderson *et al.* 1990) observed that 25 ppm formalin controlled bacterial necrosis of prawn hatchery.

Table 2. Growth response of selected bacterial species at different concentration of formalin.

Name of Bacteria	Concentration of formalin in ppm							
	0	10	20	30	50	100	200	300
Aeromonas sp.	+++	+++	+++	++	++	+	-	-
B. badius	+++	+++	+++	++	+	-	-	-
B. brevis	+++	+++	++	+	Trace	-	-	-
B. cereus	+++	+++	+++	++	+	+	-	-
B. megaterium	+++	+++	++	+	+	+	-	-
Legionella sp.	+++	+++	++	++	+	-	-	-
M. luteus	+++	+++	++	+	Trace	-	-	-
M. phenylpyruvica	+++	+++	++	+	++	-	-	-
P. aeruginosa	+++	+++	+++	++	++	+	Trace	-
S. Gallinarum	+++	+++	++	++	++	-	-	-
S. Sobrinus	+++	+++	+++	++	++	+	-	-

No. of "+" sign indicates the degree of growth and "-" sign indicates no growth.

Fabregas *et al.* (1987) used Penicillin-G and Streptomycin to control filamentous bacterial flora in prawn hatchery. New and Singholka (1985) suggested to use antibiotics to control bacterial population in prawn hatchery. A variety of chemicals and antibiotics have potential use in aquaculture (Meyer and Schnick, 1989). However, each country has different laws and regulations on the use of antibiotics or chemicals in aquaculture. Some of the chemicals and antibiotics allowed for use in the USA, Canada and in certain European countries are listed in Table 3.

Table 3. Chemicals and antibiotics authorized for use in aquaculture.

Country	Chemicals/ Antibiotics
United States	Acetic acid, Nifurpirinol, sulfadimethoxine, ormetoprim, Sulfamerazine, Tetrpcucome, Antimycin, Formalin.
Canada	Oxytetracycline, Erythromycin, Dichlovos, Oxolinic acid, Panicilin G, Sulfamerazine, Ormetoprim, Chloramine, Formalin, Malachite green.
European countries	Ampicillin, Chloramphenicol, Chlorotetracycline, Dimetridazole, Estonycine, Sulfamerazine, Oxytetracycline, Formalin.

Source: Schnick, 1991.

Further in-depth research is needed regarding the practical implication of the findings of present investigation. Future research can be carried out related to (1) safety to the target species (2) efficacy to control target pathogens (3) risks to the consumer from exposure to residues in food (4) hazards to the workers handling the chemicals and (5) potential for polluting the environment. On the contrary, considering the mass mortality of prawn larvae, doses of antibiotics and formalin at the minimum inhabitation concentration (MIC) observed in the present study may be used in different prawn hatcheries. In the present investigation, it was observed that Gentamycin, Kanamycin, Streptomycin and Tetracycline inhibit the growth of all the selected species as well

(Table 4). The findings suggested that the bacterial flora of the hatchery could be controlled by the application of these antibiotics. However, antibiotic therapy was not carried out in the hatchery.

Conclusion

The probable cause of mass mortality in prawn hatchery may be attributed to bacteria with particular reference to filamentous type. Three hundred ppm formalin was found to be effective in inhibiting all the selected bacterial species. Appropriate dose of formalin un-affecting the prawn larvae was not determined. Effective antibiotics should be used to avid mass mortality in prawn hatchery.

Table 4. Sensitivity test of the selected bacterial species with different antibiotics.

Name of Bacteria	Zone of inhibition in mm									
	AX	AM	CE	CO	ER	KA	GEN	PG	SM	TE
Aeromonas sp.	21	12	19.5	12.5	0	21.5	16	11.5	20	11
B. badius	0	0	20	0	28	22.5	23	0	21	14
B. brevis	26	18.5	29	12	17	28.5	25	11	25	26.5
B. cereus	0	0	21.5	0	30	21.5	24	0	20	18
B. megaterium	21.5	12	33	12	19.5	28	25.5	14	25	35
Legionella sp.	0	0	16.5	27	18.5	24	24	0	22	20
M. luteus	15	26	23	0	35	22.5	20	13.5	10	25
M. phenylpyruvica	28	17	30	0	0	27	26.5	0	26	27
P. aeruginosa	12	0	11	0	0	23	12.5	13	18.5	11
S. Gallinarum	18.5	0	14	24	13	20	22	0	18	18
S. Sobrinus	15	0	0	12	12	21	20	10	20	17

AX= Amoxycillin (10 µg/disc.), AM = Ampicillin (10 µg/disc), CE= Cephalexin (30 µg/disc), CO= Cotrimoxazole (25 µg/disc), ER= Erythromycin (15 µg/disc), KA=Kanamycia (30 µg/disc), GEN= Gentamycin (10 µg/disc), PG= Penicillin-G (10 µg/disc), SM= Streptomycin (10 µg/disc) and TE= Tetracycline (30 µg/disc).

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