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Microbiological Study of Costal Shrimp Aquaculture Production System of Bangladesh

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Abstract

The study was intended to identify the common pathogen from water and soil sample of some costal shrimp Hatchery and Fisheries of Bangladesh and to demonstrate the probiotic efficacy against this pathogen. 8 samples were taken from 6 individual costal shrimp Hatchery and bacterial load was determined from them. During the period of study, a total number of 20 bacterial colonies were isolated according to morphological characteristics. Among them, 7 groups of isolates were screened for detail study, based on of their morphological and cultural characteristics. The isolates were identified through biochemical and microbiological characteristics as *Aeromonas salmonicida, Vibrio parahaemolyticus, Bacillus fastidiosus, Vibrio vulnificus, E. coli, Vibrio harveyi* and *Aeromonas bestiarum*. The susceptibility of the selected isolates against traditional antibiotic was performed by agar diffusion method using antibiotic disc. Rifampicin showed inhibitory effect against almost all organisms where as ampicillin is the antibiotic which had no effect on either of the isolates and penicillin is less effective than other antibiotics used. Probiotic efficacy of *Bacillus* & *Pediococcus* against the selected isolates was performed. *Bacillus* and *Pediococcus* showed a satisfactory antimicrobial effect against the selected isolates which revealed that; they are favorable for the biocontrol of microbial flora in shrimp hatchery and aquaculture.

Keywords: Costal shrimp Hatchery, Fisheries, Pathogen, Probiotics, Biocontrol

1. Introduction

Aquaculture remains a growing, vibrant and important production sector for high protein animal food that s easily digestible and of high biological value. In respect to aquaculture, shrimp culture plays an important role in Bangladesh. One of the major constraints for the development of shrimp aquaculture is the mortality due to diseases (Lin, C.K., 1995 &

Subasinghe, R. 1997). Bacterial diseases mainly due to Vibrio have been reported in penaeid shrimp culture system implicating at least14 species and they are *V. harveyi*, *V. fishceri, V. splendidus, V. vulnificus* etc. A number of recent reports, press releases and on-going investigations have raised legitimate public concerns about the safety of antibiotic drug usage in aquaculture (Alderman, D. J, 1998). *Vibrio cholerae* is one of the important etiological agents in mass mortalities of *Penaeus monodon* rearing systems. To control microbial diseases, a number of chemotherapeutic agents including antibiotics are used in shrimp farms. This has led to problems such as antibiotic resistance (Karunasagar, I., 1994). According to WHO fact sheet 194 (World Health Organization Antimicrobial Resistance Fact Sheet 194), the massive use of antimicrobials for disease control and growth promotion in animals increases selective pressure on the microbial world and encourages the emergence of resistant bacteria which can transfer their resistance genes to other bacteria. An alternative method of controlling pathogenic bacterial strains in shrimp cultures could be supplementation of pure cultures of natural bacterial isolates (biocontrol) which might produce chemical substances inhibiting the growth of pathogens. The approach basically employs the activity of microorganism that could suppress or inhibit the growth of *V. harveyi* without causing bad impact on the equilibrium system in a particular microbial community. (Ohhira, I.,1996) This practice has recently been observed in aquatic systems such as fish culture and crustacean culture. The present study is an attempt to identify the list of pathogenic bacteria exist in natural environment as well as in hatcheries, surrounding water body (supply water and soil) and culture farm through microbial and biochemical analysis and assess the antibiotic resistance status of the pathogen due to bad practiced by the hatchery and farm. Moreover, trying to find out alternative approach to mitigate this pathogen through probiotic efficacy test.

2. Material and Method

2.1. Sampling Site

Selected sampling sites/ points were in Cox's Bazar area both for shrimp hatchery and grow-out pond operation.

- For Shrimp Hatchery: Cox's Bazar: Kolatoli , Sonapara
- For Grow-out pond operation: Kurushkul (Beximco Fisheries Ltd.).

2.2. Sampling Method

Samples from water and soil were taken at selected sampling sites at regular interval as per activity plan. In addition, feed and live samples at "different stages of production cycles' at Shrimp Hatchery and Shrimp Grow out pond were taken to check existing 'microbial load' and selected 'water quality' parameters of the respective area following Standard procedures (American Public Health Association (APHA),2002,).

2.3. Media And Techniques For Enumeration And Isolation of Bacteria

2.3.1. Media used

The following media were used for the enumeration and isolation of colonies of

microorganism
1. Nutrient agar medium
2. TCBS agar medium

2.3.2. Techniques Employed

Three different techniques were applied for the total count and isolation of bacteria: dilution plate, pour plate and spread plate methods (Sanders, E. R. ,2012). For the total count and segregation, of discrete bacterial colony serial dilution was carried out up to 10^6. The inoculated media were incubated at 37^0C for 24 to 48 hours.

2.4. Enumeration of Bacteria

After incubation, the plates having well spaced colonies were selected for counting. The selected plates were placed on a colony counter (Stuart Scientific U K) and the colonies were counted. The colonies were calculated by multiplying the average number of colonies per plate by reciprocal of the dilution. The calculated results would be as colony forming units (cfu) per ml of sample.

2.5. Isolation of Discrete Colonies

Isolation of well discrete bacterial colonies was done immediately after counting. On the basis of colony morphology, different colonies were selected for isolation. Characters of the colonies were recorded as color, form, elevation, margin, surface etc. Then the marked observed colonies were transferred to nutrient agar slant for further studies.

2.6. Purification of the Isolates

The colonies showing unique morphology were purified through consecutive pour plate and streak plate method.. Nutrient agar was used as media. When a
plate yielded only one type of colony, the organisms were considered to be pure.

2.7. Maintenance and Preservation of the selected purified Isolates

The purified isolates were then transferred to nutrient agar slants. The slants were kept in polythene bags and preserved as stock culture in refrigerator at 4^0C for further study. Sub culture of certain interval was maintained to keep the cultures in active condition characters unimpaired.

2.8. Final Selection of the Isolates

Final selection was made on the basis of their colony morphology on the agar plate, agar slant and their microscopic features under light microscopy.

2.9. Coding of the Isolates

The pure cultures of the isolates were coded according to the number of colonies and the serial of the sample used. The code numbers were maintained and followed till identification of the isolates after through characterization.

2.10. Identification of selected Isolates:

Identification of the microorganisms was a sequential process which included a series of different types of experiments. Selected Isolates. were subjected to biochemical tests and results were compared with standard description given in "Bergey's Manual of Determinative Bacteriology", 8th ed. (Buchanon RE.,1974) and 9th ed. (Holt JG,2000)**.** The tests include Gram-staining, Spore staining Acid-fast staining, starch hydrolysis , Voges Proskauer (V-P) Test, Production of hydrogen sulphide, Gelatin liquefaction test, Nitrate reduction test, Indole test Deep glucose agar test, Catalase reaction Methyl-red test, Fermentation test, Urease test Motility test, Oxidase test Cultural and physiological studies were also done.

2.11. Antibiotic Susceptibility Test (Bauer Aw, 1966)

Discs diffusion method was used for antibiotic susceptibility of the isolates. This test was performed on Mueller Hinton agar plates. Suspension of the isolates was prepared using sterile distilled water and adjusted to 0.5 McFarland standards. A 100 μl suspension of freshly grown bacterial cultures was spread on Mueller Hinton agar plates. The antibiotic discs were placed on the surface of agar and the plates were incubated at 37 ℃ for 24-48 hrs. Susceptibility pattern was assessed using Penicillin G (10 units), Chloramphenicol (30 μg), Erythromycin (15 μg), Nitrofuran (30 μg), Cephalosporin (30 μg), Streptomycin (10 μg) and Rifampicin (5 μg).

2.12. Probiotic efficacy test (Vijayan, K. K., 2006)

In performing the probiotic efficacy test by well diffusion method (Magaldi S., 2004), nutrient agar plates were heavily seeded uniformly with the selected isolates. Then hole was made in media by sterile cork borer in aseptic condition. Then one drop of malted agar was poured into hole and allowed to solidify to make a base layer. After that specific amount (0.1 ml) culture filtrate of probiotic bacteria (*Bacillus sp.* & *Pediococcus sp.) was* poured into two separate holes. For maximum diffusion, the culture plates were kept at low temperature (4 ℃) for 2-4 hours. The plates were then incubated at 37 °C for 24 hours to allow maximum growth of the organisms at inverted position. The efficacy of the probiotic was determined by measuring the zone of inhibition expressed in millimeter in diameter. The experiment was carried out more than once and mean of reading was taken.

3. Result and Discussion:

3.1.Determination of Total Bacterial Count And Vibrio Load Count of the Collected Samples

The total bacterial count and Vibrio load count of collected samples are shown in the Table 1. There is a variation in the bacterial count and Vibrio load count among different types of samples. (Figure 1)

Table 1: Total bacterial count and Vibrio load count of the collected samples at investigated sites

Sl. No.	Location	Type of Sample	Total Bacterial Count (cfu/ml)	*Vibrio* Load Count (cfu/ml)
1.	Dumping water zone at Kolatali, Cox'bazar	Water sample	7.06×10^{5}	4.58×10^{5}
		Soil sample	7.27×10^{6}	4.6×10^{6}
2.	Mixing water zone at Kolatali, Cox'bazar	Water sample	1.15×10^{3}	5.07×10^{2}
		Soil sample	5.04×10^{3}	2.74×10^{3}
3.	Diamond Hatchery, Cox'sbazar	Raw water	3.22×10^{2}	1.6×10^{2}
		Treated water	4.49×10^{1}	3.21×10^{1}
		Water from algal culture	2.94×10^{3}	1.52×10^{3}
4.	Beximco Fisheries, Cox'sbazar	Raw water	2.14×10^{3}	1.03×10^{3}
		Treated water	5.31×10^{2}	2.76×10^{2}
5.	Dumping water zone at Sonapara, Cox'bazar	Water sample	1.56×10^{6}	9.82×10^{5}
		Soil sample	1.18×10^{7}	6.84×10^{6}
6.	Mixing water zone at Sonapara, Cox'bazar	Water sample	6.54×10^{3}	3.99×10^{3}
		Soil sample	1.17×10^{4}	6.55×10^{4}
7.	Grameen Hatchery, Cox'sbazar	Raw water	3.37×10^{2}	1.65×10^{2}
		Treated water	5.6×10^{1}	3.62×10^{1}
		Water from algal culture	3.09×10^{3}	1.61×10^{3}
8.	Quality Hatchery, Cox'sbazar	Raw water	2.98×10^{2}	1.44×10^{2}
		Treated water	3.05×10^{1}	0
		Water from post larval culture	1.03×10^{2}	4.39×10^{1}

Figure 1: *Vibrio* Load Count on TCBS Agar Medium

The maximum bacterial load was found to exist in the soil sample of dumping ground and the total bacterial load at the zone of mixing point of hatchery discharged water with sea water was next to it. The waste water of hatchery discharged with poor or no treatment is supposed to be responsible for making the raw sea water contaminated. Wang and his co-workers (2005b) published their studies on total bacterial count of new and 3 years old grow out pond which was under cultivation of *Liptopenaeus vannamei*. Their finding revealed that total bacterial count of recently constructed pond was 1.11×10^6 cfu/ml, while it was 6.25×10^6 cfu/ml for 3 years old pound. According to Sung *et al.* (2001) (Ohhira, I.,1996). In the present study, the bacterial count in hatchery system was found to be less than what was demonstrated in earlier studies. Though the number bacteria represent a lower value comparing with the previous research, the possibility of presence of pathogenic microorganisms in the shrimp culture system is not out of consideration.

3.2. Isolation and Selection of the Bacteria from the Samples

During the period of study, a total number of 20 bacterial colonies were isolated according to morphological characteristics. Among them 7 groups of isolates were screened for further study on the basis of their morphological and cultural characteristics. The selected isolates were designated as AM1, AM2, AM3, AM4, AM5, AM6 and AM7.

3.3. Characterization and Identification of the Selected Isolates

Seven isolates were finally selected from seven groups for detail study. The purpose of characterization and identification was to place the microorganisms into a specific class or group so that the characteristics of these unknown organisms can be compared with others. The bacterial isolates were characterized on the basis of their morphological characteristics including size and shape of the organism, arrangement of the cells, presence or absence of the spores, regular or irregular forms, gram reaction etc.; cultural and physiological characteristics including temperature tolerance, salt tolerance, IMViC test, H_2S production, nitrate reduction, deep glucose agar test, fermentation of different carbohydrates etc. All these characteristics were then compared with the standard description of “Bergey’s Manual of Determinative Bacteriology”, 8th ed. (Buchanon RE.,1974). Out of seven isolates, three were found to belong to the genus *Vibrio*, two were *Aeromonas,* one belongs to the genus *Bacillus* and the other was found to be *Escherichia***.** The detail of the results can be seen in Table 2.

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Table 2: Morphological and biochemical test result of selected isolates.

Parameters	AM!	AM2	AM3	AM4	AM5	AM6	AM7
Vegetative cells	Short rod (0.3-1.0 μm	Curved rod (0.5-0.8 μm	Short rod (1.75-2.63 μm	Curved rod (0.5-0.8 μm	Straight rod (1.1-1.5 μm	Curved rod (1.1-1.5 μm	Straight rod (0.5-0.8 μm
Cell arrangement	Single or in pair	Single	Single, pair, short chain.	Single	Single or in pair	Single	Single or in pai
Gram staining	Gram -ve	Gram -ve	Gram +Ve	Gram -ve	Gram -ve	Gram -ve	Gram -ve
Spore staining	Non-spore former	Non-spore former	Spore former	Non-spore former	Non-spore former	Non-spore former	Non-spore former
Motility test	Motile	Motile	Non motile	Motile	Motile	Motile	Motile
Catalase test	+	+	+	+	+	-	-
Glucose broth	Turbid growth	Turbid growth	Turbid growth	Turbid growth	Turbid growth	Turbid growth	Turbid growth
Deep glucose agar test	Facultative Anaerobic	Facultative Anaerobic	Aerobic	Facultative Anaerobic	Facultative Anaerobic	Facultative Anaerobic	Facultative Anaerobic
Casein hydrolysis	-	+	-	+	+	+	-
Starch hydrolysis	-	+	+	+	+	+	-
Egg albumin test	-	-	+	-	+	+	-
Gelatin liquefaction	+	+	-	+	-	+	+
Growth in synthetic media	-	-	-	-	-	-	-
Growth in inorganic salt	+	+	-	+	-	+	+
Citrate utilization	-	+	-	+	-	+	Variable
Voges-Proskauer test	-	-	-	-	-	-	Variable
Methyl red test	+	+	-	+	+	+	+
Nitrate reduction test	+	+	+	+	+	+	+
H2S production	-	-	+	-	-	-	-
Indole test	-	Variable	-	+	+	+	+
Urease test	-	-	+	-	-	+	-
Oxidase test	+	+	-	+	-	+	+
glucose,	Acid and gas	Acid and gas	No acid and gas	Acid without gas	Acid from	Acid but no gas	Acid and gas

galactose	Acid and gas	+	Alkali without gas	Alkali without gas	Acid from	Acid and gas	Acid and gas
sucrose	Alkali without gas	-	No acid and gas	Acid and gas	Alkali without gas	Acid and gas	Acid but no gas
lactose	Alkali without gas	-	Alkali without gas	Acid without gas	Acid from	Alkali without gas	Acid and gas
xylose,	Alkali without gas	-	No acid and gas	Alkali without gas	Alkali without gas	Alkali without gas	Alkali without gas
arabinose	Alkali without gas	+	No acid and gas	Acid and gas	Acid from	Alkali without gas	Acid but no gas
maltose	Alkali without gas	+	No acid and gas	Acid without gas	Alkali without gas	Acid but no gas	Acid but no gas
mannitol	Alkali without gas	+	Alkali without gas	Acid without gas	Acid from	Acid and gas	Acid but no gas
pH 4.5	++++	++	-	-	+++	+	+
pH 6.5	++++	+++	++++	+++	+++	++	++
pH 7.5	+++	+++	+++	+++	+++	+++	+++
pH 8.5	+++	++	-	-	++	++	+++
Temperature (5 ^{0}C)	-	-	+	+	-	-	-
Temperature (10 ^{0}C)	-	+	+	+	-	-	-
Temperature (27 ^{0}C)	+++	+++	+++	+++	+++	+++	+++
Temperature (37 ^{0}C)	+++	+++	+++	+++	+++	+++	+++
Temperature (45 ^{0}C)	-	-	-	-	-	-	-

The above characteristics of selected isolate were compared with the standard description given in "Bergey's Manual of Determinative Bacteriology', 8th ed. .(Buchanon RE.,1974) and found closely related to the below species

Code of Isolates	**Name of Species**
AM1	*Aeromonas salmonicida*
AM2	*Vibrio parahaemolyticus*
AM3	*Bacillus fastidiosus*

AM4	*Vibrio vulnificus*
AM5	*E. coli*
AM6	*Vibrio harveyi*
AM7	*Aeromonas bestiarum*

During the present work, identified *Vibrio* species were *V. vulnificus, V. harveyi* and *V. parahaemolyticus* which are commonly termed as the pathogenic bacteria for shrimp larvae. The *Vibrio* species identified in the present study are considered pathogenic for shrimp larvae. The other bacteria identified have also detrimental effect in shrimp hatchery management. The root cause of these bacterial infections was the improper treatment of raw water and storage condition of the storage tank was not sufficient enough to maintain it contamination free. Moreover, in hatcheries the algal culture tank was another vital source of potential bacterial contamination where both the total bacterial count and *Vibrio* load count were higher than other sources. It is evident *V. harveyi* is the most dominant pathogenic *Vibrio* species that has got a greater effect on shrimp PL during the rearing period. Similarly in our present study, the *Vibrio* load count accounts for almost 40% of the total bacterial count. Besides, out of seven isolates identified, *Vibrio* remains dominant representing three different pathogenic species. According to Lavilla-Pitogo *et al*, 1998, Australia; (Lavilla-Pitago, C. R.,1998) and Karunasagar *et al*, 1994; (Karunasagar, I.,1994), Luminous bacteria particularly *V. harveyi* and occasionally other luminous species have become recognized as a devastating pathogen of Penaeid shrimp larvae and adults throughout South -east Asia. The salinity of this area is facilitation for pathogenic *Vibrio* growth. This environment proved congenial for harmful LB species like *Vibrio harveyi, V. fisheri, V. splendidus* and *V. vulnificus* for their survival and multiplication. In preventing disease outburst in shrimp hatcheries especially the temperature of rearing water tanks need to be maintained at optimum levels, least fluctuations in temperature would lead to luminous vibriosis. Although motile aeromonads appropriately receive much notoriety as pathogens of fish. It is important to note that these bacteria also compose part of the normal intestinal microflora of healthy fish. Therefore, the presence of these bacteria, by itself, is not indicative of disease and, consequently, stress is often considered to be a contributing factor in outbreaks of disease caused by these bacteria.. In the present study, the bacterial genus *Aeromonas* was identified as the second most dominant bacteria in shrimp culture system. The prevalence of this bacterium is an indication of its relation in pathogenic infection of cultured shrimp. Two of the other bacteria identified; *Bacillus fastidiosus* and *E. coli* also reported to be present in the shrimp culture system of which Bacillus is used as the probiotic treatment in shrimp hatcheries to control other bacterial growth. Although *E. coli* is not so much reported in shrimp culture system, the presence of *E. coli* is not unexpected due to the widespread availability of this organism which is also regarded as the pathogenic microbes affecting the shrimp growth.

3.4. Antibiotic Susceptibility of the Selected Isolates

The antibiotic susceptibility of the selected isolates was performed by disc diffusion method using the standard discs. The results of antibiotic susceptibility are shown the Figure 2. Among the antibiotic discs used Rifampicin showed inhibitory effect against almost all organisms where as ampicillin is the antibiotic which had no effect on either of the isolates and penicillin is less effective than other antibiotic used.

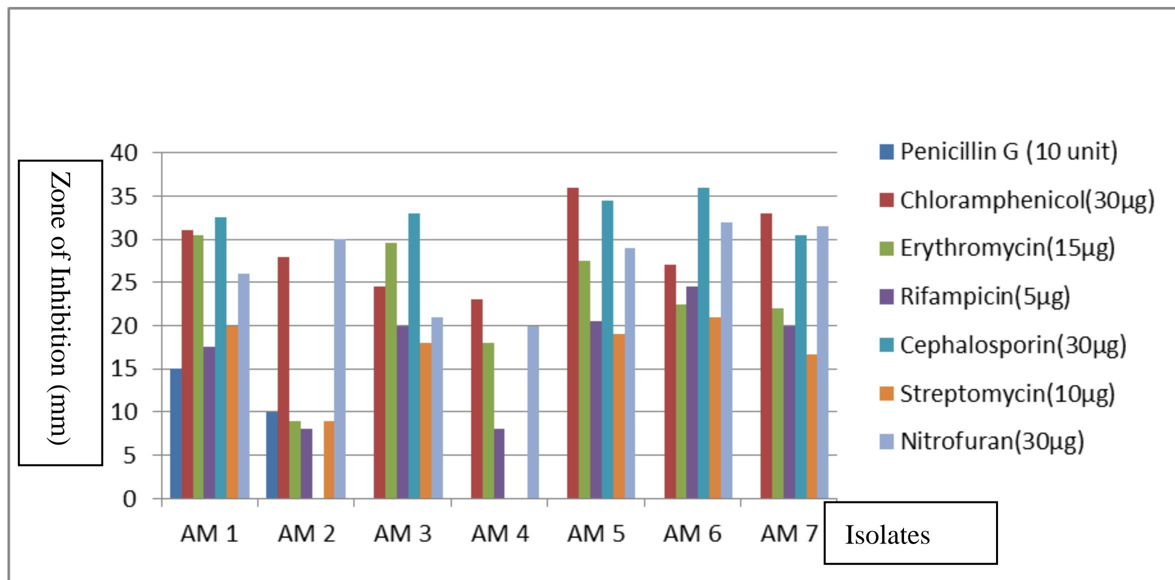

Figure 2: Antibiotic Susceptibility Test of the Selected Isolates.

All penaeid shrimp hatcheries encounter bacterial problems that impact on production. Antibiotic treatments to control pathogenic bacteria problems yields varying results. However, some of the antibiotics show effective results in controlling bacterial growth in aquaculture. Tjahjadi et al. (1994) reported that almost all of the bacteria that were isolated from seawater and hatchery-rearing water at Kalianget, East Java, including V. harveyi, were resistant to various antibiotics except rifampicin. Rifampicin, a bactericidal antibiotic, is active against gram-positive and some gram-negative bacteria. This drug interferes with transcription processes in bacteria and thus far was never used in shrimp hatchery. In the present study, a number of antibiotics were treated against the pathogenic microorganisms identified. Chloramphenicol, Erythromycin, Nitrofuran and Rifampicin are the four antibiotics that showed satisfactory inhibitory effect against all of the pathogenic microorganisms. The treatment of these antibiotics may have growth inhibitory effect upon the shrimp which requires further study.

However, with the use of antibiotics or disinfectants to kill bacteria, some bacteria survive (either strains of the pathogen or others) because they carry genes for resistance (Moriarty, D. J.,1998). These will then grow rapidly because their competitors are removed. Over a short period of time, antibiotic resistant bacterial strains develop and flourish. There is a risk of developing antibiotic resistance in these benign or beneficial bacteria, as bacteria have the capacity to transfer genetic information between themselves. A potential human or aquaculture species pathogen could acquire the genetic information required for antibiotic

resistance from the much more numerous members of the normal bacterial community. So, whether the antibiotic has high or low activity, the use of it is concerned with the shrimp growth as well as human health.

3.5. Probiotic Efficacy of Bacillus & Pediococcus on the Selected Isolates

The results of probiotic efficacy test are listed in the Figure 3. The results outlined that the probiotic organisms *Bacillus* & *Pediococcus* have highest inhibitory effect on the isolate AM1 and the isolate AM5 was less affected by it. Almost all of the isolates were sensitive to the probiotic application leaving growth inhibitory effect. Probiotics, mostly *Bacillus* strain were used and assumed that the strain produced an antibiotic that inhibit the *Vibrio* growth, then the mortality rate of *Vibrio* increased, allowing the dominance of *Bacillus*, even if the antibiotic were not produced at high concentration to kill all *Vibrio* cells directly *Bacillus* and *Pediococcus* showed a satisfactory antimicrobial effect against the selected isolates which is favourable for the biocontrol of microbial flora in shrimp hatchery and aquaculture. The probiotic bacteria *Bacillus* and *Pediococcus* have the ability to induce the antipathogenic activity by producing antimicrobial metabolites when cultured with the pathogenic microorganisms.

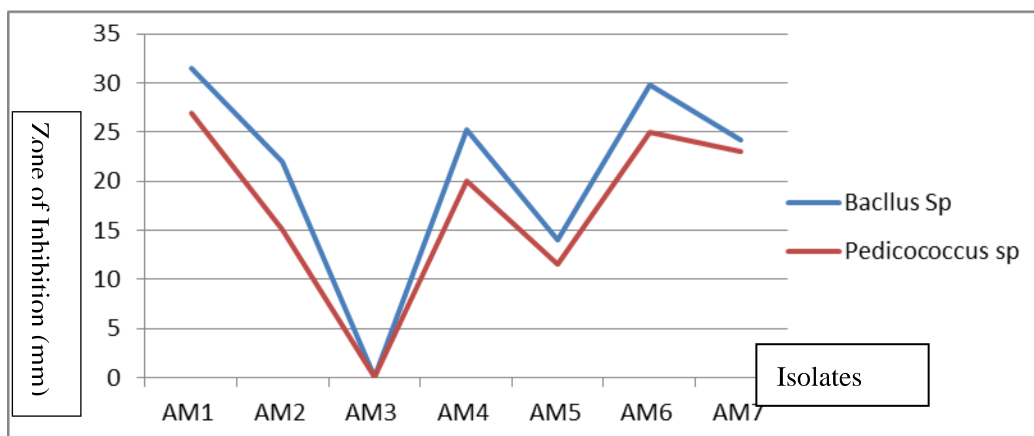

Figure 3: Probiotic efficacy test of *Bacillus* & *Pediococcus* on the selected isolates

Bacillus and *Pediococcus* showed a satisfactory antimicrobial effect against the selected isolates which is favourable for the biocontrol of microbial flora in shrimp hatchery and aquaculture. The probiotic bacteria *Bacillus* and *Pediococcus* have the ability to induce the antipathogenic activity by producing antimicrobial metabolites when cultured with the pathogenic microorganisms. In the present study, the treatment of the identified pathogens with the crude extract of *Bacillus* and *Pediococcus* produce clear zone of inhibition surrounding the area of the crude extract applied. Here, no effect was found against the isolate AM3 (*Bacillus fastidiosus*) because the isolate itself a genus of the applied probiotic bacteria. So, the presence of probiotic bacteria within the shrimp aquaculture can cause the decrease of pathogenic microorganisms by its antimicrobial action. There are many reports on the anti-*Vibrio* activity of *Bacillus* spp. Production of bacteriocin or bacteriocin-like substances have been reported from *Bacillus subtilis* (Jansen, E.F., *1944*), *Baciluus thuringiensis* (De Borjac H., 1974), , *B. licheniformis* (Bradley DE. 1967), and Use of

Bacillus spp. probiotic has been reported in piglets (Ozawa, K., *1981*) and in black tiger shrimp, *Penaeus monodon* [15]. The latter study showed that *P. monodon* larvae fed with *Bacillus* S11 showed 100% survival after challenge with pathogenic *V. harveyi*, while only 26% control animals survived. The use of *Bacillus* and *Pediococcus* as a probiotic in killing the pathogenic microorganisms present in shrimp culture and Hatchery production system is effective enough to facilitate the culture condition for shrimp larvae rearing; whereas the use of antibiotic as an antimicrobial agent may cause the growth inhibition of shrimp larvae itself. The preference for the use of *Bacillus* and *Pediococcus* as probiotic in shrimp hatchery and aquaculture is also more economical than the use of antibiotic.

4. Conclusion

Waste water discharged from shrimp hatchery and aquaculture without any or proper treatment is a potential source of microbial contamination within the shrimp culture. The untreated waste water gets mixed with sea water which is further used for hatchery operation. The representative microbial population within shrimp culture are the *Vibrio* spp., *Aeromonas* spp., *Bacillus fastidiosus* and *E. coli* among which *Vibrio* and *Aeromonas* are the pathogenic microorganisms causing disease of shrimp. The antibiotic effect upon the shrimp pathogen is strong enough to prevent microbial growth but at the same time antibiotic itself can cause inhibitory effect on shrimp growth. Alternatively, the use of probiotic bacteria to kill other bacteria is of great interest in preventing microbial contamination in shrimp culture. Finally, proper treatment of raw water before use, maintenance of personnel hygiene, treatment of waste water before discharge, use of appropriate dosage of antibiotic and probiotic treatment instead of antibiotic-are the requisites for a disease free shrimp culture system.

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