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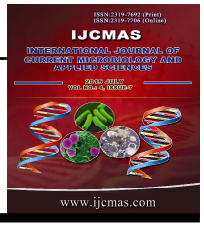

Original Research Article

Isolation of Actinomycetes from Shrimp Culture Pond and Antagonistic to Pathogenic *Vibrio* spp. and WSSV

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A B S T R A C T

The shrimp diseases in culture pond mainly accounted as vibriosis caused by *Vibrio* spp. Probiotic supplementation of live microorganisms in aquaculture aids in preventing disease, thereby increasing production and decreasing economic loss. In the present study, indigenous actinomycetes were isolated from shrimp pond sediments to control *Vibrio* infections. In this study, 56 actinomycete strains isolated from shrimp ponds were screened for their anti-vibrio activity. The pathogenic vibrios selected for the study were *Vibrio parahaemolyticus*, *V. alginolyticus* and *V. harveyi*. Based on the cell morphology, spore chain and structure of sphorophores most of the strains were found to be *Streptomyces*. Among the strains, 15 showed anti-vibrio activities within these strains VM-8, VM-15, VM-21 showed promising antagonistic activity against WSSV and extended the life span of shrimps up to three weeks.

Keywords

Marine actinomycetes, Probiotics, Vibrio, WSSV

Introduction

Shrimp farmers are suffering heavy financial losses and there is a need to find novel bioactive compounds with therapeutic potential that can be used to combat disease. *Vibrio* spp. are common in seawater and sediments, the occurrence are more in sediment than water and many of them are opportunistic pathogenic bacteria (Bhashkar *et al.,* 1998).

The proliferations of this opportunistic pathogenic *Vibrio* are the major cause for the mortality of shrimps. When the *Vibrio* load increased in the culture system it resulted in weakening of animal and ultimately facilitated the carrier of viruses**.** The virus infection first appeared among the culture ponds and was reported in Taiwan, from where it quickly spread to other

shrimp-farming countries in Southeast Asia (Kimura *et al.*, 1996). Actinomycetes are gram-positive organisms with a high G+C (>55%) content in their DNA. Actinomycetes from marine environs are important sources of novel antibiotics (Okami, 1986). Actinomycetes from marine and coastal ecosystems may provide a rich gene pool containing isolates capable of producing useful metabolites (Goodfellow and Haynes, 1984; Okami, 1986). As potential probiotic strains in shrimp culture, actinomycetes have many following advantages: (1) the production of antimicrobial and antiviral agents (Austin, 1989; Oskay *et al.*, 2004); (2) the degradation of complex biological polymers, such as starch and protein (Barcina *et al.*, 1987), lignocellulose, hemicellulose, pectin, keratin, and chitin (Williams *et al.*, 1984) which shows the potential to involve in mineralization and nutrient cycles in the culture ponds and in feed utilization and digestion once getting colonized into the host intestine; (3) the competition for nutrients, particularly iron in marine microbes (Kesarcodi *et al.*, 2008); (4) the mostly non–pathogenic to the target animals in aquaculture (Yang *et al.*, 2007); and (5) the formation heat– and desiccation–resistant spores and the retention of viability during preparation and storage.

The *Streptomyces* genus is very common in marine environments. Marine actinomycetes, especially *Streptomyces*, have long been considered chemical factories for antibiotic production (Okazaki and Okami, 1972; Ellaiah and Reddy, 1987). However, reports are scanty on the application of actinomycetes as probiotics in aquaculture. Probiotics are being used extensively in shrimp culture but imported formulations are costly. There is a need to find effective and cheaper alternatives. Considering the importance an attempt was made to screen the antagonistic actinomycetes from the shrimp culture ponds and the same was used as probiotics to control the Vibriosis and WSSV in laboratory culture.

Materials and Methods

Sample collection

Sediment samples were collected from shrimp culture ponds located in Marakkanam, South east coast of India, Tamilnadu, Collections of the samples were made at 4 shrimp culture (I, II, III and IV) ponds, Samples were collected from 2 to 6 cm depth and transported to the laboratory in sterile polythene bags and stored for further study.

Isolation of actinomycetes

Isolation media consisted of the starch casein agar (SCA) (soluble starch 10 g, casein 0.3 g, K_2HPO_4 2 g, KNO_3 2 g, NaCl 2 g, $MgSO_4$ 7g, H_2O 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4$ 7g, H_2O 0.01 g, agar 15 g, distilled water to 1 L, pH 7.6) added with filtered (0.2–μm pore size) nystatin (25 μg/l) after sterilization at 45–50 ˚C to inhibit the growth of fungi and nalidixic acid (10 μg/l) to inhibit the growth of bacteria One gram samples of dried sediments were diluted (10–2 to 10–5) in sterile saline solution (0.85% w/v NaCl). 100 μl of each dilution was plated onto isolation media in triplicate Petri dishes.

The inoculated plates were incubated at 35° C for 7 days. After incubation, actinomycetes isolates distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar were purified by streak plate method and maintained on SCA slant at 4 °C.

Isolation, identification and characterization of pathogenic *Vibrio* spp.

Vibrio spp. were isolated from sediment of Parangipettai fish landing center, South East Coast of India by spread plate method on Thiosulphate Citrate Bile Sucrose Agar (TCBS, HiMedia), at 35 ºC for 24–48 hours and kept on Tryptone Soya Agar (TSA, Becton Dickinson) slants containing 1.5% NaCl. The pathogenicity of *Vibrio* spp. to juvenile tiger shrimp (*Penaeus monodon*) was tested by injecting intramuscularly into healthy shrimps at the site between the third and fourth abdominal segments (Vera *et al.*, 1992) and maintained in plastic basins containing sterile brackish water. Shrimp inoculated with sterile saline served as control. Mortalities were recorded up to 48 h post–inoculation.

Their phylogenetic characteristics were determined based on the nucleotide sequences of 16S rRNA. DNA extraction of *Vibrio* spp. was conducted following protocol of Sambrook and Russell (2001). PCR reactions were carried out in a final volume of 25 µl containing 0.5 µl of template; 2.5 µl buffer taq (10X); 3 µl $MgCl_2$ (25 mM); 0.625 µl of each dNTP (10 mM); 1.4 µl of each primer; and 0.3 µl of Taq DNA polymerase (5U/µl). The bacterial 16S rRNA targeted primer pair consisting of 341F and 907R.

The amplifications were programmed for an initial denaturation of 5min at 95 ºC, followed by 35 cycles of 1 min at 95 ºC, 55 sec at 58 ºC and 1 min at 72 ºC, and a final extension of 7 min at 72 ºC. The 16S rRNA nucleotide sequences were compared with available 16S rRNA nucleotide sequences in GenBank using the BLAST system and submitted to DDBJ/EMBL/GenBank to get respective accession numbers.

Activities against *Vibrio* spp. of actinomycetes isolates

The activities against pathogenic *Vibrio* strains of actinomycetes isolates were determined using the double– layer agar method. The actinomycetes strains were inoculated on Petri dishes containing 15 ml SCA and incubated at 35 ˚C for 3 days. Then TCBS agar medium (HiMedia) was poured onto the basal layer containing actinomycete colonies. *Vibrio* strains were inoculated in flask containing 50 ml peptone alkaline (10 g peptone, NaCl 10 g, distilled water to 1 L, pH 8.5) at 30 °C for 24 hours. Then *Vibrio* spp. was plated onto the top layer, respectively. The inhibition zones were measured after incubation at 35 ˚C for 24 hours (You *et al.*, 2005). The actinomycetes strains with high activities against pathogenic *Vibrio* spp. were screened for further studies.

Effect of competitive interaction against *Vibrio* sp in culture tank

The 3 actinomycetes strains which showed maximum activity against the pathogenic *Vibrio* spp was taken and mass cultured in production medium (glycerol – 2.5 g, beef extract – 0.5 g, peptone – 0.5 g, yeast extract – 1.0 g, $MgSO_4$ 7g,H2O – 0.05 g, K2HPO4 – 0.05 g, $CaCO_3$ – 0.1 g, sea water – 50 ml, pH – 7) to increase the cell density. The healthy juvenile tiger shrimps (*Penaeus monodon*), collected from the shrimp culture ponds were used for this study. The experimental design was completely randomized with triplicate for each strain, each containing 20 shrimps (1± 0.3g) randomly distributed in tanks of 40 liters capacity. The actinomycetes strains were inoculated for three successive days. After 72 h of exposure a 50% water exchange was carried out. By 4^{th} day of the experiment, *Vibrio* sp. was inoculated at 10^6CFU/ml

concentrations and no water exchange performed for 24 hours. Interaction percentage was evaluated by counting the CFU/g on nutrient agar prepared in seawater, differentiating strains on morphological characteristic by Gulliana *et al*. (2004).

Activity of actinomycetes against WSSV

Three actinomycetes strains VM-8, VM-15 and VM-21 respectively were inoculated onto seed medium and then transferred to production medium to increase the cell density for increased production of bioactive compounds. After 10 days of incubation, the fermentation broth was concentrated in a vacuum evaporator (Speed Vac, Savant, USA) and 100 ml of the broth was further concentrated to 5 ml and incorporated into 10 g of the commercial pellet feed with the help of a binder (Bindex gel). Three experimental feeds were prepared with selected isolates of actinomycetes and one control feed without incorporation of actinomycetes strains.

The shrimps were acclimatized to laboratory conditions by feeding commercial pellet feed and the proper aeration The experimental design was completely randomized with triplicate for each experimental feed, the tank containing 20 shrimps (1± 0.3g) randomly distributed in tanks of 40 liters capacity.

The shrimps were fed with their respective experimental diets and control diet. After two weeks, they were challenged with WSSV through oral feeding. The results were observed between commercial pellet diet and experimental diet fed animals, as well as behavior and mortality of experimental reared animals.

Results and Discussion

Isolation of actinomycetes

In the present study, the total actinomycetes were recorded 28×10^3 g^{-1} in sediment. A total of 56 strains were screened based on colony morphology. The selected strains were showed white powdery aerial mycelium and also had the characterized leathery appearance in nutrient agar plates (Fig. 1).

Isolation, identification and characterization of pathogenic *Vibrio* spp.

Based on typical colonial morphology of *Vibrio* spp. on TCBS agar after incubating at 35 ˚C, for 24–48 hours, 11 strains were isolated from sediment of Parangipettai fish landing center samples and coded from V1 to V11. Then they were selected for the pathogenicity to juvenile tiger shrimp (*Penaeus monodon*). The severity of pathogenicity depends on strain of *Vibrio* involved. Strains V3, V8 and V11 were found to cause 100% mortality of shrimp at the infection levels of 10^6 and 10^7 CFU/g, respectively. No mortality was observed for shrimps in the control group and remains. Therefore, strains V3, V8 and V11 were used for further studies.

The 16S rRNA nucleotide sequence of strain V3, V8 and V11 were identity with the 16S rRNA nucleotide sequence of *Vibrio alginolyticus, V. parahaemolyticus* and *Vibrio harveyi.* The 16S rRNA nucleotide sequences of strains V3, V8 and V11 determined in this study have been deposited in the DDBJ/EMBL/ GenBank database with the accession numbers JQ307104, JQ307102 and JQ307110 respectively (Fig. 2, 3 & 4).

Antagonistic activity

Antagonistic activity of 56 actinomycetes strains against the pathogenic *Vibrio* was tested by double layer methods. The results revealed that 11 actinomycetes strains showed inhibitory activity against the pathogenic *Vibrio spp*. Of these strains, three strains VM-08, VM-15 and VM-21 have been showed the maximum inhibition zone and same were taken for further competitive inhibition study (Table 1).

Competitive inhibition

The competitive interaction against *Vibrio* spp. by the actinomycetes inoculated in the test tanks showed the total heterotrophic bacteria count was significantly higher in treatment tank compared to the control tank. In contrast, the total *Vibrio* count rose in the control tank and dropped in the experimental tanks. Actinomycetes colonies were isolated after 22 days from all the experimental tanks but not from the control tank (Table 2).

Actinomycetes supplied with feed.

The feed supplemented with actinomycetes was increased the survival rate of the WSSV infected shrimp when compared to the control. The survival rate of WSSV affected shrimps was increased from 11% to 35% up to 22 days of infection (with actinomycetes). In control tank (without actinomycetes) the survival rate was very less and also mass mortality was occurred within 3days.

In aquaculture systems, total heterotrophic bacteria play a significant role through mineralization and decomposition of wastes and provide supplementary feed for shrimp larvae (Sunilkumar, 1996) whereas *Vibrio*, the natural microflora of shrimp (Lightner, 1993), can cause disease and mass mortality. Actinomycete populations in estuarine and marine sediments vary in density between regions and even between sites within an ecosystem. In the present study, the highest density in sediment samples of shrimp pond was 28 x 10^3 CFU/g. In similar studies, actinomycete densities in sediment samples from marine ecosystems reached 3.29 x 10^3 CFU/g (Sahu *et al.,* 2007), 1-4 x 10^4 CFU/g (Pichavaram mangrove, Tamilnadu, India; Sivakumar *et al.,* 2005). It seems that large amounts of actinomycetes are washed from the land into the sea and connected areas where only some remain viable. Population density of *Vibrio* sp. in the marine environment is usually more because *Vibrios* can occur in a wide range of aquatic environments including estuaries, marine and coastal waters and sediments (Urakawa *et al.,* 2000; Thompson *et al.,* 2004). In the present study 11 strains were isolated from the sediments, among these strains V3, V8 and V11 were more pathogenicity to shrimps and their 16SrRNA nucleotide sequence was identity with *Vibrio alginolyticus, Vibrio parahaemolyticus* and *Vibrio harveyi*. Mahalakshmi *et al.* (2011) reported *Vibrios* contributed 72% and *E. coli* contribution only 15% contribution in the coastal areas of the Uppanar estuary, which incase higher microbial population due to *Vibrio* in the coastal environment of the Uppanar estuary.

In the present study 56 strains were isolated from the shrimp pond, of these 11 were showed strong antibacterial activity against *Vibrio* pathogens. The strains, especially VM-08, VM-15 and VM-21 which was very active against all three pathogens, should be further investigated to characterize its active compounds and as a source of potent antibiotics for combating diseases in cultured shellfish. Similar findings were recorded by Sahu *et al.* (2007), totally 41 actinomycetes were isolated, 26 strains (61%) showed antibacterial activity against shrimp pathogens and strain MKS-24 which was very active against all three pathogens.

Table.1 Antagonistic activity (inhibition zone in mm) of eleven strains of actinomycetes against *Vibrio* pathogens

Actinomycetes	Shrimp bacterial pathogens		
	Vibrio alginolyticus	*Vibrio parahaemolyticus*	*Vibrio harveyi*
VM-03	3	6	8
VM-08	16	14	15
VM-11	10	8	5
VM-14	7	12	8
VM-15	11	13	15
VM-21	15	16	12
VM-28	-	8	6
VM-31	8	9	11
VM-37	9	10	7
VM-42	5	6	9
VM-49	4	8	11

Table.2 Total Heterotrophic Bacteria and *Vibrio* (TVC) count in control and Experimental tanks

Microbial load	Control Tank (No actinomycetes)	Experimental (With actinomycetes)
Initial THB X 10^3	45	39
Final THB X 10^3	16	148
Initial TVC	180	98
Final TVC	400	22

Fig.1 Total *Actinomycetes* population in shrimp culture pond

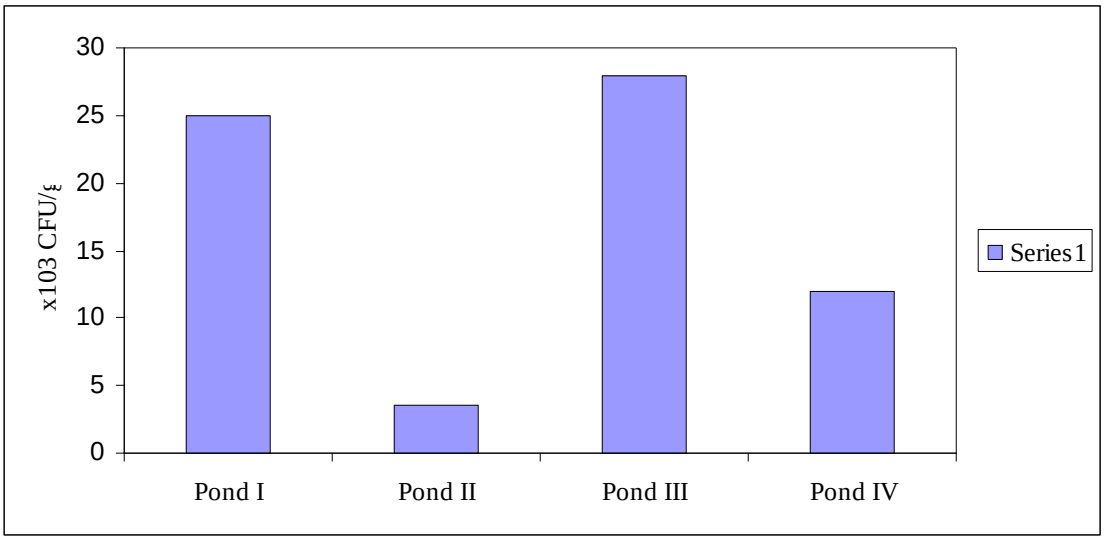

Fig.2 The strain V3 *Vibrio alginolyticus* 16S ribosomal RNA gene JQ307104

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      1 gggggggcag gcctaacaca tgcaagtcga ggggaaacga gttatctgaa ccttcgggga
61 acgataacgg ggtcgagggg gggacgggtg agtaatgcct aggaaattgc cctgatgtgg
121 gggataacca ttggaaacga tggctaatac cgcatgatgc ctacgggcca aagaggggga
181 ccttcgggcc tctcgggtca ggatatgcct aggtgggatt agctagttgg tgaggtaagg
241 gctcaccaag gggacgatcc ctagctggtc tgagaggatg atcagccaca ctggaactga
301 gacacggtcc agactcctac gggaggcagc agtggggaat attgcacaat gggggcaagc
361 ctgatgcagc catgccgggt gtgtgaagaa ggccttcggg ttgtaaagca ctttcagtcg
421 tgaggaaggt agtgttgtta atagccgcat tacttgacgt tagggacaga agaagcaccg
481 gctaactccg tgccagcagc cggggtaata cggagggtgg gagggttaat cggaattact
541 gggggtaaag ggcatgcagg tggtttgtta agtcagatgt gaaagcccgg ggctcaacct
601 cggaatagca tttgaaactg gcagactaga gtactgtaga ggggggtaga atttcaggtg
661 taggggtgaa atgggtagag atctgaagga ataccggtgg ggaagggggc cccctggaca
721 gatactgaca ctcagatggg aaagggtggg gagcaaacag gattagatac cctggtagtc
781 cacgccgtaa acgatgtcta cttggaggtt gtggccttga gccgtggctt tcggagctaa
841 cgggttaagt agaccgcctg gggagtacgg tcgcaagatt aaaactcaaa tgaattgacg
901 ggggcccgca caaggggtgg agcatgtggt ttaattcgat gcaacgggaa gaaccttacc
961 tactcttgac atccagagaa ctttccagag atggattggt gccttcggga actctgagac
1021 aggtgctgca tggctgtcgt cagctcgtgt tgtgaaatgt tgggttaagt cccgcaacga
1081 gggcaaccct tatccttgtt tgccagggag taatgtcggg aactccaggg agactgccgg
1141 tgataaaccg gaggaaggtg gggacgacgt caagtcatca tggcccttac gagtagggct
1201 acacacgtgc tacaatgggg catacagagg ggggccaact tgggaaagtg agcgaatccc
1261 aaaaagtggg tcgtagtccg gattggagtc tgcaactcga ctccatgaag tcggaatcgc
1321 tagtaatcgt ggatcagaat gccacggtga atacgttccc gggccttgta cacaccgccc
1381 gtcacaccat gggagtgggc tgcaaaagaa gtaggtagtt taaccttcgg ggggacgctt
1441 accactttgt ggttcatgac tggggtgaaa aa
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Fig.3 The strain V8 *Vibrio parahaemolyticus* 16S ribosomal RNA gene JQ307102

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      1 catggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc ggaaacgagt
61 taactgaacc ttcgggggac gataacggcg tcgagcggcg gacgggtgag taatgcctag
121 gaaattgccc tgatgtgggg gataaccatt ggaaacgatg gctaataccg catgatgcct
181 acgggccaaa gaggggacc ttcgggcctt tcgcgtcagg atatgcctag gtgggattag
241 ctagttggtg aggtaagggc tcaccaaggc gacgatccct agctggtttg agaggatgat
301 cagccacact ggaactgaga cacggtccag actcctacgg gaggcagcag tggggaatat
361 tgcacaatgg gcgcaagcct gatgcagcca tgccgcgtgt gtgaagaagg ccttcgggtt
421 gtaaagcact ttcagtcgtg aggaaggtag tgtagttaat agctgcatta tttgacgtta
481 gcgacagaag aagcaccggc taactccgtg ccagcagccg cggtaatacg gagggtgcga
541 gcgttaatcg gaattactgg gcgtaaagcg catgcaggtg gtttgttaag tcagatgtga
601 aagcccgggg ctcaacctcg gaattgcatt tgaaactggc aggctagagt actgtagagg
661 ggggtagaat ttcaggtgta gcggtgaaat gcgtagagat ttgaaggaat accggtggcg
721 aaggcggccc cctggacaga tactgacact cagatgcgaa agcgtgggga gcaaacagga
781 ttagataccc tggtagtcca cgccgtaaac gatgtttact tggaggttgt ggccttgagc
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901 aactcaaatg aattgacggg ggcccgcaca agcggtggag catgtggttt aattcgatgc
961 aacgcgaaga accttaccta cttttgacat ccagagaact ttccagagat ggattggtgc
1021 cttcgggaac tttgagacag gtgctgcatg gctgtcgtca gctcgtgttg tgaaatgttg
1081 ggttaagtcc cgcaacgagc gcaacccta tccttgtttg ccagcgagta atgtcgggaa
1141 ctccagggag actgccggtg ataaaccgga ggaaggtggg gacgacgtca agtcatcatg
1201 gcccttacga gtagggctac acacgtgcta caatggcgca tacagagggc ggccaacttg
1261 cgaaagtgag cgaatcccaa aaagtgcgtc gtagtccgga ttggagtttg caactcgact
1321 ccatgaagtc ggaatcgcta gtaatcgtgg atcagaatgc cacggtgaat acgttcccgg
1381 gccttgtaca caccgcccgt cacaccatgg gagtgggctg caaaagaagt aggtagttta
1441 accttcgggg ggacgcttac cactttgtgg ttcaaaaaaa aaaa
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Fig.4 The strain V11 *Vibrio harveyi* 16S ribosomal RNA gene JQ307110

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      1 aaaaaacgct ggcggcaggc ctaacacatg caagtcgagc ggaaacgagt tatctgaacc
61 ttcggggaac gaaaacggcg tcgagcggcg gacgggtgag taatgcctag gaaattgccc
121 tgatgtgggg gaaaaccatt ggaaacgatg gctaataccg caaaatacct tcgggtcaaa
181 gaggggacc ttcgggcctc tcgcgtcagg atatgcctag gtgggattag ctagttggtg
241 aggtaatggc tcaccaaggc gacgatccct agctggtctg agaggatgat cagccacact
301 ggaactgaga cacggtccag actcctacgg gaggcagcag tggggaatat tgcacaatgg
361 gcgcaagcct gatgcagcca tgccgcgtgt gtgaagaagg ccttcgggtt gtaaagcact
421 ttcagtcgtg aggaaggtag tgtagttaat agctgcatta tttgacgtta gcgacagaag
481 aagcaccggc taactccgtg ccagcagccg cggtaatacg gagggtgcga gcgttaatcg
541 gaattactgg gcgtaaagcg catgcaggtg gtttgttaag tcagatgtga aagcccgggg
601 ctcaacctcg gaatagcatt tgaaactggc agactagagt actgtagagg ggggtagaat
661 ttcaggtgta gcggtgaaat gcgtagagat ctgaaggaat accggtggcg aaggcggccc
721 cctggacaga tactgacact cagatgcgaa agcgtgggga gcaaacagga ttagataccc
781 tggtagtcca ccccgtaaac gatgtctact tggaggttgt ggccttgagc cgtggctttc
841 ggagctaacg cgttaagtag accccctggg gagtacggtc gcaagattaa aactcaaatg
901 aattgacggg ggcccgcaca agcggtggag catgtggttt aattcgatgc aacgcgaaga
961 accttaccta ctcttgacat ccagagaact ttccagagat ggattggtgc cttcgggaac
1021 tctgagacag gtgctgcatg gctgtcgtca gctcgtgttg tgaaatgttg ggttaagtcc
1081 cgcaacgagc gcaaccctta tccttgtttg ccagcacttc gggtgggaac tccagggaga
1141 ctgccggtga aaaaccggag gaaggtgggg acgacgtcaa gtcatcatgg cccttacgag
1201 tagggctaca cacgtgctac aatggcgcat acagagggcc cccaacttgc gagagtgagc
1261 gaatcccaaa aagtgcgtcg tagtccggat cggagtctgc aactcgactc cgtgaagtcg
1321 gaatcgctag taatcgtgga tcagaatgcc acggtgaata cgttcccggg ccttgtacac
1381 acccccgtc acaccatggg agtgggctgc aaaagaagta ggtagtttaa ccttcgggag
1441 gacgcttacc actttgtggt tcatgactgg
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So far, few active compounds isolated from marine actinomycetes are known to act as potential antibiotics with broad spectral activity (Balagurunathan, 1992). Sujatha *et al.* (2005) isolated the antagonistic actinomycetes from the Bay of Bengal, and found that promising activity against multi-drug resistant pathogens.

In the present study actinomycetes incorporated with the feed increase the THB population and lower the *Vibrio* count, perhaps due to antagonism of *Streptomyces* sp. towards *Vibrio* spp. Das *et al.* (2006) reported the *Vibrio* count decreased as the *Streptomyces* cell concentration increased. The marine *Streptomyces* can produce antibiotics *in vitro* which inhibit the growth of the human pathogenic *Vibrio cholerae* (Balagurunathan, 1992; Siva Kumar, 2001) and the finfish and shellfish pathogen *Vibrio* spp. (Moriarty, 1998; Dhevendaran and Annie, 1999; Prabhu *et al.,* 1999; Dalmin *et al.,* 2001; and Pugazhvendan *et al.,* 2010) reported the same trends in total heterotrophic bacteria and *Vibrio* when a bacterial probiont was applied in shrimp culture ponds. Reports on controlling *Vibrio* in aquaculture by applying actinomycetes are meager. The present study it is postulated that marine *Streptomyces* may produce antibiotics that prevent the shrimp pathogenic *Vibrio* in the culture system. Thus, the actinomycetes cells applied to the feed reached the intestine of the animals and improved their health.

Flegel (2001) reviewed the response of shrimps to viral pathogens and suggested that their defence system can be stimulated for a limited length of time, which is often much shorter than that in vertebrates. In the present study control WSSV infected animals was lost their life within three days, but actinomycetes supplemented WSSV infected animal was extended their life time

maximum up to 22 days. Kumar *et al.*, (2006) studied the actinomycetes were available as feed additives to the post-larvae of the black tiger shrimp *Penaeus monodon* for two weeks and challenged with WSSV, the post challenge survival showed variations from 11 to 83%. All the previous studies indicated that bioactive compounds produced by the actinomycetes function as antiviral antibiotics. Das *et al.* (2006) reported a preliminary study on the effect of probiotic supplementation of *Streptomyces* on the growth and aids in preventing disease of black tiger shrimp, *Penaeus monodon* (Fabricius). You *et al.* (2007) recommended the use of actinomycetes to prevent the disease caused by *Vibrio* spp. The results obtained in the present study are in good agreement with these findings. Thus, it leads to the obvious conclusion that isolates of actinomycetes in the culture broth may have produced bioactive compounds that possess potent the feds, lowered WSSV infection in shrimps. Isolation of these compounds and their characterization are essential to further the findings of this study, which would lead to the possibility of developing antiviral agent against white spot disease in shrimps and vibriosis.

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