Research Paper

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A Study on Genetic Variability of The Intestinal Bacteria in The Gastrointestinal Tract of Penaeus Monodon and Rapd Analysis in a Selected Bacterium

Zoology

KEYWORDS : Bacillus, Pseudomonas, Penaeus monodon, PCR-RAPD techniques.

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ABSTRACT

The present study was intended to separate, typify and scrutinize the probiotic chattels of Bacillus and Pseudomonas from Penaeus monodon. The total of twelve Bacillus strains and one Pseudomonas strain isolated from gastrointestinal tract of Penaeus monodon. The strain was appraised under in vitro intestinal condition based on resistance to antibiotics susceptibilities and various exo-enzymes namely , amylase, caseinase, gelatinase, urease and lipase were tested for their activity in isolated microbes from the gut. Bio chemical characterization comprising of variety of tests, was also done for the strains. PCR-RAPD analysis was undertaken to show the relationship of different strains to locate in the probiotic category. The positive outcome of the study illustrates that further experiments in Prawn and could strengthen the probiotic knowledge and it can ameliorate the aquaculture scope.

Introduction.

Aquaculture is the fastest growing food-producing sector in the world at an average rate of 8.9% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (Subasinghe, 2005).

The first application of probiotics occurred in 1986, to test their ability to increase growth of hydrobionts (organisms that live in water). Later, probiotics were used to improve water quality and control of bacterial infections. Nowadays, there is documented evidence that probiotics can improve the digestibility of nutrients, increase tolerance to stress, and encourage reproduction. Currently, there are commercial probiotic products prepared from various bacterial species such as Bacillus sp., Lactobacillus sp., Enterococcus sp., Carnobacterium sp., and the yeast Saccharomyces cerevisiae among others, and their use is regulated by careful management recommendations.(Patricia Martínez Cruz et al,2012). Pseudomonas aeruginosa can also be used as probiotic and it has been observed to show specific inhibition against V.harveyi by Torrento and Torres (1996) and Chaiythanya et al. (2002)

The gut microbiota with the epithelium and mucosal immune system orchestrate a network of immunological and nonimmunological defenses, providing both protection against pathogens and tolerance to commensal bacteria and harmless antigens (Sanz and Palma, 2009)As for the aquatic animals such as fish and shrimp, the colonization of the gastrointestinal tract starts immediately after hatching and is completed within a few hours to modulate expression of genes in the digestive tract, thus creating a favorable habitat for them and preventing invasion by other bacteria introduced later into the ecosystem (Balcázar et al 2006)

Bacillus sp. (B. coagulans), on growth performance and digestive enzyme activity of the shrimp, *Penaeus vannamei,* was investigated and the results showed that the effects were related with supplementation concentrations of probiotics and thus use of a 10 g/kg (wet weight) supplement of probiotics in shrimp diet was recommended to stimulate productive performance (Wang, 2007). A mixture of Bacillusprobiotic bacteria (Bacillus subtilis, Bacillus licheniformis and Bacillus pumilus) was also evaluated in the gilthead sea bream (Sparus aurata) larviculture focusing on their effects on survival, growth and general welfare (Avella et al, 2010). The data generated in this study show the benefit of the administration of Bacillus probiotic mixture in terms of stress response and growth and provide scientific and technical support for the implementation of sustainable development of sea bream aquaculture.,

Three general modes of probiotics actions have been classified and presented by Oelschlaeger (2010) as follow: (1) Probiotics might be able to modulate the host's gut defences including the innate as well as the acquired immune system and this mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of Inflammation of the digestive tract or parts thereof. (2) Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones and this principle is in many cases of importance for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut. (3) Finally, probiotic effects may be based on actions affecting microbial products, host products and food ingredients and such actions may result in inactivation of toxins and detoxification of host and food components in the gut.

Hence, with view to identify and characterize the beneficial bacteria associated with crustacean intestine, this work was carried out on the gut micro flora of *Penaeus monodon* The microflora where biochemically characterized and their numerical changes in the gut where monitored. RAPD analysis was carried out in selected Pseudomonas bacteria from the different gut regions of marine tiger prawn, *P.monodon* .

Material and Methods

Penaeus monodon young ones about 6-12 cm length were procured from Cuddalore district, Tamilnadu, India . Animals were fed with commercial compounded feed two times a day and acclimatized to laboratory conditions in large scale plastic tubs. Anatomical description of the digestive system of *penaeus monodon* is based on the personal observation and guided by the earlier descriptions.

Prawan's ventral surface sterilized with distilled water pursued by 80% ethanol and 2% glutaraldehyde. Normal sterile condition was being given to dissect the animal and homogenized with 10% saline solution.

The homogenate solution held in reserve in a boiling water bath at 90 °C for 15 minutes and kept in normal water.

The homogenate solution applied as inoculums which was undergone for serial dilution method used to decrease the concentration of stock solution by the same quantity in each successive step until to obtain the concentration of 10^{-5}. Hereafter, solution plated on both *Nutrient agar* medium and *Tryptic soya agar* and incubated at 37 °C for 24 h. Plates were examined for total heterotrophic count according to different medium.

The purified isolated colonies were tested for gram staining, motility, indole production test, Methyl red test, Voges Proskauer test, citrate utilization test, urease production test, nitrate reduction test, decarboxylation of lysine, ornithine and arginine test, phenylanine test, triple sugar ions agar test, carbohydrate fermentation.

For the further study of extraction of genomic DNA required PCR- RAPD analysis. Bacteria samples were well ground and mixed with CTAB extraction buffer. This homogenate was incubated with 150 µl/ml proteinase K at 50 ºC for 4-12 hours. It was then extracted with equal volume of phenol : Chloroform (1:1). It was then centrifuged at 10,000 rpm for 5min. The upper aqueous was collected and chloroform : isoamyl alcohol (24:1) was added. Contents were centrifuged and the upper aqueous phase was collected.DNA was precipitated with cold absolute ethanol. The contents were centrifuged at 5,000 rpm for few minutes the pellet was then dissolved in 400 ul of 1Ml Nacl. To this 2 µl of RNAse was added and kept at 37 ºC for 30 min. To this 1 ml cold absolute ethanol was added and kept at -20 ºC for 30 min. The sample was centrifuged and the supernatant was discarded, the pellet was washed with 70% ethanol and the centrifugation was repeated.. The pellet was collected; the ethanol content was evaporated and dissolved in 1x TE buffer.

PCR amplification was described by Muyzer et al (1993). One synthetic oligonucleotide primer (IDT, New Delhi) was used for amplification of DNA through using primer code (A03) with 5′ to 3′ sequence (5′ AGTCAGCCAC 3'). The PCR cycles were performed in a Programmable Thermal Controller PTC-150 (MJ Research, USA with initial denaturation of DNA at 94 ºC. Following 40 cycles of denaturation at 94 ºC for 45 sec. and extension at 72 ºC for 90 sec. Final extension was given for 7 min at 72 ºC. The amplified samples were then fractionated by DNA gel electrophoresis using 1.7% gel agarose.The gel was viewed on a Ultra Violet transilluminator and photographed using orange filter and the data analysis was performed by using Diversity database software (Bio-Rad).

Antimicrobial Susceptibility

Agar volume. The NCCLS protocol calls for plates of Mueller-Hinton agar that are 3.5 mm deep . With the petri plates used, that was equivalent to just under 25 ml. Twenty four plates of Mueller-Hinton agar were prepared for each volume of 20, 25, or 30 ml. Average weights of the plates were 37 g, 40 g, and 45g, respectively. Plates were inoculated with Bacillus suspension adjusted to a 0.5 McFarland turbidity standard and Amphicilin, Penicillin,gentamicin,tetracycline disks were placed on each plate between 5 and 15 minutes after inoculation. After 18 hours of incubation at 37ºC,the diameters of the zones of inhibition were recorded (Table 3.1 and 3.2).

Exo enzyme production

Isolates were checked for their ability to produce enzymes, as mentioned below

Amylase

Amylase production was tested for their ability tested on agar medium formulated by Harrigan and McCance (1972) , supplemented with starch as the substrate.. The production of amylase was tested by flooding the plates with gram's iodine solution. Starch, when not hydrolysed formed a blue colour with iodine. The amylolytic colonies developed clear zones around them.

Caseinase

Caseinase production by different cultures was detected by employing casein agar medium of Harrigan and McCance(1972) .Caseinase enzyme production is detected by the presence of clear zones around colonies.

Gelatinase

Gelatinase production by different cultures is detected by employing gelatin agar medium of Harrigan and McCance (1972). The plates are flooded with 8-10 ml the test agent. Gelatin hydrolysis is identified by clear halos around around the colonies.

Lipase

Lipase production was detected by employing Tween agar medium of Harrigan and McCance (1972). Lipase production is detected by the appearance of opaque zone around colonies. Appearance of a waxy material around the colonies is the indication of the liberation of insoluble oleic acid formed as a result of lipase action.

All these enzyme production activity cultures are inoculated by surface streaking on the media and incubated at room temperature (28+ 2 ºC) for 7 days.

Staistics

The computed similarities among the isolates were estimated by means of the dice coefficient . The program calculates all the Pearson Correlation Coefficients between pairs of variables, transforms these coefficients into distances and makes a clustering using the Underweighted Pair Group Method with Arithmatic mean (UPGMA) . Dendrograms were produced on the UPGMA.

Results

Total Heterotropic Bacteria (THB)

Gut microflora of prawns were enumerated in different region of gut. Among these, foregut had 1.80^{10}, midgut, 1.50^{10} and the hind gut , 1.86^{10} CFU/ml of microbial colonies in prawns collected and cultured in Nutrient Agar Medium. In gut analysis of prawns cultured in TSI agar, foregut had 2.64^{10} , midgut, 1.52^{10} and the hindgut , 1.84^{10} CFU/ml of microbial colonies (Table 1.1)

Table 1.1- Total heterotropic count of Gut microflora of Penaeus monodon in nutrient agar and Tryptic soya agar

S. No	Region of Gut	Dilution	Number of colony forming units (Nutrient agar)	Number of colony forming units (Tryptic soya agar)
1	Foregut	10^{-1}	180	264
2	Foregut	10^{-3}	96	210
3	Foregut	10^{-5}	48	170
4	Midgut	10^{-1}	156	152
5	Midgut	10^{-3}	92	124
6	Midgut	10^{-5}	59	44
7	Hind-gut	10^{-1}	186	184
8	Hind-gut	10^{-3}	108	112
9	Hind-gut	10^{-5}	82	76

Average= 130.1667		
Correlation = 0.603714		
SD= 61.11344		
Pearson Coefficient Correlation = 0.603714		

Identification of Bacterial Strains

Bacterial colonies were identified based on their physical characteristics, biochemical tests and exoenzyme production (Bergey's manual, 1989). It showed twelve strains of Bacillus namely, B.firms, B.licheniformes, B.laterosporus, B.Sphearicus, B.circulans, B.mycoides, B.alvei, B. pumilus, B.brevis., B.coagulans. Apart from Bacillus species Pseudomonas was also represented in gut microflora (Table 2.1)

Characteristics

Different characteristics futures of microbes such as spore position, shape , sporangium and biochemical characterization were tabulated in table -1.4.

Bacillus

Gram positive rods, colonies are light brown colour with size of 0.5-1.55 mm diameter, with a convex shape and appreciable transparency. New colonies could be grown from the spores following subculture procedures. Bacillus tested positive for amylase, caseinase, gelatinase. It is the positive for Voges-Proskaeur test. Methyl red, and citrate utilization test. They were found to hydrolase glucose, sucrose and tested negative for other sugars.

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Pseudomonas

They were gram negative rods, colonies were smooth , translucent, low convex and 2-3 diameter. The colonies were fluorescent greenish blue. Pseudomonas tested positive for amylase, caseinase, gelatinase, cellulose and protease activity. The strains were non haemolytic. Biochemical tests revealed the pseudomonas strains were glucose fermenting, catalase and oxidase positive and hydrolyzing starch. Pseudomonas also used citrate as an energy source.

Bacillus cereus

It is an endemic , soil dwelling, gram positive rod shaped bacteria, beta hemolytic bacteria that cause food borne illness . B.cereus bacteria are facultative aerobes and produce protective endospores.

Bacillus licheniformes

It is a gram positive , thermophilic bacterium. Optimal growth temperature is around 50°C. Optimal enzyme secretion is 37°C. It can exist in spore form to resist harsh environs, or in avegetative state when conditions are good.

Bacillus subtilis

B.subtilis is a gram positive , catalase positive bacterium found in soil. It form a tough, protective endospore, B.subtilis has historically been classified as an obligate aerobe, though recent research has demonstrated that this not strictly correct.

Bacillus coagulans

B.Coagulans is a gram positive, spore forming, motile rod, aerobic to microaerophilic and as all other species in genus Bacillus, forms endospores, which are resistant to chemical and physical agents. It may appear gram negative when entering the stationary phase of growth. The temperature optimum for growth is 37°C. On activation of spore formation in the acidic environment of the stomach, this organism can germinate and proliferate in the intestine, producing the favored L (+) optical isomer of lactic acid, potentially the growth of numerous bacterial and fungal pathogens.

Bacillus sphaericus

Bacillus sphaericus is an obligate aerobe bacterium used as a larvicide for mosquito control. Form spherical endospores.

Bacillus mycoides

Bacilus mycoides forms chain of cells, is non-motile, and can form acid from glucose .It's cell body does not swell when sporulating,and is ususlly larger than 3 micrometeres. Using the test Voges-Proskauer, Bacilus mycoides produces a positive result. It can also hydrolyse starch.

Table 2.1 – Identification of gut microflora of P.monodon by biochemical characterization.

S. no	Morphology	FTM	Shape				Spore position	S.S	Blood Agar	Mac	Egg yolk	Skim Milk	Species
			BCS										
1	Flat,dry, NLF	+/-		C			S	Y	Dry	Dry	-	-	B.circulans
2	Smal, NLF	+/-					-	-	Yellow	Yellow	-	+	B.coagulans
3.	Dry,NLF	+/+		C			PC	Y	Dry	Dry	+	+	B.licheniformes
4	Dry	+/-		C			S	Y	Dry	Dry	-	-	B.circulans
5	Tiny,small,moist	-	-	-	-		-	-	-	-	-	+	P. aeuroginosa
6	Haemolytic,dry	+/+		C			PC	-	Dry	Dry	-	+	B. licheniformes
7	Tiny,NLF	+/+					S		-	-	-	+/-	Pseudomonas
8	Big,Dry	+/-		C			PC	-	Dry	Dry	+	+	B. cereus
9	Small,Dry	+/-		C			PC	-	Dry	Dry	-	+	B. firmus
10	OPLF	++/+		C			PC	-	Dry	Dry	-	+	B. subtilis
11	Small	+/++		C			PC	-	Dry	Dry	+	+	B. cereus
12	Dry	+/+		C			PC	-	Dry	Dry	+	+	B. cereus
13	Dry,small	+/-		C			PC	-	Dry	Dry	-	+	B. pumilis
14	Small,NLF	+/-		C			PC	-	Yellow	Moist	-	+	B. firmus
15	Flat,dry	+/+		C			PC	-	Dry	Dry	+	+	B.mycoides
16	Dry,haemol-ytic	+/+		C			PC	-	Dry	Dry	-	+	B. pumilus
17	Haemolytic,dry	+/-		R			S	+	Dry	Dry	-	+	B. sphericus
18	Dry	+/-		C			PC	+	Dry	Dry	-	+	B. laterosporus
19	Dry,white	+/-		C			S	+	Dry	Dry	-	+	B. alvei
20	Small,dry	+/+		C			PC	-	Moist	Moist	+	+	B. cereus
21	Haemolytic,dry,white	+/-		C			S	+	Dry	Dry	-	+	B.circulans
22	Small,NLF	+/+		C			PC	-	Moist	Moist	-	+	B. pumilus
23	Dry,Small, NLF	+/-		C			S	+	Moist	Moist	-	-	B.brevis

S.No	Arginine	Inulin	Mannitol	Trehalose	Salicin	Indole	TSI	Starch	Species
1	+	-	-	-	+	-	Ak/Ak	-	B.circulans
2	+	-	-	-	+	-	Ak/Ak	-	B.coagulans
3.	-	-	+	+	+	-	Ak/Ak	-	B.licheniformes
4	+	-	+	+	+	-	Ak/A	-	B.circulans
5	-	-	-	-	-	-	Ak/Ak	-	P. aeuroginosa
6	+	-	+	-	+	-	Ak/A	-	B. licheniformes
7						-	Ak/A	-	Pseudomonas
8	-	-	-	+	-	-	A/A	-	B. cereus

9	+	-	+	-	-		Ak/Ak	+	B. firmus
10	+	-	-	+	+	-	Ak/Ak	+	B. subtilis
11	-	-	-	+	-	-	A/A	-	B. cereus
12	-	-	-	+	+/-	-	A/A	-	B. cereus
13	-	-	-	+/-	-	-	A/Ak	-	B. pumilis
14	-	-	-	-	-	-	Ak/Ak	-	B. firmus
15	+	-	-	+	-	-	Ak/Ak	-	B.mycoides
16	+	-	+/-	+	+	-	Ak/A	-	B. pumilus
17	+	-	-	+	-	-	Ak/Ak	-	B. sphericus
18	+	-	+	+/-	+	-	Ak/A	-	B. laterosporus
19	-	-	-	-	-	-	A/A	+	B. alvei
20	-	-	-	+	-	-	A/Ak	-	B. cereus
21	-	-	+	+	+	-	Ak/A	+	B.circulans
22	-	-	+	+	+	-	Ak/A	-	B. pumilus
23	+	-	-	-	-	-	Ak/Ak	+	B.brevis

S.S –spore position, C-circular; A-Acid production; Mac- Mac Conkey agar; FNLF- non lactose fermentation; OPLF- opaque lactose fermentation; FTM- fermentation medium.

Bacillus Spp. exhibited resistance to cell wall inhibitors like penicillin G and Ampicillin. Antimicrobial susceptibility result shows protein synthesis inhibitor predominantly inhibits the growth of selected bacterium. Gentamicin was able to prevent the growth of all Bacillusspecies tried. This study revealed that all the isolates of Bacillus spp. (except one isolate of B.alvei) were sensitive to protein synthesis inhibitor tetracycline, which is commonly used antibiotic in aquaculture in India (S.Balakrishnan et al, 2003).

Table 3.1 shows antimicrobial susceptibilities of selected Bacterium in cell wall inhibitor

Cell wall Inhibitor	Bacillus cereus	Bacillus licheniformes	Bacillus subtilis	Bacillus coagulans	Bacillus sphaericus	Bacillus mycoides
	(Zone of inhibition-Diameter in Cm)	-	-	-	-	-
Penicillin G	4.5 (MS)	5 (MS)	4 (MS)	4.5 (MS)	4 (MS)	3 (R)
Ampicillin	3 (R)	4 (MS)	9 (S)	3 (R)	6 (MS)	2.5 (R)

Average= 4.375
STDDEV= 1.759173
Correlation=-0.00977

Table 3.2 shows antimicrobial susceptibility of selected bacterium in protein synthesis inhibitor

Protein synthesis inhibitor	Bacillus cereus	Bacillus licheniformes	Bacillus subtilis	Bacillus coagulans	Bacillus sphaericus	Bacillus mycoides
	(Zone of inhibition-Diameter in Cm)	-	-	-	-	-
Gentamycin	6.5 (S)	7 (S)	18 (S)	6 (S)	6 (S)	7 (S)
Tetracycline	8 (S)	7 (S)	16.5 (S)	5.5 (S)	6 (S)	6.5 (S)

Average= 8.33333	
STDEV= 4.338332	
Correlation = 0.983946	

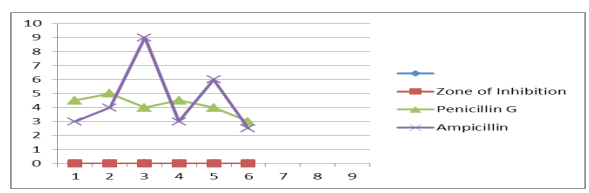

Fig.1.1. Selected bacterium shows resistant and moderately sensitive to Cell wall inhibitor

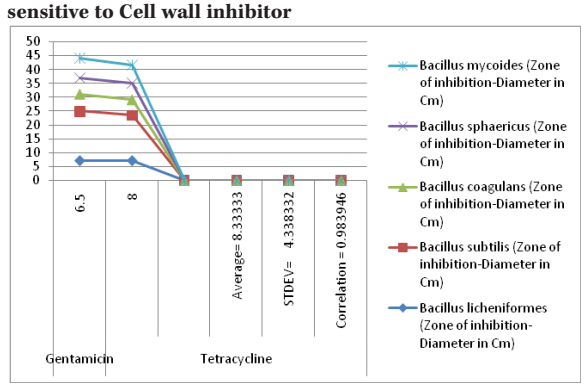

Fig 1.2 . Selected bacterium shows highly sensitive to Gentamicin and Tetracycline.

PCR-RAPD studies

The RAPD procedure was used in the present study because of its simplicity and speed in identifying the genetic polymorphisms within the species level. DNA samples of Pseudomonas were screened by using the arbitrary 10-mer primers.

RAPD promers (kit A1 to A20) were obtained from IDT, New Delhi. About 20 random primers were tested and primer A 03 alone gave reproducible, consistent and scorable fragments. Following is the sequence of the primer A 03 which gave results

Primer code	Sequence
A 03	5” AGT CAG CCAC 3”

The pattern of RAPD profile for Pseudomonas in this study revealed characteristics of genetic variability of each population. PCR pattern of genetic variability shown in Fig.2a

Similarly indices and dendrograms analysis were also computed

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and presented in Fig 2b and Fig.2c. Amplified fragments ranged from 982 bp to 289 bp. Species specific fragments were identified at 674 bp (Fig. 2.1). An interesting finding of this study is 100% similarity do not exist among the six populations studied (Fig. 2.2). The similarity index and the dendrogram constructed by UPGMA, clearly supported PCR pattern of genetic variability in the population. A maximum similarity index was exhibited among isolates 2 and 3 (i.e Pseudomonas isolated from foregut and hindgut of the prawn, P. monodon).

The UPGMA based dendrogram analysis grouped isolated from foregut and hind gut of prawn, P.monodon (isolates 1,2,3 and 5) in one cluster and isolates fore gut, mid gut and hind gut in another group. This cluster was further sub grouped which contain pseudomonas species from foregut and hindgut in one group, and foregut and midgut into another. Even though Pseudomonas species isolates were grouped under different stirs, in general one can say genetic variations exist in all the microbial populations.

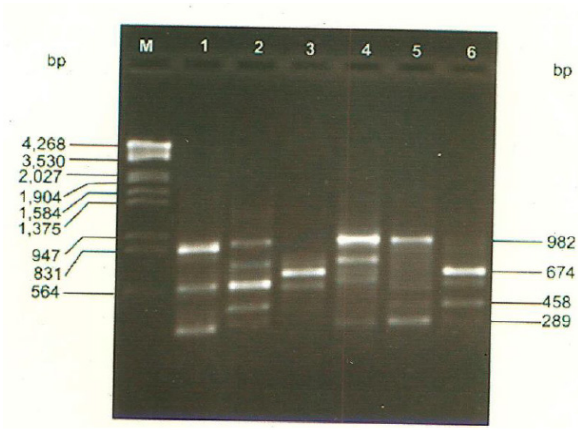

Figure -2.1

	1	2	3	4	5	6
1	**1**					
2	0.8	**1**				
3	0.5	0.4	**1**			
4	0.4	0.33	0.29	1		
5	0.8	0.67	0.4	0.67	**1**	
6	0.33	0.57	0.29	0.57	0.29	**1**

Figure – 2.2

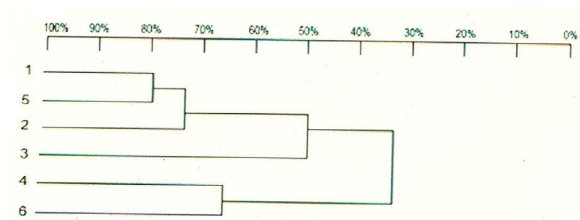

Figure 2.3

Discussion

Present investigation being select putative probiont from gut bacteria microflora of P.monodon it was necessary to find a vehicle for their entry in to the animals. Earlier investigators adopted several methods such as exclusive live bacterial feed or their spores, where the intention was chiefly utilize its nutritive value (Nayak and Savan, 1999). Several other methods have been also suggested , such as (i) addition to the formulated feed , (ii) addition to the culture water (Verschuere et al, 1999), (iii) Bathing the animals in bacterial suspension (Smith and Davey, 1993, Bruno et al, 1993) and (iv) addition through enriched or encapsulated live food (Dhert et al., 1990 Gomez- Gil et al., 1998., Robels et al., 1998). Use of formulated feeds is the regular feeding method in Prawn and marine shrimp farms, and also a convenient method for regular monitoring. Hence, in the present study for the probiont delivery into the gut of the prawn, the bacteria were incorporated in a commercially available diet, taking care to destroy the bacteria already existing in them . Gildberg and Mikkelson (1998) also following such method of fortifying commercial feed with probiotic candidate to feed the Atlantic cod, Gadus morhua.

Most of the earlier works advise against the use of 100% bacteria biomass as food, as the recipient species may be deprived of some essential nutrients like specific aminoacids or fattyacids, which may be absent in the bacteria given. Also the high level of nucleic acids in bacteria may cause accumulation of uric acid in vertebrates (Prescott et al., 1990) . Several acceptable dietary levels of bacterial SCP have been suggested earlier, and these dietary levels range from 0 to 50% (Gehra et al., 1985 ; Murray and Merchant, 1986 ; settens et all., 1988). In studies involving Bacillus and Pseudomonas strains as replacement for regular micro algal diet for Penaeus monodon larva, less than 10% substitution could give appreciable level of survival (> 60%) and good rate of growth (Mohamed, 1996)

Coating was done with help of agar. Agar was used as a binder in the present study. Several other binders including fish oil (Robertson et al., 2000) , have been used for bacterial incorporation in the feed. However coating with agar was quick and convenient under laboratory circumstances, and it in no way deteriorated water quality. Even with 3% incorporation of bacterial cells, large number of bacteria could be bound to the feed (5.67 x 10^{23} cells/g feed in the case of B.subtilis.

During the feeding experiments, it could be observed that animals were quite active and showed no rejection of the bacteria incorporate close to the established interval of 21 days (Vijayaraman, 1993), showing regularity in their growth. Mortality was infrequent and could not be related to any specific experimental situation. Live bacteria coated diet hada remarkable influence on the glycogen content. B. Subtilis could bring forth three increase in the whole animal.

The biochemical changes induced by stress may lead to disturbance in metabolism. Changes such as reduction in protein and globulin content of the hemolymph and inhibition of activity of certain important enzymes at cellular level lead to retardation of growth, reduction in the fecundity and longevity at organism (Tilak et al. 2000; Acharya et al.2004).

Fournier et al., (1998) have made extensive studies using PCR-DGGE approach to find out genetic evidence for highly diversified bacterial populations in waste watersludge. PCR fingerprinting was used by Wiedmann-Al-Ahmad et al (1994) for the differentiation of closely related strains of Acinetobacter. Tjernberg and Ursing (1989) have undertaken DNA-DNA hybridization studies to find out genetic variation in Acinetobcter. Welsh and McClelland (1900) have made similar studies using PCR with arbitrary primers. The PCR fingerprinting method, which has been established in recent years as universal method for identifying genetic variation in bacterial species and other organisms (Welsh et al., 1990; Williams et al., 1990) was found to be reliable. Hence in the present study an attempt has been made to find out the genetic variation in Pseudomonas population isolated from gut microflora of P.monodon. It revealed that there was genetic variation between the different gut regions of prawn.

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