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Probiotic activity of *Pseudomonas aeruginosa* (PIC-4) isolated from Visakhapatnam coast, Bay of Bengal, India, against *Vibrio harveyi* in *Penaeus monodon*

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Abstract
Pseudomonas aeruginosa (PIC 4), isolated from coastal waters of Visakhapatnam (Gen Bank Accession no: KF803248) was tested for its antagonistic activity against *Vibrio harveyi* as probiotic in cultured *Penaeus monodon. Pseudomonas aeruginosa* PIC 4 has proved to be non-pathogenic to the shrimp by pathogenicity tests. *Vibrio* counts in probiotic fed shrimp and the surrounding water medium were significantly lower when compared to the control group of shrimp and water during 50 days of culture. Mean weight of probiont fed shrimps after 50 days of culture was (2.21 $\pm$ 0.15 g) , significantly higher than that of normal diet fed ones (1.33$\pm$0.18 g). Survival percent was also significantly higher in probiont fed shrimp (47.33% $\pm$ 5.55%) than that of the control diet fed shrimp (26.33% $\pm$ 7%). Percent survival in probiotic fed and normal diet fed shrimp after the challenge with *V. harveyi* was 93.04 and 38.87 respectively.

Keywords: Probiotc, *Pseudomonas aeruginosa*, *Vibrio harveyi*, *Penaeus monodon.*

1. Introduction
The pathogenic *Vibrio* spp. have been implicated as major cause of bacterial infections in shrimp aquaculture [1]. *Vibrio harveyi*, a luminous species and commonly isolated from marine source, has been recognized to be pathogenic for fish and several Crustaceans, particularly, *Penaeus* spp. [2, 3, 4]. As antibiotic resistant strains are becoming more prevalent and difficult to treat, alternative methods of controlling the microbial environment are gaining significance [5]. Several alternative strategies to the use of antimicrobials in disease control have been proposed and applied very successfully in aquaculture [6]. A number of preventive approaches such as the use of vaccines, immunostimulants, and probiotics have been explored in order to reduce the losses due to diseases and mortality of cultured stock. A successful alternative method to antibiotic treatment is the application of probiotics. Probiotics have been proved to enhance specific, non-specific immunity and also improve water quality [7, 8, 9]. A variety of microbes have been investigated for use as probiotics in aquaculture such as Gram positive, Gram negative bacteria, yeast and unicellular algae [10, 11]. *Pseudomonas aeruginosa* isolated from Visakhapatnam coast was used as an alternative to the existing probiotic bacteria to fight against the *V. harveyi* infections in the cultured shrimp *P. monodon*.

2. Materials and Methods:
2.1 Selection of isolate & testing of antagonistic activity
Pseudomonas aeruginosa (PIC 4) isolated from Visakhapatnam coast (NCBI GenBank Accession no: KF803248) has been selected to test as probiotic bacterium against *V. harveyi* (MTCC 3438) in cultured shrimp (*Penaeus monodon*). Antagonistic activity of the isolate PIC 4 was tested by cross streak and agar well diffusion methods [12].

2.2 Pathogenicity Experiment
Pathogenicity of *Pseudomonas aeruginosa* (PI C4) was tested on the postlarvae (PL) of *Penaeus monodon* (stage PL15) obtained from a commercial hatchery. The postlarvae tested negative for white spot syndrome virus (WSSV) by nested PCR (WSSV Detection Kit supplied by Genei Bangalore, India) were acclimatised in laboratory for two days before conducting the experiment. One hundred Postlarvae (PL 15) of *P. monodon* were placed in each plastic tub of 8

litre capacity containing 4 litres of sterile sea water of 25 ppt salinity. The experiment was carried out with three replications and a control tank.

Pseudomonas aeruginosa (PI C4) cultured in LB Broth medium supplemented with 1% NaCl was harvested and washed in Phosphate Buffered saline (PBS) pH 7.8. Bacterial concentration level adjusted in PBS to the OD of 1.0 at 600 nm; corresponding to 5×10^8 CFU/ml according to Vijayan *et al.*, [11]. Postlarvae were bath challenged at 10^7 CFU/ml concentration with the *Pseudomonas aeruginosa* culture (PI C4). Survival rate was monitored at every 24 hrs for a period of seven days.

2.3 LD_{50} of *Vibrio harveyi* against post larvae of *Penaeus monodon*

This experiment was conducted to determine the dose of *V. harveyi* to be given in the challenge infections [13] to the postlarvae of *P. monodon*. *V. harveyi* cultured in Tryptone Soya Broth (TSA) was taken, centrifuged at 5000 rpm and washed and re-suspended with sterile saline. Postlarvae (PL 15) obtained from a local hatchery of Visakhapatnam were acclimatised for 3 days in four fibre troughs (8 litres capacity) with four litres of sterile marine water each tank containing 100 PLs. Temperature was maintained at 28 ^{0}C and pH at 8.2-8.5 and PLs were bath challenged with *V. harveyi* at different doses such as 10^4, 10^5, 10^6 and 10^7 CFU/ml. Mortality rate of PLs was noted at every 12 hrs interval up to 48 hrs [2, 14]. This experiment was conducted with three replications and a control. LD_{50} value was determined based on the 50% of the mortality attained by the postlarvae at 48 hrs after bath challenge.

2.4 Experiment with *Pseudomonas aeruginosa* (PIC 4) as feed probiont

Based on the results obtained in the pathogenicity experiment, isolate of *Pseudomonas aeruginosa* (PIC 4) was selected as a probiotic bacterium as it proved to be non-pathogenic to the post larvae of *P. monodon.* Bacterial culture (PIC 4) of *P. aeruginosa* was prepared as a feed additive (probiotic) to find out the effect on survival and growth of *P.monodon* and also resistance against *Vibrio harveyi.*

2.4a Probiotic mixed shrimp feed preparation

Commercially available shrimp feed was altered by mixing with the Bacterial culture of *Pseudomonas aeruginosa* (PIC 4) following the standard protocols [15]. Pure isolate of *Pseudomonas aeruginosa* (*PI C4*) was cultured in LB broth supplemented with 1% w/v NaCl in an orbital shaker incubator at 200 rpm, 28 ^{0}C for 24 hrs. Bacterial cells were harvested by centrifugation at 7000 rpm and washed in Phosphate Buffered saline (pH 7.8) for two times and re-centrifuged at 7000 rpm. These bacterial cells at the concentration of 10^7 CFU/g were mixed with pelleted shrimp feed (Classic shrimp feeds India starter II. Composition: protein 32-33%, fat 3.5%, fibre 4%, Moisture 11%) in 1:3 ratio (1part bacterial culture and 3 parts of feed by weight). The bacterial suspension in PBS was mixed with feed thoroughly so that the bacterial suspension formed a probiotic layer over the feed pellets and covered by a protein gel binder. Such probiotic coated feed was dried at room temperature and then stored at -20 ^{0}C for further use.

2.4b Experimental setup

Post larvae of *Peneaus monodon* (PL 20) were obtained from a commercial shrimp hatchery of Visakhapatnam, AP, and India. Postlarvae were tested negative for WSSV by nested PCR (WSSV Detection Kit supplied by Genei Bangalore India) were acclimatised to the laboratory conditions in FRP tanks (measuring 1.5x 0.5x0.75 m) for one week and fed with normal pelleted shrimp feed three times in a day at 10 % of the body weight. Experimental animals were fed three times a day by splitting the daily ration (Morning 25%, afternoon 25% and night 50%). Experiment was conducted in seven identical tanks containig 50 litres of sterile marine water with salinity 25 ppt and pH 8.2, having 100 animals in each tank on the first day of the experiment. Shrimp larvae in four culture tanks (control tanks C1-C4) were fed with commercial feed and those in three tanks (P1-P3) were fed with probiotic mixed feed for a period of 50days.

2.5 Bacterial and water quality analysis

Total bacterial counts of water as well as shrimp from each tank was enumerated by pour plate method on Zobell's Marine Agar(ZMA), *Vibrio* counts were enumerated on Thiosulphate Citrate Bile salt Sucrose agar (TCBS) agar and *Pseudomonas* counts on *Pseudomonas* isolation agar . Whole animal was sacrificed for the enumeration of bacterial counts up to four weeks and gut alone was taken from 5th week onwards to find out the effect of probiont on the gut flora. Water quality parameters such as Dissolved oxygen, Nitrate, Nitrite and Ammonia were also tested at weekly intervals. Growth and survival of the shrimp in all the experimental tanks were also monitored at weekly intervals.

2.6 Experimental challenge of *Vibrio harveyi* on the Postlarvae of *Peneaus monodon*

Vibrio harveyi (MTCC 3438) was harvested from LB broth by centrifugation and suspended in phosphate buffered saline (PBS) as per the protocol given by the Rengpipat *et al*., [15]. Shrimp in three tanks of probiotic fed group and three normal diet fed group (control tanks) were bath challenged with *Vibrio harveyi* @ $10^{7\ CFU}$ /ml on 50th day of the experiment. One control tank (normal diet fed) was left as unchallenged control (UC). Second challenge was given after four days of the first challenge with the same dose. Total bacterial, *Vibrio* and *Pseudomonas* counts of the water and shrimp respectively were enumerated on 1st, 4th, 8th and 12th day of the post challenge.

2.7 Statistical analysis

Data on growth and survival of shrimp and bacterial counts during pre and post challenge period were tested by ANOVA to find out significance using SPSS (Version 21.0).

3. Results

3.1 Antagonistic activity: Isolate PIC 4 of *P. aeruginosa* showed inhibitory zone in cross streak as well as agar well diffusion methods (36 mm in dia). (Fig 1 & 2)

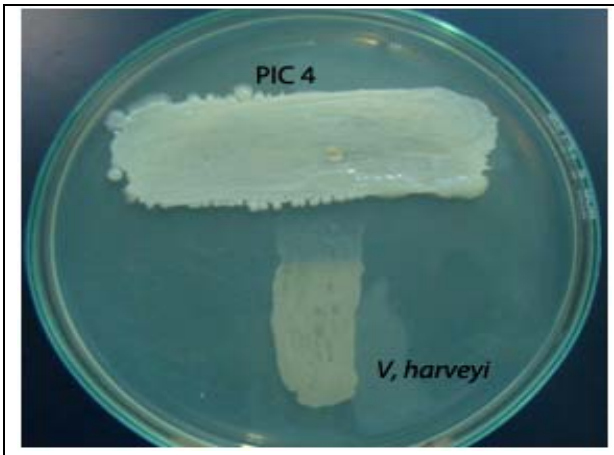


Fig 1: Petri dish showing cross streak between *Pseudomonas aeruginosa* (PIC 4) and *Vibrio harveyi.*

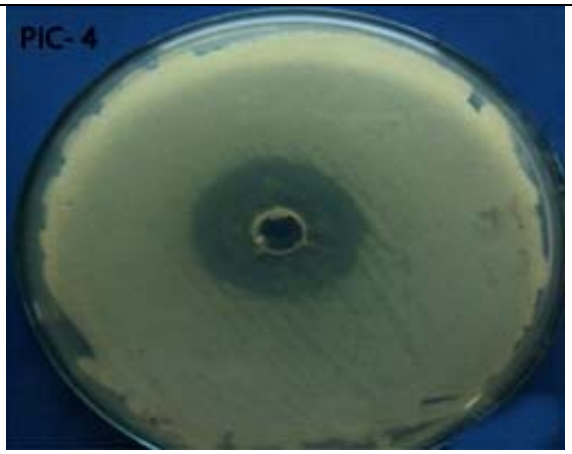


Fig 2: Petri dish showing Agar well diffusion of *Pseudomonas aeruginosa* (PIC 4) on *Vibrio harveyi.*

3.2 Pathogenicity test

Survival rate of post larvae of *P. monodon* was monitored for seven days after the bath challenge with the selected isolate of *Pseudomonas aeruginosa* (PIC4). The mean percent survival was 92.6, indicating very less mortality rate in a period of seven days similar to that of mortality rate in control (93% survival) (Table: 1).

Table 1: Survival rate of postlarvae challenged with the isolate PIC 4 *Pseudomonas aeruginosa* .

Day	% survival of Post larvae			
	Tank 1	Tank2	Tank3	Control
1	100	100	100	100
2	100	100	100	100
3	100	99	100	99
4	99	98	98	98
5	96	95	97	96
6	95	94	95	95
7	94	92	92	93

3.3 LD 50 values

The lethal dose (LD_{50}) of *V. harveyi* to the postlarvae of *Penaeus monodon* was found to be 10^6 CFU/ml at 48 hrs (Table: 2). Hence, the next higher concentration i.e. 10^7cfu/ml was applied as a challenge dose for the succeeding experiments.

Table 2: Percent mortality of postlarvae of *P. monodon* challenged with *Vibrio harveyi*

Dose of *Vibrio harveyi* (CFU/ml)	Cumulative Mortality rate						Mean % of mortality
	Hrs. →	0	12	24	36	48	
10^8	Tank 1	0/100	35/100	60/100	95/100	100/100	98.33
	Tank 2	0/100	35/100	65/100	100/100	100/100	
	Tank 3	0/100	30/100	55/100	90/100	95/100	
10^7	Tank 1	0/100	23/100	42/100	62/100	86/100	82.67
	Tank 2	0/100	21/100	38/100	61/100	79/100	
	Tank 3	0/100	25/100	41/100	63/100	83/100	
10^6	Tank 1	0/100	15/100	25/100	45/100	52/100	51.33
	Tank 2	0/100	10/100	25/100	40/100	49/100	
	Tank 3	0/100	15/100	20/100	45/100	53/100	
10^5	Tank 1	0/100	5/100	15/100	30/100	35/100	36.66
	Tank 2	0/100	10/100	10/100	35/100	40/100	
	Tank 3	0/100	5/100	10/100	25/100	35/100	
10^4	Tank 1	0/100	0/100	5/100	15/100	25/100	23.33
	Tank 2	0/100	0/100	0/100	10/100	20/100	
	Tank 3	0/100	5/100	5/100	15/100	25/100	
Control		0/100	0/100	0/100	0/100	0/100	0

3.4 Bacterial analysis in probiotic experiment

3.4a Bacterial counts of water in the experimental tanks

Mean of Total bacterial count (TBC) of water from four control tanks was initially $6.9x10^2$ and gradually increased to $3.27 x10^7$ CFU/ml on 50^{th} day. TBC of probiotic treated tank water was $7.7x10^2$ CFU/ml on the first day, increased to $1.7x10^7$ CFU/ml on the 50^{th} day of feeding. There was no significant difference in TBC of probiotic and control tanks ($P>0.05$). Mean of Total *Vibrio* counts (TVC) in the water of control tanks was $0.13x10^2$ CFU/ml on first day, raised to $2 x 10^4$ CFU/ml on 50^{th} day contributing major portion to TBC. *Vibrio* counts in probiotic fed tanks were $0.15x10^2$ CFU/ml on the first day, increased to $1.2x10^2$ CFU/ml on 50^{th} day, contributing very less portion to their TBC. *Vibrio* counts in the water medium of probiotic fed shrimp tanks were significantly lesser than those in water of control diet fed tanks ($P<0.05$). Mean of Total *Pseudomonas* counts (TPC) of water in probiotic fed tanks was $1.14x10^2$ CFU/ml on first day of the

experiment, reached to $7.1x10^6$ CFU/ml on 50th day. TPC of water in control diet fed tanks was $0.03x10^2$ CFU/ml on the first day of the experiment and reached $5.08x10^2$CFU/ml on 50th day, these values were significantly lesser than those of probiotic fed tanks ($P<0.05$) (Fig. 3-9).

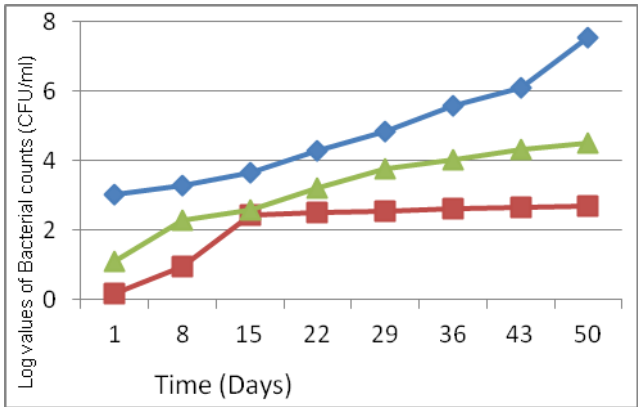


Fig: 3 (C 1)

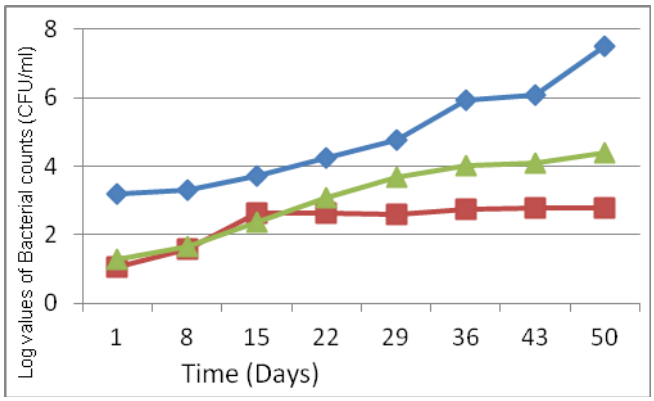


Fig: 4 (C 2)

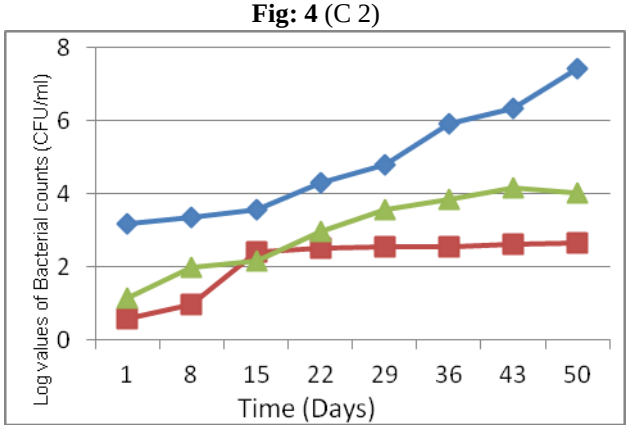


Fig 5 (C 3)

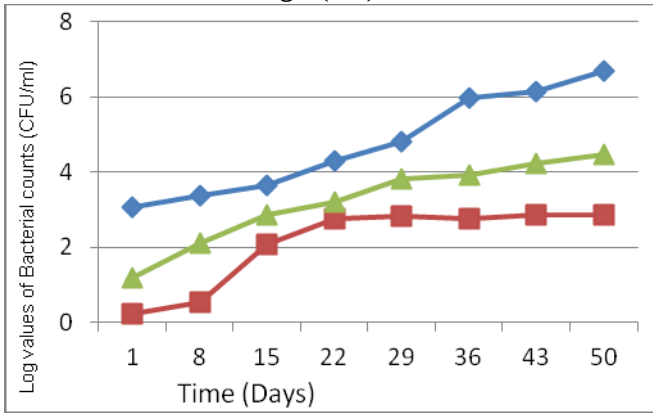


Fig: 6(C 4)

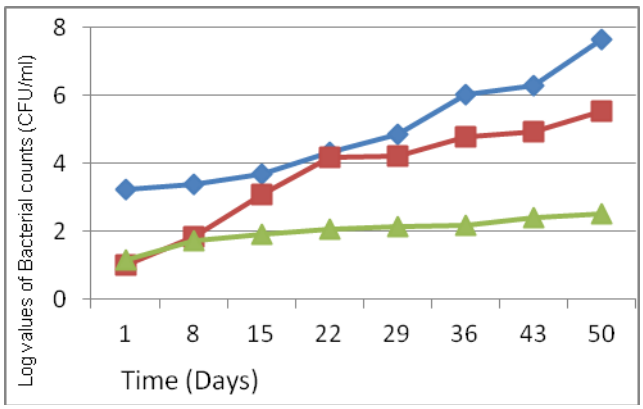


Fig: 7 (P 1)

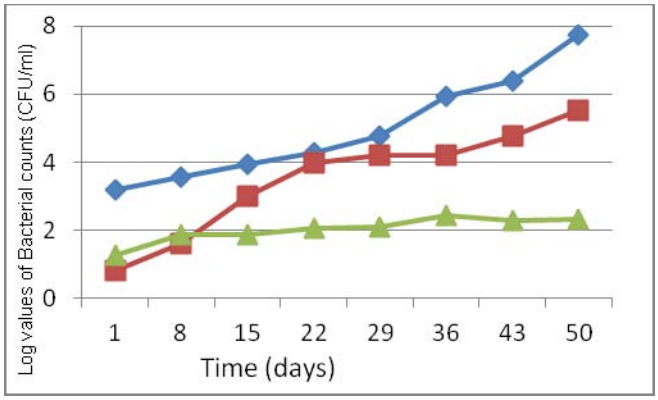


Fig: 8 (P 2)

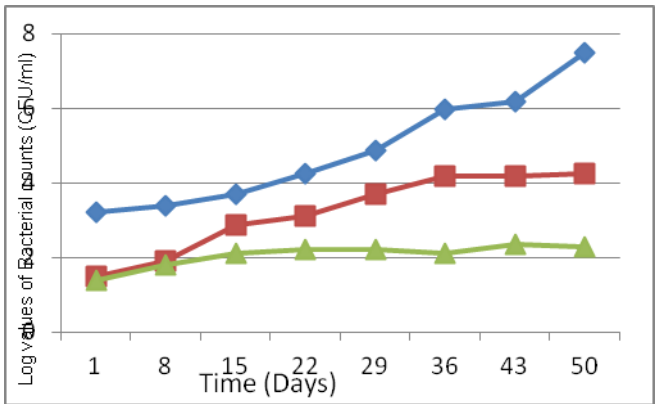
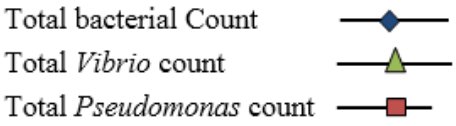


Fig 9 (P 3)

Figs. 3-6: Bacterial counts of water in control diet fed tanks (C1 to C4),
Figs. 7-9: Bacterial counts of water in Probiotic diet fed tanks (P1 to P3).



3.4b Bacterial counts of Shrimp

Mean total bacterial count (TBC) in the normal diet fed shrimps was 1.2 $x10^3$ CFU/g on the first day and increased to 3.6 $x10^7$ CFU/g by 50th day of the experiment. TBC of probiotic fed ones was 1.5 $x10^3$ CFU/g on the first day and reached 3.7$x10^7$ CFU/g by the 50th day. There was no significant difference in TBC of shrimp in control and probiotic fed tanks (P>0.05). Mean value of the Total *Vibrio* count (TVC) in shrimp of control diet fed tanks was 0.14$x10^2$ CFU/g on the first day and 2 $x10^4$ CFU/g on the 50th day. The mean TVC of shrimp in probiotic fed tanks was 0.18$x10^2$ CFU/g on the first day, increased gradually to 2.1$x10^2$ CFU/g by 50th day. TVC in control diet fed shrimp were significantly higher than that of probiotic fed shrimp (P<0.05). Mean of Total *Pseudomonas* counts in control diet fed shrimp was 0.03$x10^2$ CFU/g on the first day and 5.6$x10^2$CFU/g on 50th day. Total *Pseudomonas* counts (TPC) in shrimp of probiotic fed tanks were 0.11 $x10^2$ CFU/g on the first day of the experiment, increased to 4.6$x10^5$ CFU/g by 50th day. *Pseudomonas* counts in probiotic fed shrimp were significantly higher than those of the control diet fed (P<0.05) (Fig. 10-16).

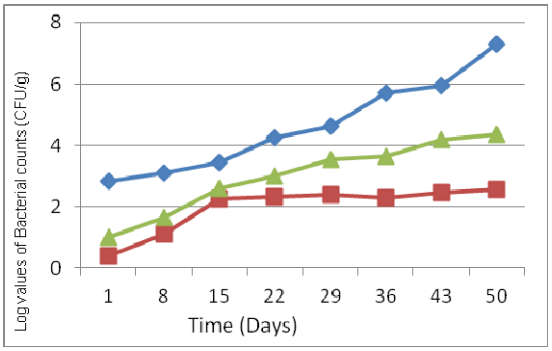


Fig 10: (C1)

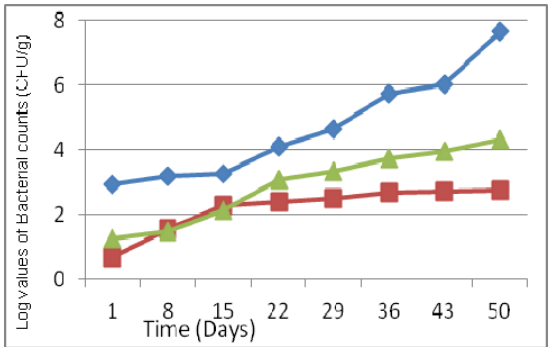


Fig: 11 (C2)

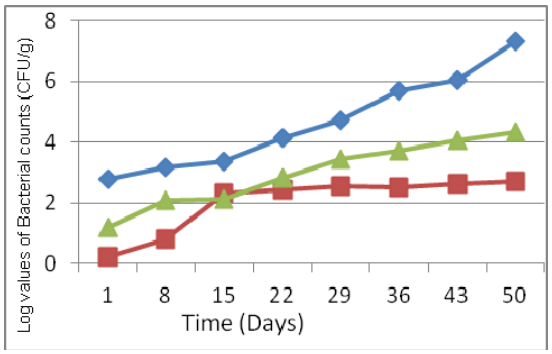


Fig: 12 (C3)

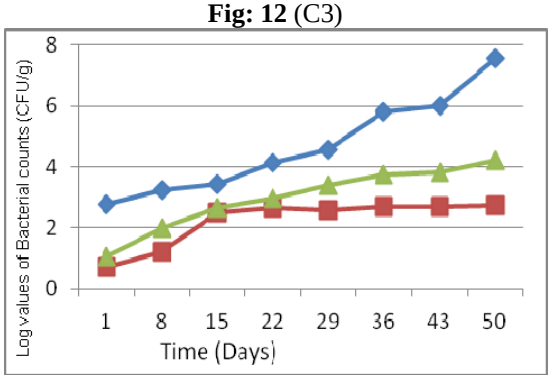


Fig: 13 (C4)

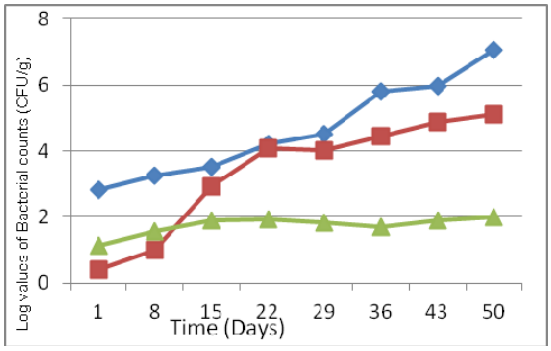


Fig: 14 (P1)

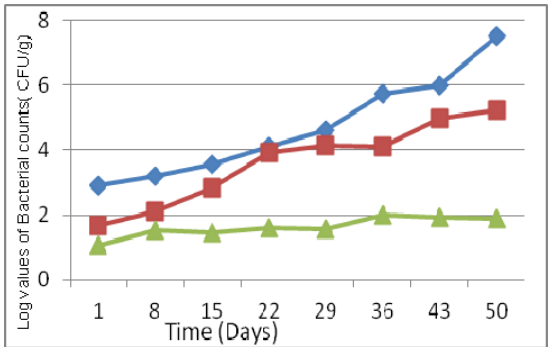


Fig: 15 (P2)

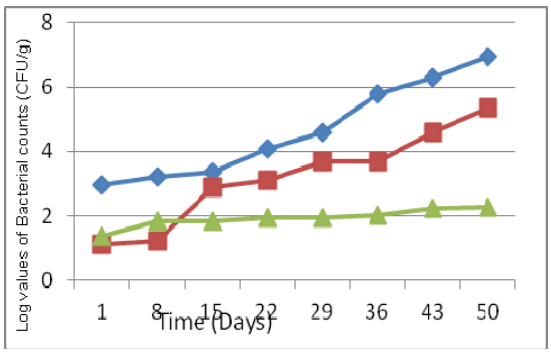
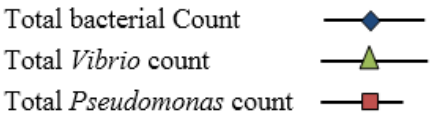


Fig: 16 (P3)



Figs 10-13: Bacterial counts of shrimp in control diet fed tanks (C1 to C4),
Figs 14-16: Bacterial counts of shrimp in Probiotic diet fed tanks (P1 to P3)

3.5 Water quality analysis

The mean values of dissolved oxygen, nitrate, nitrite and ammonia concentrations in control and probiotic applied tanks were 5 to 6.4 mg/l, 0.6 to 2 mg/l, 1.7 to 3 mg/l, 0.12 to 0.69 mg/l and 5.2 to 6.6 mg/l, 0.6 to 1.5mg/l, 1.6 to 2.4 mg/l, 0.1 to 0.5 mg/l respectively. There was no considerable difference observed in water quality parameters between probiotic and normal diet fed tanks.

3.6 Growth and survival of shrimp

Mean weight after the 50 days of experimental period in all probiont fed shrimps was (2.21 ± 0.15g) significantly higher (Fig. 17) than that of normal diet fed shrimp (1.33±0.18 g) ($P<0.05$). However there was no significant difference within these two groups individually ($p>0.05$). Mean survival percent during the 50 days of experimental period in probiont fed shrimp (47.33% ± 5.55%) was significantly higher (Fig. 18) than that of the control diet fed shrimp (26.33% ± 7%) ($P<0.05$). However, there was no significant difference within these two groups individualy ($p>0.05$).

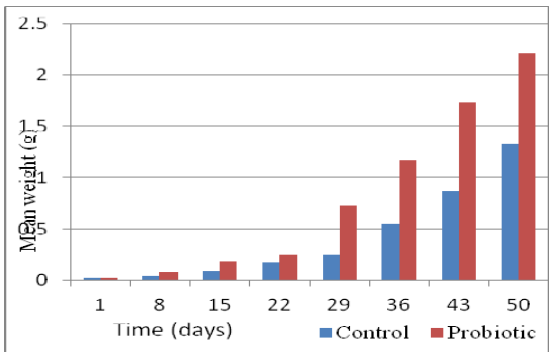


Fig 17: Weight gain (g) of shrimp in the experimental tanks.

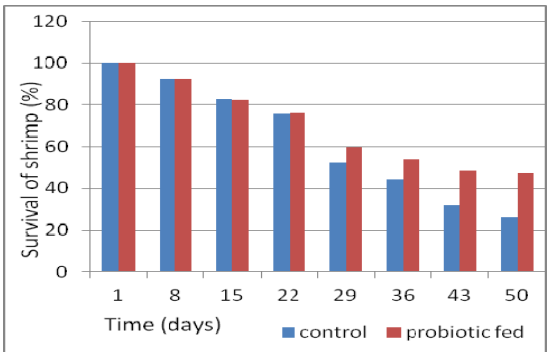


Fig 18: Percent shrimp survival in the experimental tanks

3.7 Post - Challenge observations

3.7a Bacterial counts of water

Total bacterial count (TBC) of water in both control and probiotic diet fed tanks increased up to 10^8CFU/ml after second challenge with *V. harveyi*, and gradually reduced to 10^7 by the 12th day. In control diet fed tanks, total *Vibrio* count raised after second challenge, and reached up to 10^6 CFU/ml by 12th day whereas, the values in probiotic fed tanks were $7.2x10^5$ CFU/ml on 4th day (after second challenge), gradually decreased to $2.51x10^3$ CFU/ml by 12th day. Similar values of TVC were observed in unchallenged control also ($1.58x10^3$ CFU/ml) (Fig.19-21).

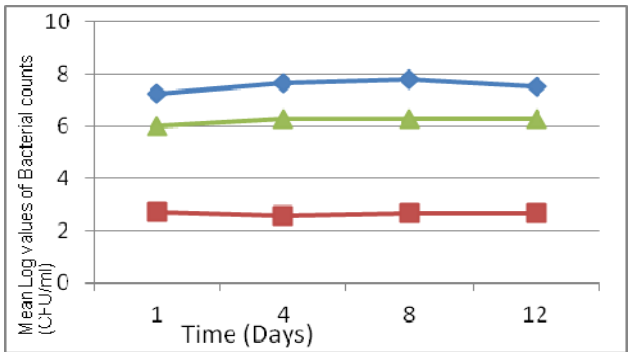


Fig:19 (CF)

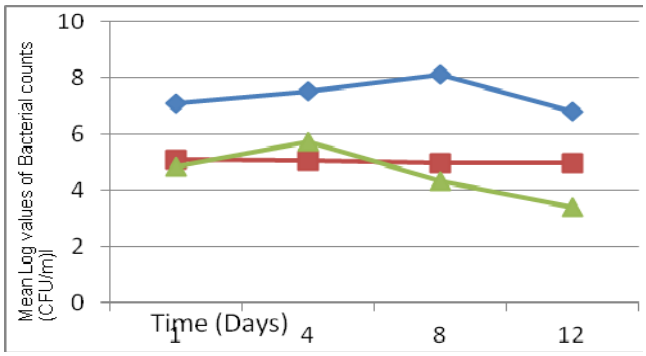


Fig: 21 (PF)

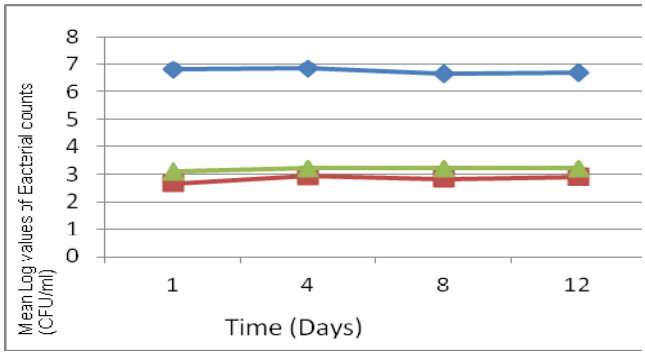


Fig: 20 (UC)

Fig. 19-21 Mean bacterial counts of water in Control diet fed tanks (CF), Probiotic fed tanks (PF) and in unchallenged control (UC) during the challenge with *Vibrio harveyi*

Total bacterial Count
Total *Vibrio* count
Total *Pseudomonas* count

(Mean values of bacterial counts three probiotic tanks and three control tanks were presented to simplify the results)

3.7b Bacterial counts in experimental Shrimp

TBC of control diet fed and probiotic shrimps were raised to a maximum of 10^8 CFU/g and 10^7 CFU/g respectively after second challenge. *Vibrio* count was also increased with TBC in control fed shrimp from 1.7 x10^6 CFU/g on 4th day (after second challenge) to 1.3x10^7 CFU/g by 12th day. The TVC of probiotic fed shrimp was recorded as 6.6 x10^4 CFU/g on 4th day and gradually decreased to 1.3x10^3 by 12th day of challenge. This value was lesser than the TVC of unchallenged control 2.24x10^4CFU/g. *Pseudomonas* remained stable around 3x10^2 CFU/g in control shrimp and 7x10^4 CFU/g in probiotic fed shrimp during the 12 days of challenge (fig 22-24).

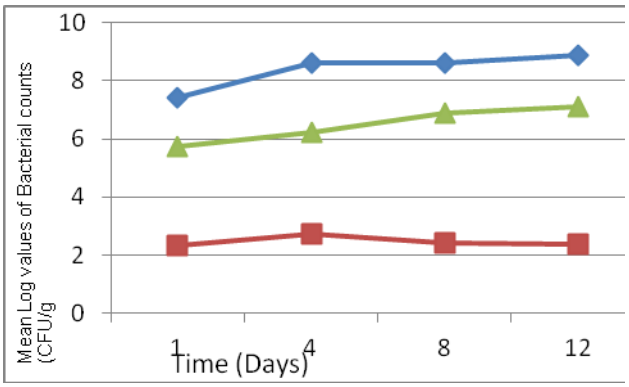


Fig: 22 (CF)

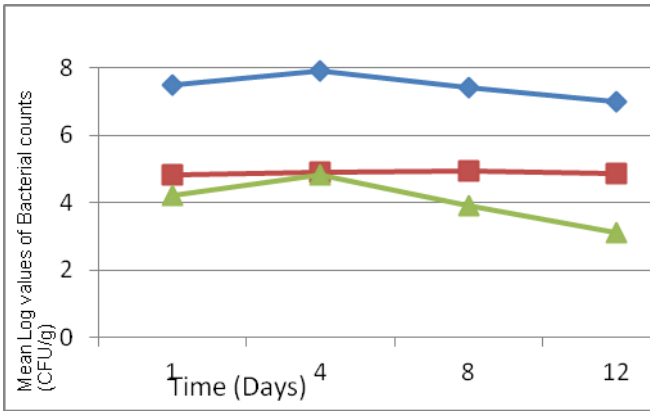


Fig: 24 (PF)

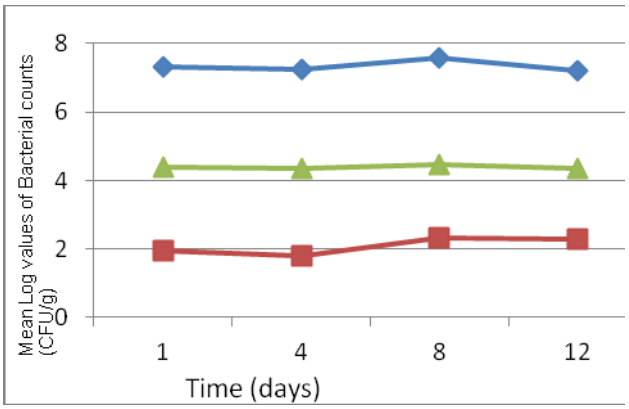


Fig: 23 (UC)

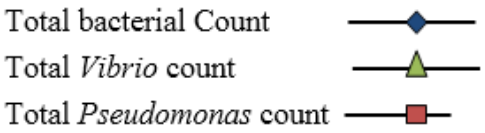


Fig. 22-24: Mean bacterial counts in shrimp from the Control diet fed tanks (CF), Probiotic fed tanks (PF) and in unchallenged control (UC) when challenged with *Vibrio harveyi.*

(Mean values of bacterial counts three probiotic tanks and three control tanks were presented to simplify the results).

3.8 Survival of shrimp after challenge with *V. harveyi*

Survival of the shrimp in experimental tanks after the post challenge has been represented graphically (Fig: 25). No mortality was recorded in all the three groups of shrimp up to 4 days of first challenge with *Vibrio harveyi* at 10^7CFU/ml. Hence, a second challenge dose (10^7CFU/ml) was given on 4th day.

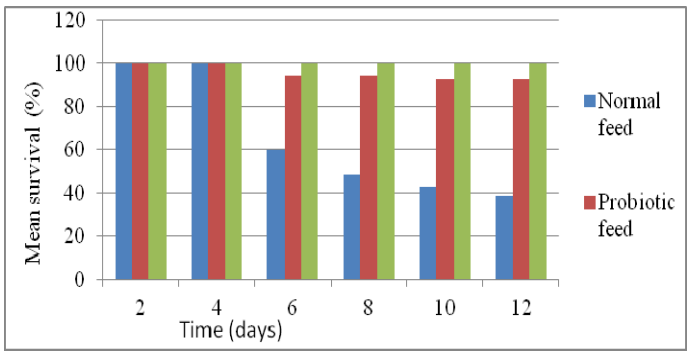


Fig 25: Percent survival of shrimp during the experimental challenge.

Survival rate in control diet fed shrimp was 59.75 % by the 6th day, decreased to 48.66% on 8th day, 43.28% on 10th day and finally reached 38.87% on 12th day. Survival percent in probiotic diet fed shrimp was 94.44 % in 8 days. Percent survival did not fall much from 10th to 12th (ie 93.04%) which was significantly greater than that of the control diet fed shrimp (38.87%) ($P<0.05$). Shrimp survival was 100% in unchallenged control group.

4. Discussion

Application of probiotics for intensive shrimp cultivation is the most promising preventive method developed to fight against diseases caused by *V. anguillarum*, *V. vulnificus*, *V. alginolyticus* and *V. harveyi*. Many probiotics including *LactoBacillus* sp [16, 17] *Bacillus* sp. [18], yeast [19, 20] have been reported to have effectively inhibited *Vibrio*s in shrimp culture. A *Pseudomonas* sp, for example, isolated from a brackish water lagoon showed significant probiotic activity against a number of shrimp pathogenic *Vibrio*s, while its safety in a mammalian system was also found satisfactory [11]. These so-called beneficial bacteria are not therapeutic agents but will alter directly or indirectly the composition of the microbial community in the rearing environment and in the shrimp gut [6, 21]. Antagonostic activity of *P. aeruginosa* on *V. harveyi* in our experiment has proved to be satisfactory to

proceed further. *Pseudomonas aeruginosa* inhibited the growth of *Vibrio* in both water as well as in shrimp. Similar results were obtained in a probiotic experiment with *Bacillus* conducted by Regipepat *et al*., [15]. Pathogencity test in our experiment revealed that *Pseudomonas aeruginosa* (PIC4) was non-pathogenic to the shrimp larvae. Similar results were obtained by Vijayan *et al*., [11]. The lethal dose (LD_{50}) of *V. harveyi* to the Post-larvae of *Penaeus monodon* was found to be 10^6 CFU/ml at 48 hrs (Table: 2). Hence, the next higher concentration i.e. 10^7cfu/ml was chosen as a challenge dose for the succeeding experiments.

During the 50 days of feeding, TBC in both control and probiotic of tank water were recorded as 10^7 CFU/ml, these counts were similar to those of normal bacterial count (10^7 CFU/ml) in regular shrimp culture pond water. [22, 23, 24, 25, 26]. TBC in control and probiotic shrimp has also reached up to 10^7 which were similar to TBC of shrimp in culture ponds, [24, 27] . *Vibrio* counts in culture tanks (10^7 CFU/$_{g \text{ or } ml}$) were similar to those of shrimp culture ponds reported by earlier workers [23, 24, 28] .

Lesser *Vibrio* counts were recorded on 50th day in probiotic diet fed shrimps indicating that presence of *Pseudomonas aeruginosa* inhibited the growth of *Vibrio* in both water as well as in shrimp. Similar results were obtained in a probiotic experiment conducted by Regipepat (15).

Water quality parameters such as pH temperature salinity and ammonia of both control and probiotic tanks were under the range of safe shrimp culture practices in ponds and in hatcheries (24, 29,30,31,32,33,34,35). Similar values were also observed in probiotic experiment conducted by Rengpipat *et al*., (15,36).

Probiotics in the form of Bacterial cells or their products have proved to be growth enhancers and promoters of resistance against pathogens, (15, 31, 37). In our experiment survival rate in probiotic fed shrimp was more due to exclusion of *Vibrio*s both in shrimp as well as in the water, and our results are in concurrence with the earlier studies conducted by Khanitta *et al*., (1). *Pseudomonas* counts in shrimp guts evidenced indirectly that probiotic *Pseudomonas* colonised in the guts of probiotic fed shrimp and there by reduced the *Vibrio* count.

Vibrio count in probiotic tanks after challenge with high dose of *V. harveyi* has gradually reduced from 10^6 CFU/ml to 10^3 CFU/ml by 12th day, these counts were equal to the TVC in normal shrimp culture ponds as reported by Jawahar and Debasis [24] and in hatcheries of P. monodon by Rajeshwari *et al*., [37].

Mortality rate in P. monodon after challenge with *Vibrio harveyi* in probiont fed tanks was significantly less compared to the normal control diet fed tanks indicating the protection due to presence of probiont *Pseudomonas* (PIC 4), it is evident that the *Pseudomonas aeruginosa* (PIC4) is capable of inhibiting the growth of *Vibrio harveyi* in the guts as well as in the water medium. Several researchers have already reported the important role of probiotics in disease control and growth enhancement in aquaculture animals [31, 38] particularly against *Vibrio harveyi* in shrimp cultures, [15, 33, 36] The present finding adds a probable probiotic to already existing ones and may prove useful to control *Vibrio harveyi* in shrimp culture ponds.

5. Conclusions

Antagonistic activity of *Pseudomonas aeruginosa* (PIC-4) against the pathogenic *Vibrio harveyi* has been established. The isolate (PIC-4) of *P. aeruginosa* has been proved to be non- pathogenic to the shrimp larvae through challenge infections and also reduced the total *Vibrio* counts in both shrimp as well as in the medium. The probiotic isolate has also increased the percent survival and growth rate in tiger shrimp. The present finding is a suitable probiotic to control *Vibrio harveyi* in shrimp culture ponds

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