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Effect of Probiotics on Growth and Survival of *Penaeus Monodon* (Fabricius, 1798) Post Larvae Infected With Swollen Hindgut Syndrome (SHG) With Better Management Practices

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Abstract: *The culture of shrimps received maximum importance due to its unique taste, high nutritive value and persistent demand in the world market. In recent years, the diseases of shrimp slowed down the development of shrimp culture. Swollen hind gut syndrome (SHG) of tiger shrimp, Penaeus monodon postlarvae is common problem in shrimp hatcheries. Post larvae infected with SHG are generally rejected by the hatcheries and farmers, as stocking of SHG seeds or postlarvae are supposed to cause several problems such as size variation, white fecal disease. Loose shell syndrome etc., resulting is severe loss in form production and earning. In the present study an attempt carried to culture the giant tiger shrimp, P.monodon by using post larvae having swollen hindgut syndrome (SHG). The use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture has a glorious future. In this present study has been made to evaluate the growth and survival of SHG infected post larvae by using probiotics viz., Gut actin (gut probiotic), Sanolife MIC (water probiotic) and Super Ps (soil probiotic) was applied in culture ponds A and B. A section of P. monodon was stocked with SHG infected post was applied with probiotic and it was compared with "B" section without probiotic treatment (control). Culture pond C and D are stocked with disease free SHG post larvae. Culture pond "C section" was applied with probiotics same as section A and it was compared to "D section" without probiotic treatment (control). Growth and survival rate of the ponds which was applied ("A" and C sections) with probiotics was higher than that of "B and D section ponds. The bacterial population decreased at the end of culture in "A" section and "C" section ponds, but in "B" and "D" sections the bacterial population was not showing a significant decrease. Bacterial necrosis, black gill, white gut and fungal diseases were recorded in "B" and "D" section ponds. But these diseases were not that much reported in probiotic treated (A and C section) ponds. The results of the present study showed that there is significant difference (P<0.05) in growth and survival and FCR between the SHG infected postlarvae and disease free postlarvae treated with probiotics and without probiotic culture. The general conclusion obtained from the present study is that the probiotic plays a vital role in growth, survival and disease resistance (not virus) of the animal by maintaining good water quality parameters throughout the culture period. It is clear from the bacterial colony data that green colony dominated in the "B and D" section ponds. So it can be recommended that the probiotics can be very well utilized for the shrimp ponds to get maximum growth and survival.*

Key words: *Penaeus monodon, swollen hind gut syndrome, gut actin, sanolife MIC, super ps, survival.*

I. Introduction

Marine shrimp farming is the most important aquaculture in the world and is reported by FIGIS (2007) about 75% of farmed shrimp produced in Asia where China, India, Malaysia, Thailand and Indonesia. In India, shrimp culture has grown enormous revenue in terms of foreign exchange, lot of employment and uplifted the living standards of many people involved directly or indirectly with the industry and reduction of over tropical and sub-tropical regions (Ramanathan *et al.*, 20005). The boom period of shrimp culture in India started in 1990 and the bust came in 1995-96. The growth of the industry off late witnessed two major setbacks. The first important bottleneck is viral disease outbreak (Karuna *et al.*, 1994), which withered the confidence of entrepreneurs and financial institutions. Another very important problem is pollution. The organic load in term of unutilized feed due to excessive feeding (due to over feeding), faecal matter released by shrimps and dead algae, settle at the bottom of the pond contribute pollution of the pond bottom (Yew- Hu, 1992). The success of a crop is greatly depends on the quality of seeds for stocked in ponds. Shrimp seed for stocking are mostly procured by the farmers from the commercial shrimp hatcheries. Farmers collect seed samples from hatcheries and get them tested at commercial seed testing or evaluation from PCR laboratories before stocking. These labs rate the seed based on several parameters such as size, appetite, muscle-gut ratio, stress tests, viral tests etc and advice the farmers to either stock or reject the seed. One such seed quality parameter is the presence of Swollen Hindgut Syndrome (SHG). Over the last few years, millions of seed affected by Swollen Hindgut Syndrome

(SHG) from hatcheries all across the country were rejected by labs and farmers. Swollen Hindgut Syndrome (SHG) is a morphological deformity that tends to occur at later larval (PL) stage, typically after PL-15 and was first reported by Lavilla-Pistogo *et al.,* (2002) in *P.monodon* postlarvae. SHG mainly affected the hindgut and to some extent, the posterior midgut. Postlarvae infected with SHG show enlargement and distension of the hind gut folds and its junction with the midgut. This abnormality affects the rhythmic movements in the rectal region in the shrimps resulting in difficulty in expelling the faecal pellets (SEAFDCE/AQD, 1994). Aftabuddin and Akter (2011) also reported that unlike WSSV, though SHG does not show mass mortality of *P. monodon* larvae, it is still considered to be a nascent problem of concern to many shrimp farmers in India. Most SHG infected seeds are healthy in all other respects included the absence of the deadly WSSV, they are still drained causing severe losses to hatchery operations as well as the shrimp aquaculture industry on the whole.

India is a large country with great potential for aquaculture, but the application and development of the probiotics in Indian aquaculture is very limited when compared to other countries. In recent years, the diseases of shrimp over-involved the development of shrimp culture. Based on the previous research results on probiotics suggest that the use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture have a brilliant future. The role of probiotics bacteria in small culture is studied but commercial level is not that much reported especially in giant tiger shrimp *P. monodon*. Hence the beneficial effect of probiotics on the commercial culture of Indian major candidate shrimp, *P. monodon* is very much need of the hour. Therefore, the present study was selected to examine the effect of a probiotics on SHG infected and SHG free post larvae by applying probiotics viz., Gut actin, Sanolife MIC (INVE-Thailand Ltd) and super Ps (C.P aquaculture India Pvt Ltd) on the shrimp, *P.monodon* culture was studied.

II. Materials And Methods

This experimental study was carried out in farm site located in Karlapalem village. The farm is situated about 20 km away from Guntur town, Andhra Pradesh, South India. . The total area covered is 9.0 ha of which water spread is about 8.5 ha. Totally twelve ponds, each with a 0.7 ha was selected for this operation. The ponds are rectangular in shape. The culture is semi intensive type with stocking densities of 12 PL's/m2. The depth of pond was 1.5 m. The culture was carried out from May to July 2011. Initially all the selected ponds was allowed to dry and splinter to increase the capacity of oxidation of Hydrogen Sulphide ($H_2S\uparrow$) and to eliminate the fish eggs, crab larvae and other unwanted predators. Then pond bottom was scrapped 3 to 5 cm by using a tractor blade to avoid top soil. Then the pond bottom was ploughed horizontally and vertically a depth of 30 cm to remove the obnoxious gases, oxygenate the bottom soil and remove the hydrogen sulphide odor and to increase the fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. For increases the availability of nutrients, required amount of lime was applied to neutralize the acid soil, condition of the soil based on the average pH level of the pond.

Water management is one of the most important practices during the culture period. Indeed, if the PLs are stocked into a pond with poor algal populations, they will become stressed. That not only greatly reduces PL growth, but weakens the animals, making them much more prone to disease and subsequent death. For blooming, the pond is fertilized with inorganic or organic fertilizers. The initial water levels in all ponds were maintained at 120 cm level. After filling, one day was allowed for sedimentation of turbid particles. After sedimentation process, chlorination was done for all ponds (dosage for 1 ha for every 1 meter water level/500 kg chlorine). Blooming process was started after 48 hrs of chlorination. The organic fertilizers such as groundnut oil cake, dry cow dung and rice bran powder was flooded overnight and applied the extract to all the ponds. Then water level was maintained to 120 cm of the ponds and added urea and super phosphate to improve the primary production. Fertilization enhanced the optimal algal bloom in the ponds and the transparency in the ponds ranged from 30 to 35 cm.

The SHG infected and SHG free *P. monodon* (PL-20) seeds were purchased from local hatchery, Bapatla and were transported in oxygenated double layered polythene bags. The seeds were brought to the farm site and bags were kept in the pond for acclimatization. Subsequently the seeds were released slowly in to the ponds. The stocking density per pond was 12/m2 (84,000 PLs / pond). Along with the present study, the representative samples of 100 infected and normal postlarvae (PL) were placed in mesh hapas of 1m X 1m X1m dimensions for each pond to assess the initial survival in ponds as well as to examine the presence of SHG. The postlarvae in both the experiment as well as control ponds were fed with gut probiotics and vitamin C right from the beginning of the study to improve their immunity and growth. The postlarvae from the hapas were observed for the presence of SHG, 48 hours after stocking. Thereafter, the juveniles were routinely observed to check health status and growth on weekly basis. Water quality parameters for all the ponds were constantly maintained at best possible level with good management practices. The total area of the farm was divided in to four experimental ponds; they are section A, B, C and D. The "A" section ponds (A1, A2 and A3) was stocked with SHG infected postlarvae and treated with both water and soil probiotics (Sanolife MIC–water (INVE, Thailand) and super Ps – soil probiotics, manufactured by C.P aquaculture India Pvt. Ltd, Chennai). For "B" section ponds (B1, B2, and B3) were stocked with SHG infected post larvae and these are free from probiotics treatment (control pond). The "C" section ponds (C1, C2 and C3) was stocked with SHG free normal postlarvae and treated with both water and soil probiotics (same procedure like section A). For "D" section ponds (D1, D2, and D3) were stocked with SHG free normal post larvae and these are free from probiotics treatment (control pond). For water quality management 1.5 kg of Sanolife MIC mixed with 200 liter water (8 hour fermentation process was done by aerating through aquarium aerator). It was broadcasted throughout the pond during morning hours in an interval of 15 days. However, it was only 10 days once after 50th DOC (days of culture) onwards. For bottom soil quality management 10 liter super Ps probiotic was mixed in 50 kg of dry sand then it was broadcasted throughout the pond during morning hours in an interval of 15 days. However, it was only 10 days once after 50th DOC onwards. The water quality parameters were recorded in control and probiotics treated ponds regularly. Water temperature was measured in the pond itself using a standard centigrade thermometer. The pH of the pond water was measured by using electronic pH pen manufactured by Hanna Instrumental Company, Japan. The water salinity was measured by using a hand refract meter (Erma-Japan). Dissolved oxygen meter estimated the dissolved oxygen. The water level was measured by using a standard scale with cm marking. Transparency was measured in terms of light penetration using a secchi disc. During the first 3-4 weeks of culture, water exchange is not required. Subsequently the water was exchanged once in a weak or depends upon the water and shrimp quality. Water quality parameters maintained during the present study was given in Table 1.

Feed management plays a vital role in the shrimp culture. Avanti (Pingtoi) feed was used during the entire cycle and distributed manually with the help of a boat. During the first month after stocking, feeding rates were based on estimated survival and feeding tables and distributed five times per day. After 30th DOC, daily rations were adjusted using feed trays and increased to six times per day thereafter. The use of feed trays is extremely important in the control of feeding. They provide information regarding the feed consumption, the health and survival of the shrimp and also the condition of the pond bottom. If the shrimp consumed all the feed within the given time, we have to reduce the feed to prevent over feeding. Left over feed can cause the pond bottom to decay and water becomes deteriorated easily, the shrimp will be weak and stressed. They will also avoid feeding and easily get sick and eventually die. As in water and soil probiotics feed probiotics were also used in the present study. The dosage and duration details of the feed probiotics are presented in Table 2. Cast net was used collect shrimp from the ponds and measure the growth rate and survival of shrimps. The first sampling was taken after 40th days of culture and number of individuals and the average body weights were recorded in each sampling. Five hauls were made in each pond in different corner of the ponds. Healthiness, survival rate, average body weight (ABW), average daily growth (ADG) and food conversion ratio (FCR) of the animals was estimated. Sampling was regularly performed every one weak until harvest. Ammonia level was monitored regularly.

For microbial analysis, the water and sediment samples were collected separately from different parts of the pond in sterile conical flask and were mixed to make a single sample. This procedure was repeated for every pond and the final samples were brought to the laboratory immediately and were analyzed for microbial counts. For enumeration of Total Heterotrophic Bacteria (THB), Zobell marine agar medium (Hi-media, Mumbai) was used. For enumeration of *vibrio* spp TCBS media was obtained from Hi-media, Mumbai. Enumeration of the microbes was done by adopting spread plate method. In this method, sterile media were poured into petri dishes aseptically and allowed to solidify. One milliliter of serially diluted sample was pipette out into sterile petridish. It was made spread in the plate first by rotating it in clockwise and then anti-clockwise directions for three times and then spread with the help of a 'L'-rod. The plates were incubated in an inverted position at 28±2ºC. After the incubation period of 24 hrs colonies were counted. The plates were examined and counted the number of colonies per plate. The microbial load in the given sample was calculated using the following formula and it is expressed as Colony Forming Units (CFU) per gram of the sample. The shrimps were reared for a period of 170 ±5 days and the data obtained were subjected to student *t-test* and ANOVA to determine the difference between the treatments and the data were analyzed with corresponding statistical analysis.

III. Results

The water quality parameters maintained during the present study was described in Table 1. The salinity was recorded maximum (28 ppt) in the month of June and minimum (10 ppt) was during March. In general minimum salinity was recorded in probiotics treated ponds and maximum was in control ponds. The pH was recorded during the culture period from Feb to June 2011. The average pH was between 7.6 to 8.2. The pH was minimum in all months of the probiotics treated ponds. However, it was maximum in control ponds. The dissolved oxygen was recorded maximum (6.5 ppm) during the month of March and minimum (3.6 ppm) was during June. It is maximum in probiotics treated ponds and minimum in control ponds. The temperature did not show much variation in probiotics treated and control (26- 30ºC) ponds. The transparency also did not show much difference in probiotics and control ponds (35-55 cm). The ammonia was totally absent in probiotics treated ponds of all months. But the ammonia was recorded maximum (0.3) in the month of June and minimum

(0.1) was during May in control ponds. Survival rate of probiotics treated ponds was higher than that of control ponds. The dosage and duration details of the feed and probiotics are described in Table 2. At the end of culture period the survival rate of probiotics treated ponds was maximum (98.7 % in Pond-A and 92.5% in Pond-C) and minimum in control ponds (55.91% in Pond-B and 87.2% in Pond-D). Average growth and survival of the shrimp was presented in Table-3 and 4. Maximum growth was observed in probiotics treated ponds during each sampling interval and by the end of the experiment than control ponds. The bacterial population changed during every sampling. In general, the bacterial population of sediment was higher than that of water in both “A” and “B” section ponds. The bacterial population decreased at the end of culture in probiotics treated ponds, but the load of *Vibrio* spp when compared with THB in control ponds was not showing a significant decrease. *Vibrio* sp. was contributing much in the THB load of the control ponds. In general, the probiotics treated ponds were showing a reduced count of *Vibrio* sp.

Table 1: Water quality parameters recorded from culture ponds (A, B, C and D)

Parameter	Range
Temperature (^oC)	26-30
Salinity (ppt)	12-28
pH (ppm)	7.6-8.2
Dissolved oxygen (ppm)	3.6-6.5
Transparency (cm)	35-55
Ammonia	0.1-0.3

Table 2: Application of probiotics in culture ponds (A and C)

Date of culture (DOC)	Gut actin (gr/kg feed)	Sanolife (MIC) kg/ha
20-30	20	1.5 kg
31-40	20	1.5 kg
41-50	20	1.5 kg
51-60	20	1.5 kg
61-70	20	1.5 kg
71-80	20	1.5 kg
81-90	20	1.5 kg
91-100	10	1.5 kg
101-110	10	1.5 kg
111-120	10	1.5 kg
121-130	10	1.5 kg
131-140	10	1.5 kg
141-150	10	1.5 kg
151-160	10	1.5 kg
161-170	10	1.5 kg

Table 3: Performance of SHG infected and normal *P.monodon* seeds (Probiotic treated ponds Vs control ponds) Mean ± S.E, n=20

Parameter	SHG infected and Probiotic treated	SHG infected and without probiotic treated (control)	SHG free and Probiotic treated	SHG free and without probiotic treated (control)
24 hrs after stocking	75.4 ±0.18	65.7± 0.14	90.2 ±0.16	91.5 ±0.14
ABW at harvest	46.72± 0.45	28.21± 0.012	48.25± 0.12	46.82 ±025
Survival at Harvest	98.71± 1.32	55.91± 0.062	92.5±.0.18	87.2± 0.006
FCR	1.75 ±0.07	1.54 ±0.06	1.83 ±0.04	1.81 ±0.11

Table 4: Average weight of shrimp at different culture periods of both probiotic and control ponds

Days of culture (DOC)	Average weight (g)			
	Pond-A (g)	Pond-B (g)	Pond-C (g)	Pond-D (g)
40	7.2	5.2	7.5	6.8
50	10.5	8.2	11.5	10.2
60	12.5	9.5	13.2	12.2
70	15.5	10.4	15.6	14.2
80	18.1	12.2	19.2	15.6
90	21.2	13.5	22.4	16.5
100	22.6	16.2	26.7	22.4
110	26.5	20.1	28.5	25.2
120	28.5	21.5	31.4	26.3
130	31.2	23.5	32.6	30.4
140	34.2	26.5	37.5	32.5
150	33.8	28.4	42.2	34.8
160	34.7	29.5	48.5	38.2
170	35.2	30.2	50.2	42.8

IV. Discussion

There has been a considerable increase in the culture of brackish water shrimp due to its taste, market demand both national and international markets. In order to prevent many problems due to shrimp culture, sustainable shrimp farming is the need of the hour. Ideal pond size for shrimp culture was 1 or less than 1 ha (Ramanathan *et al*., 2005). In the present investigation also 12 ponds were used for shrimp culture and each pond size was 0.6 ha. Even though shrimps are bottom dwelling organisms, the depth and volume of water in a pond has certain physical and biological consequences. The present study was undertaken to ascertain the effectiveness of probiotics (Gut actin, Sanolife MIC, Super Ps) on the growth and survival of the most important cultivable shrimp species, *P. monodon* in addition to its influence on important water quality parameters. Important water quality parameters monitored during the study were, temperature, salinity, dissolved oxygen, pH, transparency and ammonia levels. The volumes of water perform like a buffer, which prevents weather fluctuations from influencing the environment in which shrimp lives. The ideal water depth is between 0.8 to 1.5 m depending upon the stage of culture. It is recommended that a minimum depth of 1m be maintained at operational level. In the present study 100 cm water level was maintained in all ponds throughout the culture period. W hen a pond is ready for operation, the optimum stocking density of seeds in a pond determined in accordance with the production capacity of the pond and the culture system, which included the soil and water quality, food availability and seasonal variations, target production and farmers experience (Ramanathan *at al*., 2005). The stocking density between 10-20 PLs/m2 is ideal for successful shrimp farms (Ramanathan *et al*., 2005; Soundarapandian and Gunalan, 2008, Sundarapandian *et al.,* 2010). In the present study the seeds were stocked at the stocking density of 12/ m^2 in all ponds.

The maintenance of good water quality is essential for optimum growth and survival of shrimps. The levels of physical, chemical and biological parameters control the quality of pond waters. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Excess feed, faecal matter and metabolites will exert tremendous influence on the water quality of the shrimp ponds. Hence critical water quality parameters are to be monitored carefully as adverse conditions may be disastrous effect on the growing shrimps (Ramanathan *et al*., 2005; Soundarapandian and Gunalan, 2008, Jaideep *et al*., 2011).The optimum range of temperature for the black tiger shrimp is between 28 to 30ºC (Ramanathan *et al*., 2005). The temperature in the present study was 26 to 30ºC and the low temperature 25ºC was observed due to cloudy weather. The optimum range of temperature of *P. monodon* was between 26 to 33ºC (Boyd, 1995; Soundarapandian and Gunalan, 2008) and temperature range of 28 to 33ºC supports normal growth as observed in the present study. Salinity is important parameters to control growth and survival of shrimps. Even though, *P. monodon* is euryhaline animals; it is comfortable when exposed to optimum salinity. At high salinity the shrimps will grow slowly but they are healthy and resistance to diseases. If the salinity is low the shell will be weak and prone to diseases. The salinity of the present study was maintained 12-28 ppt in all ponds. Muthu (1980), Soundarapandian and Gunalan (2008) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture. While Chanratchkool *et al*. (1994) maintained the salinity of 10-30 ppt. Chen (1980) opined that salinity ranges of 15-20 ppt are optimal for culture of *P. monodon*. There are few reports (Shivappa and Hambry, 1997; Ramakrishna, 2000; Collins and Russel, 2003; Jaganmohan and Prasad, 2010), which stated that *P. monodon* adapted quite well in freshwater conditions also because of its wide range of salinity tolerance. Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Oxygen affects the solubility and availability of many nutrients. Low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. Lack of dissolved oxygen can be directly harmful to shrimps and cause a substantial increase in the level of toxic metabolites. Low-level of oxygen tension hampers metabolic performances in shrimp and can are reduce growth and moulting and cause mortality. The dissolved oxygen in all the culture ponds in the present study was ranging between 3.6 to 6.5 ppm. Water temperature is probably the most important environmental variables in shrimp cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature affects the shrimp immune system.

pH is one of the imperative environmental characteristics, which decides the survival and growth of shrimp under culture; it also affects the metabolism and other physiological process of shrimps. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production (Ramanathan *et al.*, 2005). In the present study pH was ranging between7.6 to 8.2 for the probiotics treated and control ponds. Jaideep *et al.*, (2011) observed that 8.2 to 9.0 is the optimal pH for shrimp culture. Saha *et al*. (1999) noticed the pH of 8.11 to 8.67 in low saline ponds. Ramakrishna (2000) and Soundarapandian and Gunalan (2008) was recommended pH of 7.6 to 8.2 for *P. monodon* culture. The pH of pond water is influenced by many factors, including pH of source waters and acidity of bottom soil and shrimp culture inputs and biological activity. The most common cause of low pH in water is acidic bottom soil, liming can be used to reduce soil acidity. In most common cause of high pH is high rate of photosynthesis by dense phytoplankton blooms. When pH is high water exchange will be better choice (Boyd, 2001). The transparency is mainly depends on the presence of phytoplankton. The optimum range of secchi disc reading is in between 30 to 55 cm at the juvenile stage and between 25 to 55 cm to the sub adult and final stage. The transparency of the present study is 35 to 45 cm. Ramakrishna (2000) and Soundarapandian and Gunalan (2008) also observed similar transparencies (25-50 cm) for their studies on shrimp culture. The reading less than 30 cm mean that the phytoplankton density is high. If it is more than 40 cm indicates, low population of phytoplankton. For the growth of phytoplankton adequate quality of sunlight is needed. Due to low intensity of light during the culture period, the plankton bloom was less.

In the present study, the level of ammonia was well below this mark in “B and D” ponds. But in the “A and C” section ponds no ammonia problem was encountered. This is mainly due to the microorganisms (*Nitosomonas*) present in the probiotics, which initiate nitrosification. Due to this process ammonia is converted into nitrite, which is further acted upon by the nitrobacter and converted as nitrate through the process nitrification. In “B and D” section ponds the shrimps were affected by black gill and tail rot diseases, however this is absent in probiotic used “A” section ponds. This is mainly due to the absence of probiotic in the “B” section ponds. Ravi *et al*. (1998) already described the benefits of probiotics in maintaining water quality and enhancing growth rate in Indian white shrimp, *P. indicus.* Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. Antibiotics, which have been used in large quantities, are in many cases ineffective, or result in increases in virulence of pathogens and, furthermore, are cause for concern in promoting transfer of antibiotic resistance to human pathogens. Probiotic technology provides a solution to these problems. The microbial species composition in hatchery tanks or adding selected bacterial species to displace deleterious normal bacteria can change large aquaculture ponds. Virulence of luminous of *Vibrio* species can be controlled in this manner. Abundance of luminous *Vibrio* strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm on Negros, in the Philippines, which had been devastated by luminous Vibrio disease while using heavy doses of antibiotic in feed, achieved survival of 80-100% of shrimp in all ponds, treated with probiotics (Moriarty, 1996). Ruangpan (1991) reported in their study that the high abundance of luminescent *Vibrio* is consistent with occurrence of disease and poor or zero harvest results. *V. harveyi*, a pathogen of *P. monodon* that causes severe losses (Baticados *et al.*, 1990). The farm, which used the superbiotic probiotic bacteria, had either a very low abundance or a complete absence of luminous *Vibrio* in pond water and very good harvest result. This consistent and high productivity occurred, even though the proportion of luminescent *Vibrio* in the pond water was high in the sea water source, and the abundance of total green colony in the pond water was higher than in the water source. Furthermore, luminescent *Vibrio* were completely absent at all stages of grow out from the pond sediment in the presence of the super biotic *Bacillius* species.

In the present study also the shrimps were recovered in ponds with Gut actin, Sanolife and Super Ps. The probiotics treated (“A and C” section) ponds in the present study had either a very low abundance or a complete absence of luminous bacteria and very good survival was achieved. This result is comparable with the study of Dalmin *et al*. (2001). Colonization of the gastrointestinal tract of animals by probiotics is only after birth, and before the definitive installation of a very competitive indigenous micro biota (Gilles, 2001). After this fixing, only the addition of doses of probiotic provokes its artificial and temporary dominance. In mature animals, the population of probiotic organisms in the gastrointestinal tract shows a sharp decrease (Fuller, 1992). Application of microbial supplement in the probiotic ponds hindered the growth of Vibrio spp, like *V.*

alginolyticus and V. harveyi because of the colonization of the beneficial microbes like *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Saccharomuces* sp. in the shrimp gut. Since the shrimps in the "B and D" section ponds were dominated with green colony, which caused Vibriosis can be attributed as the reason for low survival when compared with the "A" section ponds. This was evident from the presence of higher load of green colony, in the water and sediment of control ponds than in the probiotics used ponds. The occurrence of green colony in the "B" section ponds was concluded by presence of luminescence in the nighttime and occurrence of dead animals in the check tray. In the present study white gut disease was also reported in "B" section ponds, which ultimately leads fungal diseases, naturally animal's activities slowed down and become sluggishness. The white gut and fungal disease are not observed in probiotics treated ("A" section) ponds.

Microscopic observation of the post larvae in the experimental ponds of SHG infection is only 48% of seeds on an average was suffered. On the execution of the experiment (170 ±5 days of the culture), the SHG infected shrimp of average size of 35.2±0.06 g were harvested in A-pond (Probiotic treated) with the average daily growth rate achieved was 0.201 g d^{-1}.The SHG infected post larvae in pond-B (without probiotic treatment) achieved 30.2 ±0.05 g was achieved. the SHG free shrimp of average size of 50.2±0.75 g d^{-1} g were harvested in C-pond (Probiotic treated) with the average daily growth rate achieved was 0.256 g d^{-1}.The SHG free post larvae in pond-D (without probiotic treatment) achieved 42.8 ±0.12 g d^{-1} was achieved. The probiotic treated normal post larvae was significantly higher than the other treatments especially compared with SHG infected (Pond-A) post larvae. The mean survival of SHG infected seed was recorded as 75.4±0.18% in probiotic treated ponds (Pond-A) and 65.7±0.14% in without probiotic treated ponds (Pond-B). But in normal (SHG free) postlarvae was recorded as 92.5±0.18% in probiotic ponds (Pond-C) and 87.2±0.06% in without probiotic treated pond (Pond-D). The survival rate in between the treatment also significantly different ($P<0.005$) from SHG infected and normal post larvae based on probiotic treatment (Table3 and 4). The FCR of the SHG affected shrimps and the normal in different treatment was evaluated as 1.75±0.07 (Pond-A), 1.54±0.12 (Pond-B), 1.83±0.04 (Pond-C) and 1.81±0.11 (Pond-D).

The general conclusion based on probiotics obtained from the present study is that the probiotics plays a critical role in growth, survival and disease resistance of the animal by maintaining good water quality parameters throughout the culture period. It is clear from the microbial load data that green colony is dominant in the "B and D" section (without probiotics) ponds. Besides green colony, the shrimps in the "B and D" section ponds also affected by black gill, white gut and fungal diseases. Based on the effect of SHG the above result revealed that the SHG infected *P.monodon* postlarvae can be cultured and harvested as similar to the normal seed provided the best farm management practices are followed by the shrimp farmer. By using different types of probiotics, the harvesting time was reduced and good production was obtain from normal post larvae and also SHG infected post larvae.

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