A STUDY ON THE RELATION BETWEEN DIFFERENT COMMERCIAL PROBIOTICS AND SURVIVAL OF SHRIMP LARVAE IN DIFFERENT HATCHERIES OF BAPATLA COAST

By

M ASHA RANI
T LAKHSMI THIRUPATHAMMA
SANGALA RADHIKA
RAMPATHOTI MADHAVILATHA
SHAIK VAZEED

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Research Supervisors

K.BHANU PRAKASH

B ASHOK KUMAR

Department ofZoology

DEPARTMENT OF ZOOLOGY

K R K GOVT DEGREE & PG COLLEGE, ADDANKI

PRAKASAM DISTRICT, ANDHRA PRADESH

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DECLARATION

We declare that this thesis entitled **"A Study On The Relation Between Different Commercial Probiotics And Survival Of Shrimp Larvae In Different Hatcheries Of Bapatla Coast"** is composed by us and has not been published or submitted in part or in full for award of any degree.

Station: Addanki

Date : February 2018

M.S Rani

T.L. thirupatamma

S. Radhika

R. madhavilatha

Sk. Vazeed.

CERTIFICATE

This is to certify that the work incorporated in this thesis entitled, **"A Study On The Relation Between Different Commercial Probiotics And Survival Of Shrimp Larvae In Different Hatcheries Of Bapatla Coast"** is the bonafide work carried out by my students under our supervision.

K Bhanu Prakash

B Ashok Kumar

Research Supervisors

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ABBREVIATIONS:

FAO	: Food and Agricultural Organization
MPEDA	: Marine Products Export Development Authority
CAA	: Coastal Aquaculture Authority
ANOVA	: Analysis of Variance
PL	: Post Larva
RGCA	: Rajiv Gandhi Centre for Aquaculture
SR	: Survival Rate
SPF	: Specific Pathogen Free

INTRODUCTION

Riding on a robust demand for its frozen shrimp and frozen fish in international markets, India exported 11, 34,948 MT of seafood worth an all time high of US$ 5.78 billion (Rs 37, 870.90 crore) in 2016-17 as against 9, 45,892 tons and 4.69 billion dollars a year earlier, with USA and South East Asia continuing to be the major importers while the demand from the European Union (EU) grew substantially during the period. According to the FAO, India is one of the top exporters of the Aqua products in the world.

The overall export of shrimp during 2016-17 was pegged at 4, 34,484 MT worth USD 3,726.36 million. USA was the largest import market for frozen shrimp (1, 65,827 MT). The export of Vannamei shrimp, a major seafood delicacy, improved from 2, 56,699 MT to 3, 29,766 MT in 2016-17, registering a growth of 28.46 per cent in quantity. In value terms, 49.55 per cent of total Vannamei shrimp was exported to USA (MPEDA).

Shrimp aquaculture is the fastest growing animal food producing sectors in the world due to its high demand in developed countries; however, they are affected by diseases mainly caused by opportunistic pathogens results in huge economic losses. Today preventive and management measures are central concern to overcome such outbreak of diseases. The adverse effect of chemotherapeutics in shrimp farming has drawn attention for the development of non-antibiotic and environment friendly agents, which is one of the key factors for health management in shrimp aquaculture.

In recent years, application of Probiotics against virus and bacteria in shrimp farming had been a novel and safe approach since they promote the innate immune response. Probiotics for bacterial diseases like vibriosis is well reported, but for viral diseases, the authentic strains still need research. Apart from the discovery of new or better formulations, improvement in the

probiotic benefits may be helpful. Thus, better and economic production methods, administration ways or combination with other preventive/therapeutic measures are welcomed. Further studies are still necessary to increase the knowledge about use of Probiotics to control bacterial infections in shrimp but much more efforts are needed in the case of viral diseases.

Bacterial problems of penaeid larval stages are typically described as follows, "In zoea syndrome, larvae stop feeding at zoea 2 stage and spend like 4-5 days without moulting to the next stage. Larvae don't eat, have no faeces, and are totally white and without lipids. Mortalities can go up to 80-90%. It's caused by a *Vibrio spp.* Some of the prevention methods used in Ecuador are reducing the stocking period of the hatchery to no more than 3 days, Probiotics and strong asepsis (sanitization). Some of the treatments used (mostly with not very good results) are antibiotics, and sacrifice." (Marcillo, 2003). I have experience bacterial outbreaks where initially healthy zoea 1 is all dead within 12 hours.

Probiotics as live micro-organisms that, "...when administered in adequate amounts, confer a health benefit on the host."

The term Probiotics was first used by Lilly & Stillwell in 1965.

Probiotics was defined as the microbiological origin factor that stimulates the growth of other organisms.

In 1989 Roy Fuller introduced the idea that Probiotics generate a beneficial effect to the host. He defined Probiotics as live microorganisms which, when administered in adequate amounts, confer benefit to the host's health, improving the balance of the microbiota in the intestine.

Probiotics in shrimp larval rearing:

The defense mechanisms of crustaceans are less developed than those in finfish and other vertebrates as they do not have the ability to produce immunoglobulins, so they apparently depend only on the innate defense system of phenol oxidase enzymes (Roch, 1999; GonzalezSantoyo and Cordoba-Aguilar, 2012). Keeping this in view, Rodriguez and Le Moullac (2000) elucidated the application of immunological tools to evaluate those of an inherent health marker, that is, PO and radical oxygen intermediates, and the clinical significance of responses in shrimp.

The ever-growing intensification of shrimp culture ponds has been posing water quality problems which predispose the culture crustacean for many opportunistic diseases. In order to overcome this problem, the farmer started the application of antibiotics and other chemicals which reach man through the food-chain. The export products with residual antibiotics and other forbidden chemicals faced rejection in the European countries in the recent past.

This has necessitated the search for alternative nutrients to improve the health of the culture animals. These Probiotics were proved to improve growth, digestion, water quality of the culture crustacean. They also inhibit the pathogens that cause harm to the animal by correcting the balance of the microbiota in the gut of the animal being cultured.

The present study has been carried out with the objective of assessing the relationship between the Survival Rate (SR) and application of different commercial Probiotics in 3 different CAA approved shrimp hatcheries in coastal Bapatla, Guntur District.

Objectives of the study:

1. To assess the efficacy of probiotics on the percentage of survival in the larvae of *L.vannamei.*
2. To study if there is any effect of different commercial probiotics on the survival of the larvae of L.vannamei.

REVIEW OF LITERATURE

Shrimp farming plays a pivotal role in the socio-economic condition of the coastal population of India by way of contributing to foreign exchange earnings and livelihood options. The export of Vannamei shrimp, a major seafood delicacy, improved from 2, 56,699 MT to 3, 29,766 MT in 2016-17, registering a growth of 28.46 per cent in quantity. In value terms, 49.55 per cent of total Vannamei shrimp was exported to USA (MPEDA). Diseases occur in hatcheries. Some of the treatments used (mostly with not very good results) are antibiotics, and sacrifice." (Marcillo, 2003).

Some of the researchers who worked on shrimp defense system are: Roch (1999); GonzalezSantoyo and Cordoba-Aguilar, (2012); Lilly & Stillwell (1965) Rodriguez and Le Moullac (2000); Crocos and Coman (1997); beneficial effects of Probiotic bacteria was elucidated by Neish et al.,(2009); Zherdmant et al.,(1997); Gómez-Gil et al.,(2000). Srinivas et al. (2010) showed that traditional practices (large exchange of water, application of disinfectants and antimicrobials, or both) are required to successfully complete the larval cycle. Luis-Villaseñor et al.,(2011) worked on probiotics and development of larvae. The researchers worked on the cultivation of larval crustaceans (Balcázar et al., 2007b; Garriques and Arevalo, (1995); Gómez et al.,(2008); Guo et al., (2009); Nogami and Maeda, (1992) and bivalves Douillet and Langdon (1993, 1994); Riquelme et al.,(1996, 1997, 2001).

MATERIALS & METHODS

The following Commercial Probiotics were selected for study in different hatcheries:

Table 1:

S.No	Name of the Commercial Probiotic	Company	Hatchery
1.	Super PS	CP Aqua India(Pvt) Ltd	Hatchery 1
2.	Bio Com Plus	VXL	Hatchery 2
3.	Biostabil	Biomin	Hatchery 3

Fig.1

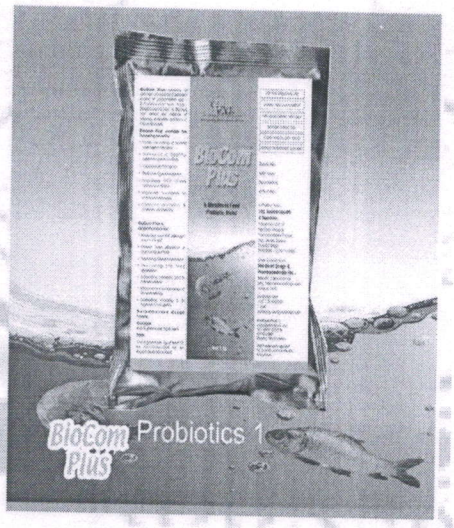


Fig. 2:

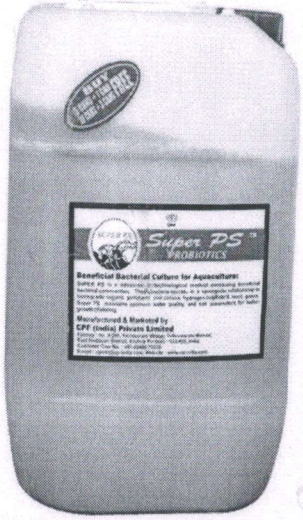
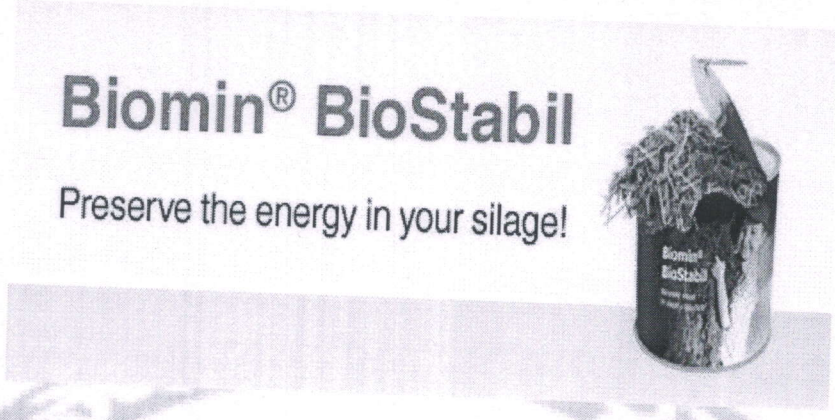


Fig.3:



The shrimp hatchery was using 5 production tanks and three production hatcheries were selected for study. The three hatcheries used SPF Brood stocks purchased from the Rajiv Gandhi Centre for Aquaculture (RGCA), Chennai. The water probiotics were applied in the tanks daily throughout the production cycle of the shrimp, *L.vannamei.* The number of eggs was estimated approximately and the Post-larvae were counted at the end of the cycle. The three hatcheries having 5 production tanks each were observed throughout the production period. Each hatchery used one brand of probiotic. And the influence of the three different brands of probiotics on the total survival percentage was assessed. The number of Post-larvae survived at the end of the production cycle was recorded for each tank and the survival rate was calculated.

$$\text{Survival } \% = \frac{Number\ at\ Harvest}{Total\ number\ of\ eggs} \text{ x } 100$$

Fig 4: Nauplius stage of Shrimp larva

Fig 5:Mysis stage of Shrimp larvae

Fig 6: Zoea Stage of Shrimp larvae

Fig 7: Shrimp Brooders- Male & Female

Fig 8: At Shrimp Post Larva packaging unit in Sai Hatchery,Bapatla.

Fig 9: Post larvae ready to be packed

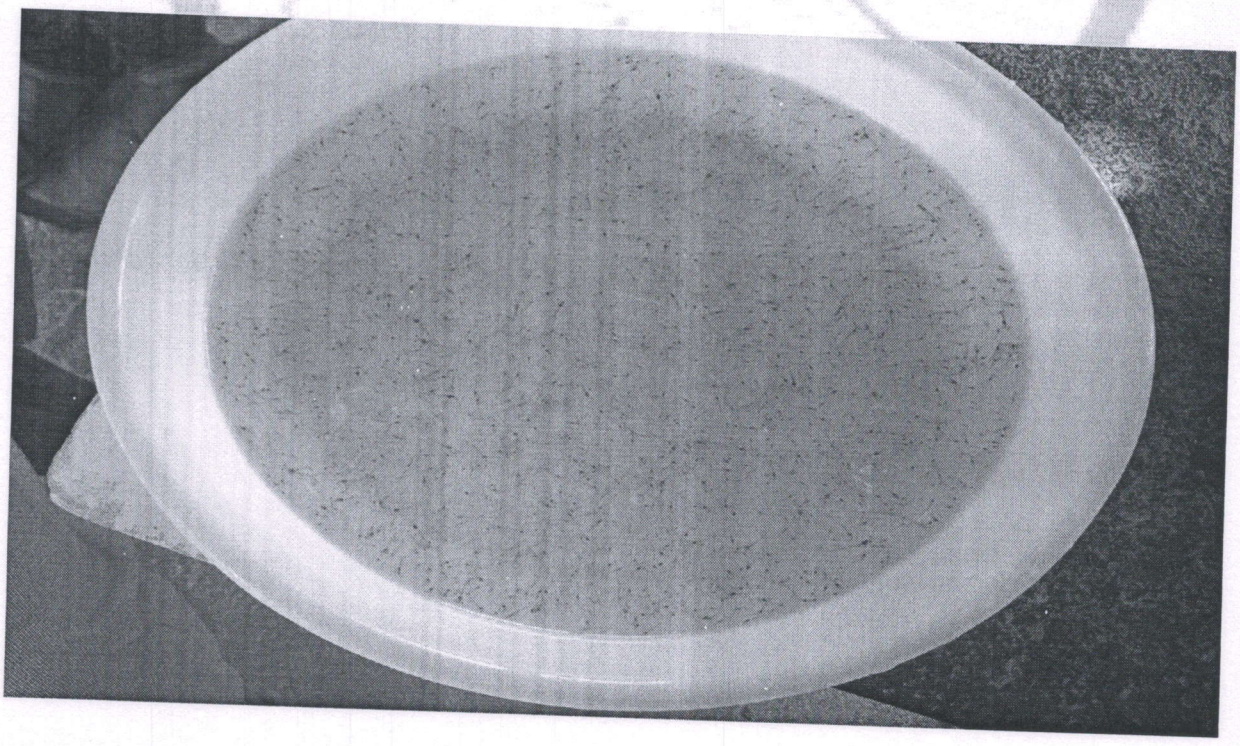


Fig 10: Biosecurity strategy.

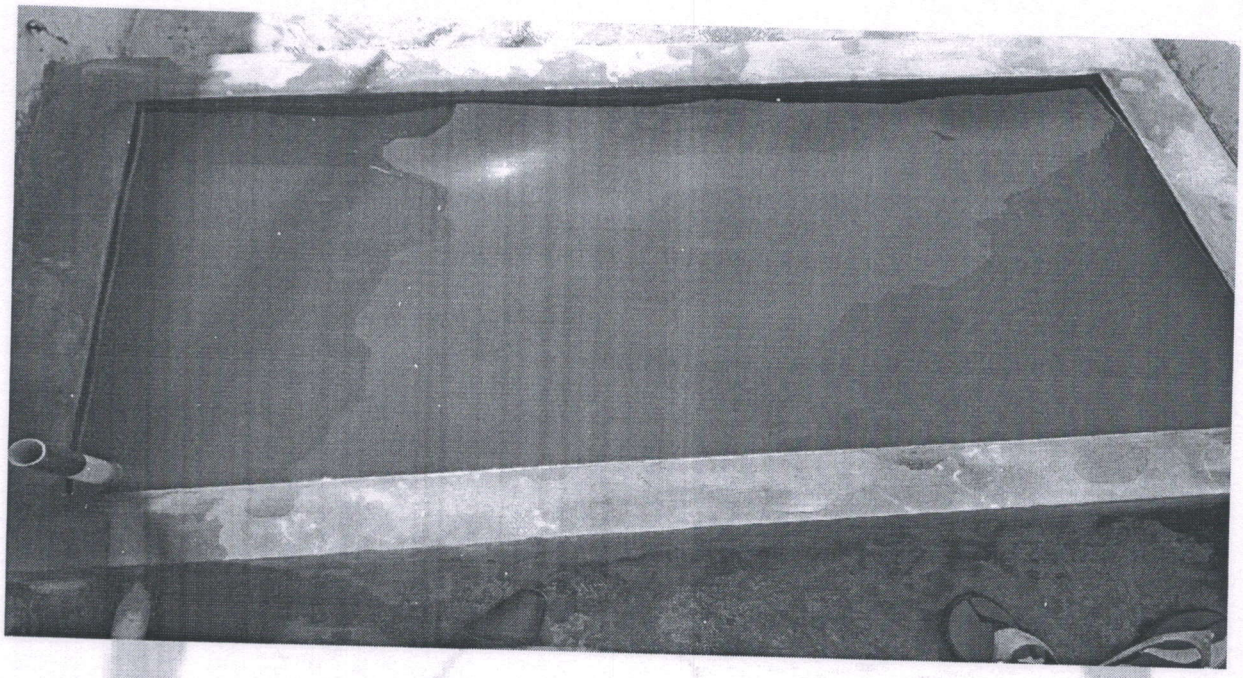
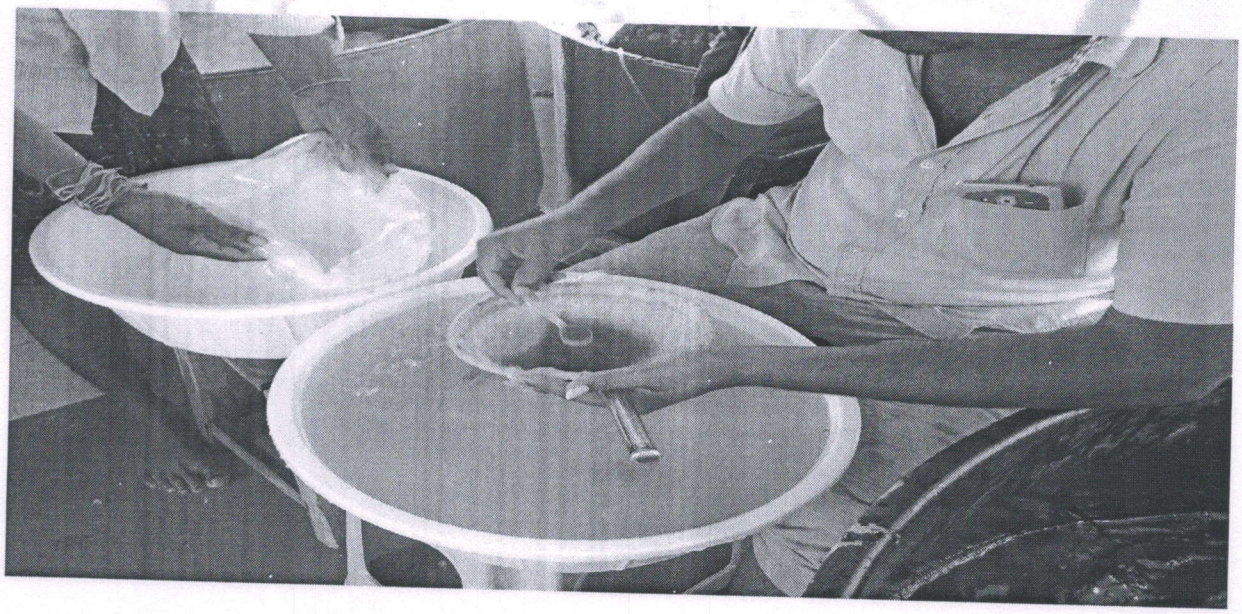


Fig 11: Packing of Post larva



Statistical Treatment:

The statistical tool One-way ANOVA was employed to see if there is any significant difference between the groups and among the groups with respect to the survival % and the probiotics.

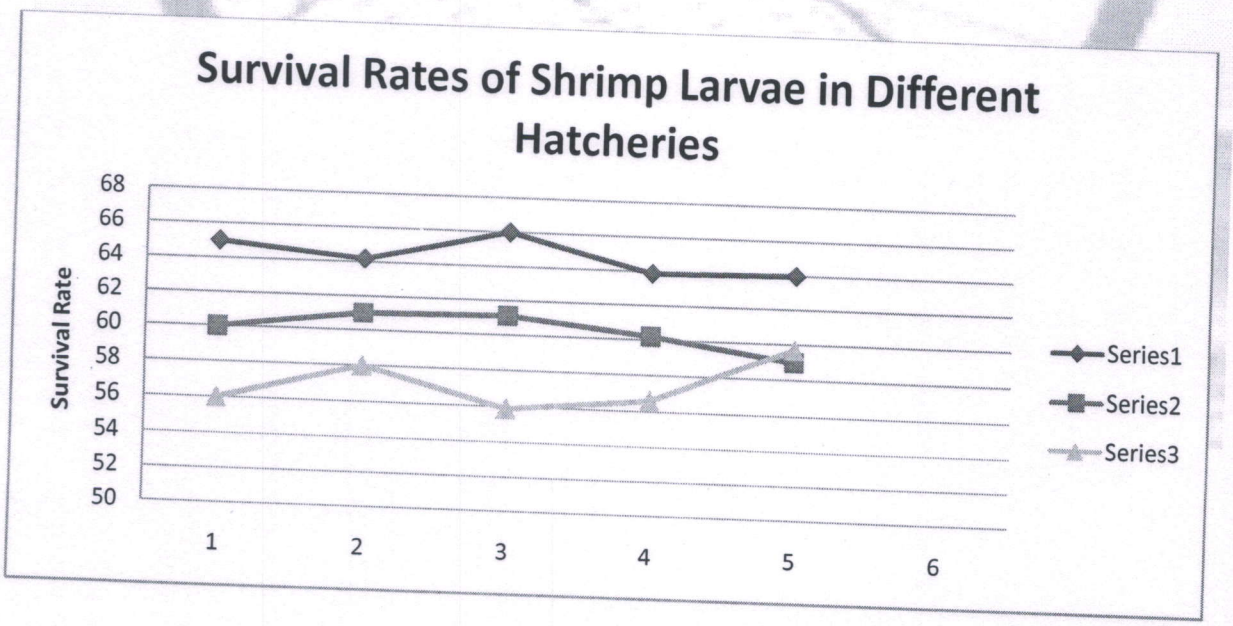
RESULT & DISCUSSION

Table 2:

Production Tanks	Bio-com Plus (Hatchery 1)	Super PS (Hatchery 2)	Biostabil (Hatchery 3)
Tank 1	65.0 ± 0.5	60.0 ± 0.3	56.0 ± 0.1
Tank 2	64.2 ± 0.4	61.0 ± 0.2	58.0 ± 0.3
Tank 3	66.0 ± 0.4	61.1 ± 0.6	55.9 ± 0.4
Tank 4	63.9 ± 0.5	60.2 ± 0.5	56.6 ± 0.2
Tank 5	64.0 ± 0.2	59.0 ± 0.1	59.7 ± 0.2

The survival % of Post-Larvae for the probiotics used in 3 different hatcheries in shrimp larval production

Fig.12:



Statistical Analysis of the Survival Data of the three Hatcheries for three different Commercial Probiotics

Table 3:

	1	2	3	4
N	5	5	5	15
$\sum X$	323.1	301.3	286.2	910.6
MEAN	64.62	60.26	57.24	60.7067
$\sum X^2$	20881.85	18159.25	16392.46	55433.56
Std.Dev.	0.8843	0.8532	1.6103	3.3174

RESULTS

TABLE4 :-

SOURCE	SS	df	MS	
Between-treatments	137.6573	2	68.8287	$F = 50.32562$
Within-treatments	16.412	12	1.3677	
Total	154.0693	14		

Discussion:

This study examined the effects of a commercial probiotic on the digestive survival of the *L.vannamei* larvae at various ontogenetic stages in three separate experiments from Naplius stage to Post-larvae (PL). They were exposed to probiotic added directly to the water. The shrimp hatchery that used Bio com plus has seen the highest survival percentage when compared to the other brands of commercial probiotics used in 1st and 3rd Hatcheries.

In the present study, *Chaetoceros* and *Artemia* nauplii were used as feed for the growing larval stages of *L.vannamei.* Crocos and Coman (1997) concluded that microalgae improve the growth and survival of *Penaeus* sp. Many disease-causing microorganisms may be present in the water. But they cause disease only when larvae become weak due stress and other adverse environmental factors. The probiotic that has high *Bacillus* Count may be the probiotic used in the 2nd hatchery. Hence the probiotics might have improved the water quality parameters and caused healthy larval metamorphosis and molting. This might have resulted in the better survival than the survival registered in other hatcheries.

Shrimp probiotics with higher *Bacillus* counts than did shrimp administered with low probiotic count in the water causes improvement in water quality parameters. Because, improved water quality has been associated with the *Bacillus* sp. The better parameters of water quality are important for the normal metabolism of the rearing animals. If the water quality indicators fluctuate from the optimum values, the culture animals face stress which ultimately leads to opportunistic diseases. This further causes death and also decline in the survival percentage of the shrimp larvae.

Intestinal bacteria thrive in a stable, nutrient rich environment but serve beneficial function to the host including energy salvage of otherwise indigestible complex carbohydrates, vitamin and micronutrient synthesis, activation of immune response, development and competitive exclusion of pathogenic microorganisms (Neish et al., 2009).

Previous studies showed that inoculation with a probiotic strain during cultivation of larval L. vannamei (nauplii stage V) prevented colonization by a pathogenic strain, because the probiotic succeeds in colonizing the gut of the larvae (Zherdmant et al., 1997; Gómez-Gil et al.,

2000). In this study, the effects of the probiotic strains cultured alone or mixed in the larval culture were evaluated. Larvae inoculated with potential probiotic isolates at a density of 1×10^5 CFU mL–1 had significantly better survival than the control.

The highest larval survival, compared to the control (4.9%) was inoculated with isolate YC5-2 (67.3%) and the commercial probiotic Alibio™ (57.4%). The low survival of the control shrimp (5%) in the second trial reinforced the view that probiotics are highly effective for increasing survival of larvae. Srinivas et al. (2010) showed that traditional practices (large exchange of water, application of disinfectants and antimicrobials, or both) are required to successfully complete the larval cycle. The probiotics act as alternatives for disinfectants and antimicrobials, or both. The onset of exogenous feeding by larvae of penaeid shrimp is a critical phase in survival, growth, and development because the larval gut is exposed to microbes at the transition from nauplii 5 to zoea I (Jones et al., 1997). In our study, Bacillus tequilensis (strain YC5-2), B. endophyticus (strains C2-2 and YC3- b), and B. amyloliquefaciens (strain YC2- a) significantly increased development of larvae (Luis-Villaseñor et al., 2011).

Using probiotics, modification of bacterial communities in tank water improves cultivation of larval crustaceans (Balcázar et al., 2007b; Garriques and Arevalo, 1995; Gómez et al., 2008; Guo et al., 2009; Nogami and Maeda, 1992) and bivalves Douillet and Langdon (1993, 1994); Riquelme et al., 1996, 1997, 2001). Our study advances previous work demonstrating that probiotics maintain a balanced and natural bacterial community that improves production of shrimp larvae, which is also reflected in the rate of development, as demonstrated in our two bioassays with Bacillus spp.

Summary & Conclusion:

It is clear that probiotics can support aquatic animals during the vulnerable hatchery phase by controlling pathogens, improving disease resistance and alleviating environmental stressors (e.g. ammonia, low salinity). This results in improved survival, increased production and ultimately higher profitability of hatchery operations.

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