SEAMEO-SEARCA Professorial Chair Lecture

Probiotic Bacteria from Tilapia green water and Bio floc culture systems: *An Eco-friendly Approach in the Prevention and Management of Early Mortality Syndrome (EMS) Disease affecting the Philippine Shrimp Aquaculture*

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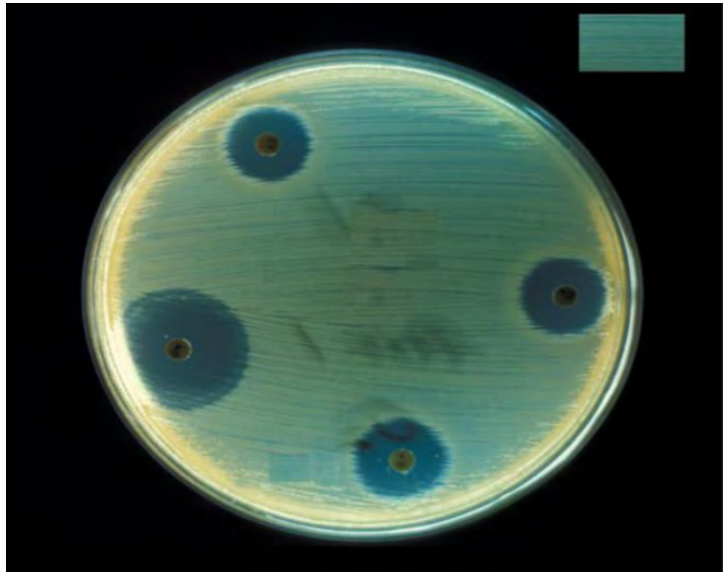
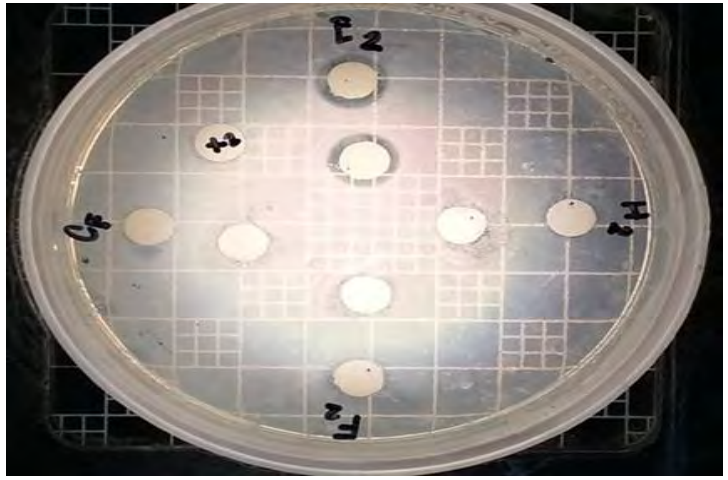
Topics to Discuss/Presentation Coverage

1. Introduction to Probiotics and its application in Aquaculture
2. Mode of actions of Probiotics in Aquaculture
3. Techniques of probiotic screening & selection
4. The Philippine shrimp industry and Early Mortality Syndrome Disease
5. Anti-vibrio actions of Tilapia green water and Biofloc culture systems
6. Development of Probiotics (Actions and Mechanisms) from Tilapia green water and Biofloc systems to inhibit *Vibrio parahaemolyticus*, the pathogenic agent of Early Mortality Syndrome in shrimp aquaculture.
7. Application of the probiotics in actual pond production trials
8. Conclusion and future works
9. Open discussion

Probiotics



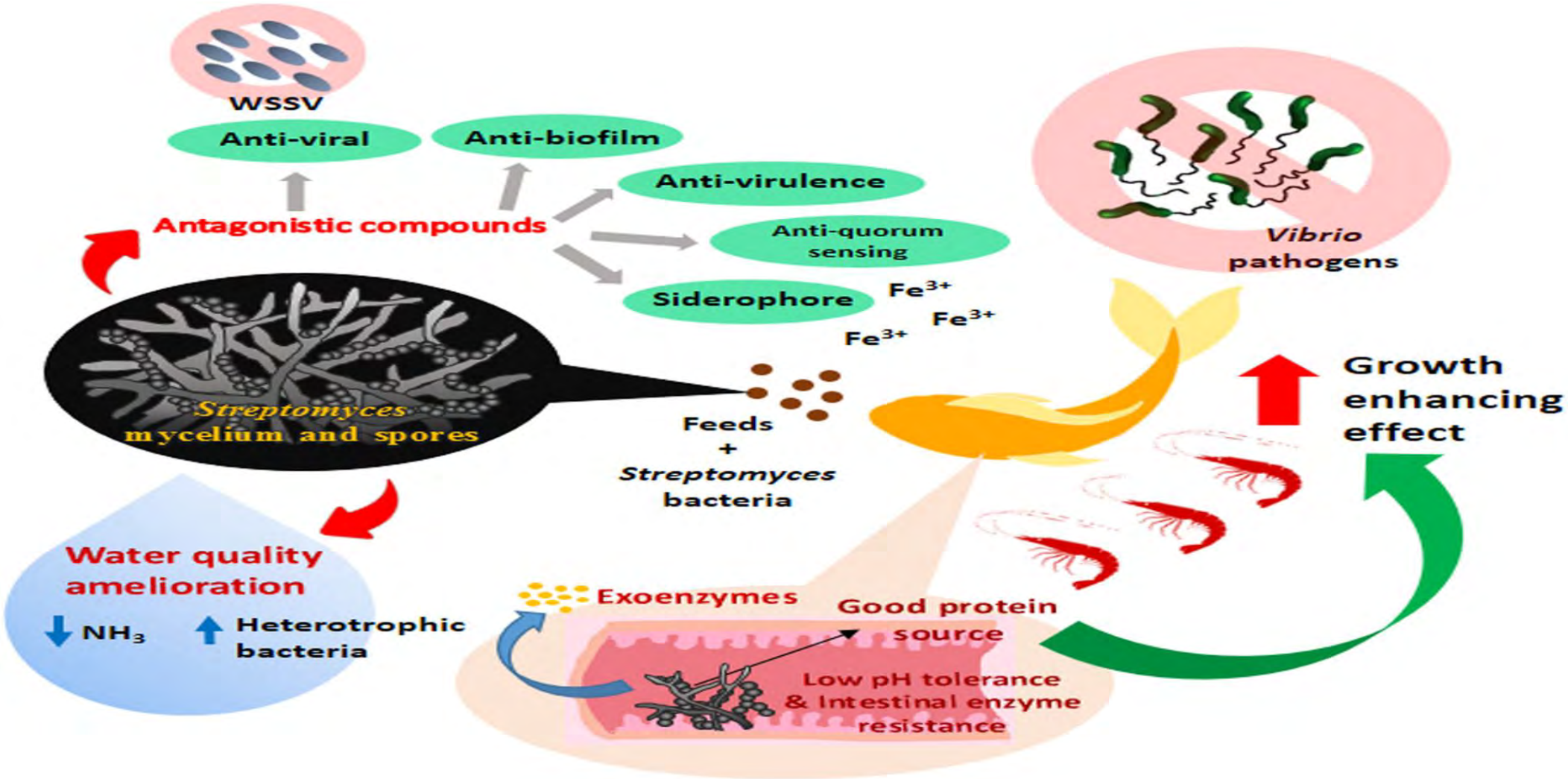


- **Definition** " Greek word, Pro & bios, " for Life"

- *"...a live microbial feed supplement* **which beneficially affects the host animal** *by improving its intestinal balance"* **(Fuller, 1989).**

- *"... components of microbial cells or products from microbes that beneficially affect the health and immune system of the host. (* **Irianto and Austin ,2002)**

Overview of Probiotic Effects on Host



I. Competition

Bacterial antagonism: "beneficial" vs. pathogenic

= **production of antibiotic/antiviral compounds**

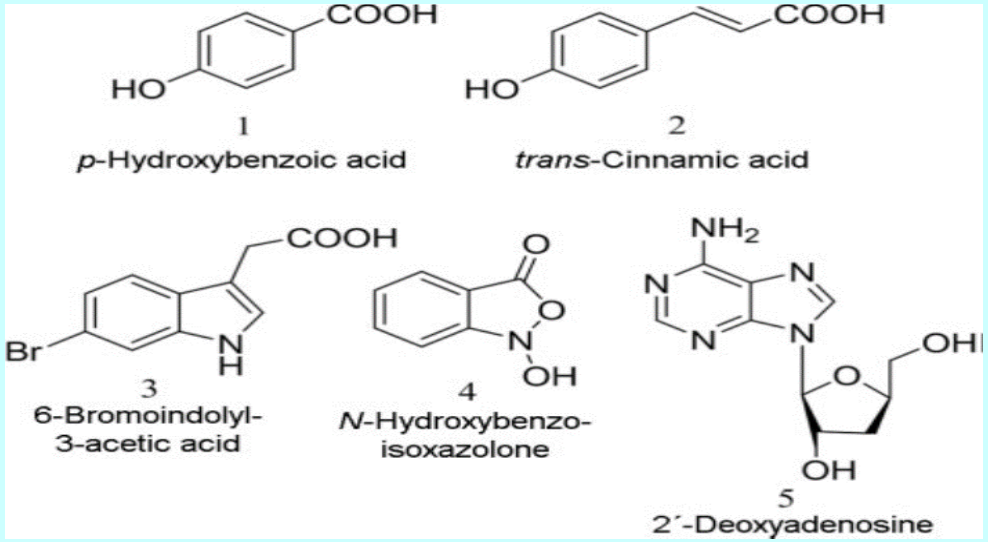


Fig. 3. Structures of the five compounds isolated from ethyl acetate extracts of strain JG1.

= Pseudomonas sp., Vibrios sp., Aeromonas sp, coryneforms isolated from salmonid hatcheries,
= showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) > 50% plaque reduction (Kamei et al., 1988)

I. Competition

Bacterial antagonism: "beneficial" vs. pathogenic

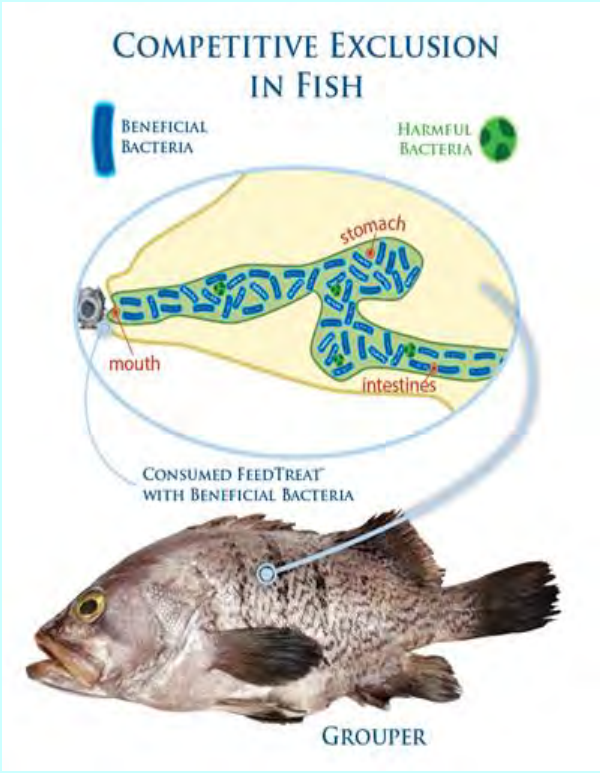
= **fast growth rate (colonization)**

rate of reproduction > rate of expulsion

Thalassobacter utilis vs. *Vibrio anguillarum*
(increased survival of crab larvae; reduced *Vibrio* sp. in rearing water

Bacterial strains from intestine and skin mucus of adult marine turbot and Bacillus sp. vs. *V.anguillarum*

***V. alginolyticus alginolyticus* for *L.vannamei* improves survival and growth against *V. harveyi* (Ecuador).**



I. Competition

Bacterial antagonism: “beneficial” vs. pathogenic

= **Nutrient utilization Competition** ***(siderophore, quorum sensing inhibition, AHL)***

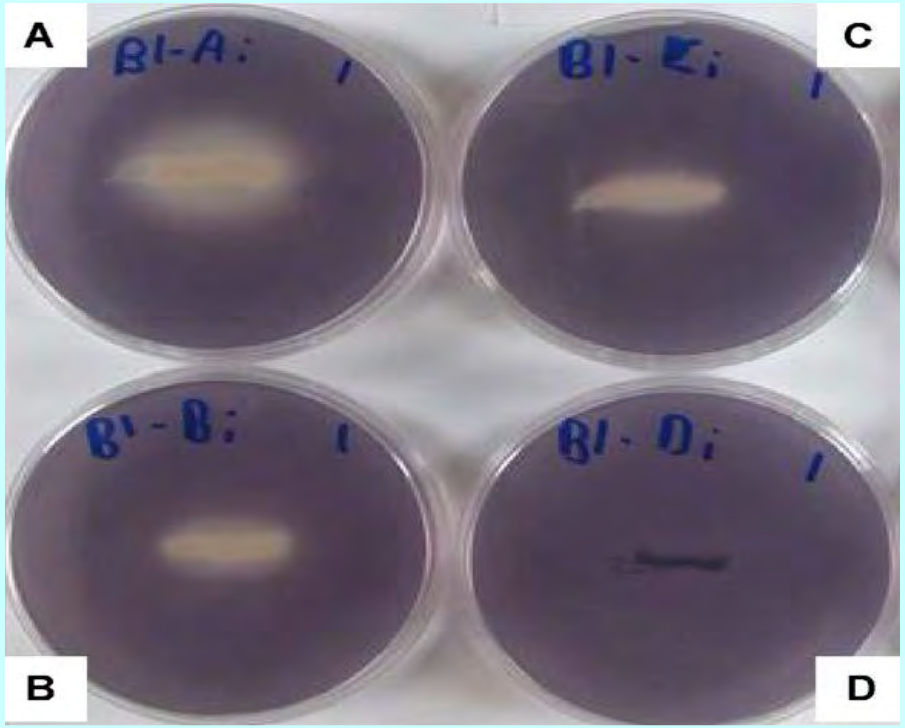


Fig. 2. P. aeruginosa AHL production is mainly responsible for pigmentation inhibition of C. violaceum ATCC 12472. P. aeruginosa PAO-1 (A) producing both 3-oxo-C12 HSL and C4-HSL gives the largest zone of pigmentation inhibition. Limited pigmentation inhibition is seen with P. aeruginosa strains PDO-100 (rhlI) (B) and PAO-MW1 (C) (rhlI, lasI). No pigmentation inhibition is observed when C. violaceum ATCC 12472 is present (D) (negative control).

II. Nutrient Source and Digestion aid

- **Source of Nutrients (*bacterial fermentation, prebiotics*)**
  = ***Fatty acids, protein, carbohydrate and vitamins***
- **Act as digestion aid (secretes enzymes for digestion)**

- Ex. ***Bacteroides, Clostridium*** **sp. (fatty acids, vitamins); (Sakata 1990)**

Penaeus chinensis**, complement of enzymes for digestion , synthesize compounds assimilated by animal (Wang et al., 2000)**

digestion processes of bivalves, extra-cellular enzymes (proteases, lipases, other necessary growth factors); (Prieur et al1990)

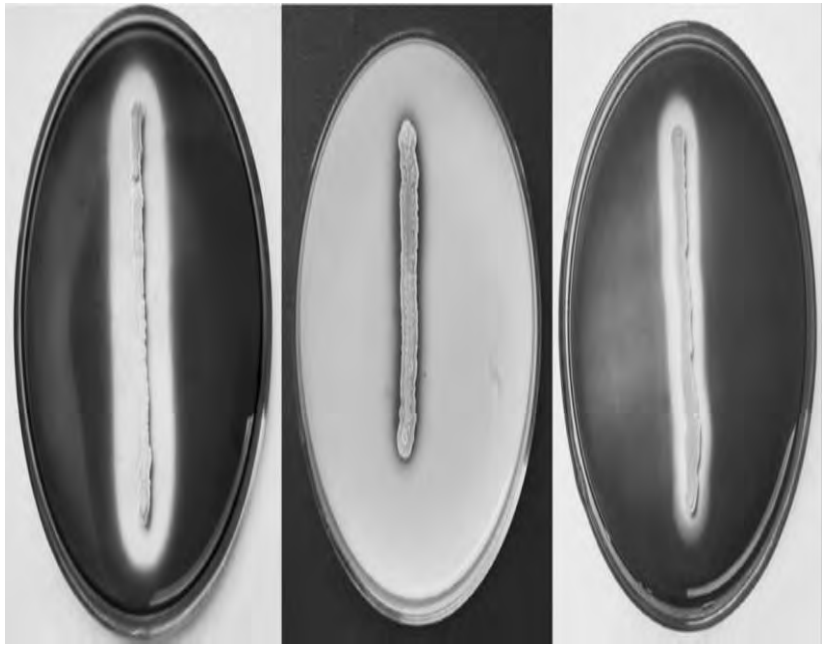


Fig. 10. Degradation of starch (left), casein (middle), cellulose (right) of *Streptomyces* sp. A1.

III. Improves Water Quality

= Aids in Nitrification process (Mineralization)

= Digests Organic Matter into Carbon Dioxide

= Converts host metabolites into bacterial biomass

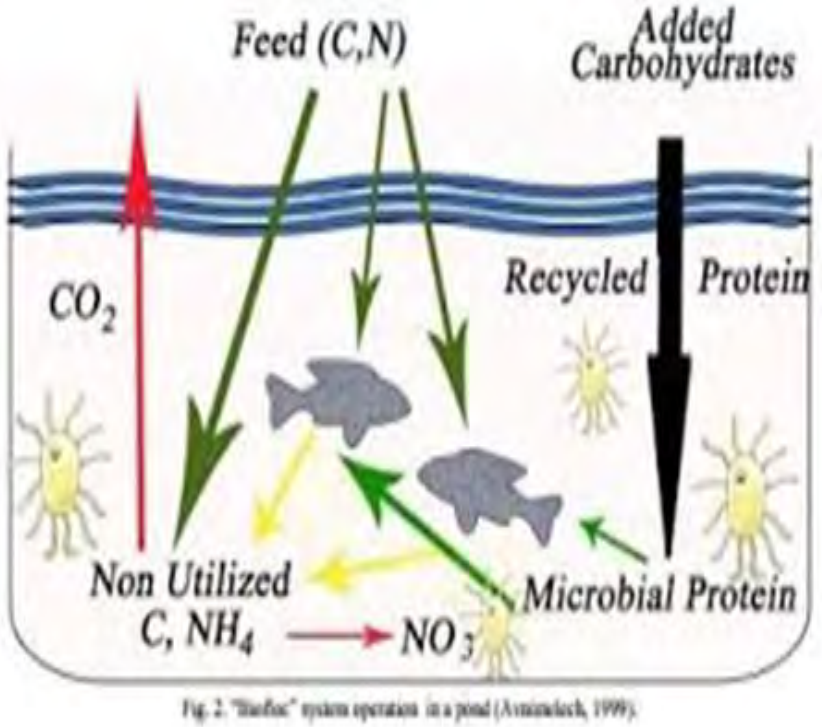


Fig. 2. "Biofloc" system operation in a pond (Avnimelech, 1999).

Examples

= Gram$^+$ bacteria: better converters of OM to CO_2 vs. Gram ^{negative} (Balcazar, 2006)

= *Bacillus* sp. for water quality, survival and growth rates, increased health status (*P.monodon)*, reduced *Vibrio* counts; Dalmin et al., 2001

IV. Act as an Immunostimulant

= Activates gastric tract immune cells

Examples

= *Clostridium butyricum* to rainbow trout, increased resistance to Vibriosis (phagocytic activity of leucocytes)

=*Bacillus* sp. S11 to *P.monodon,* disease protection (activated cellular and humoral immune defenses)

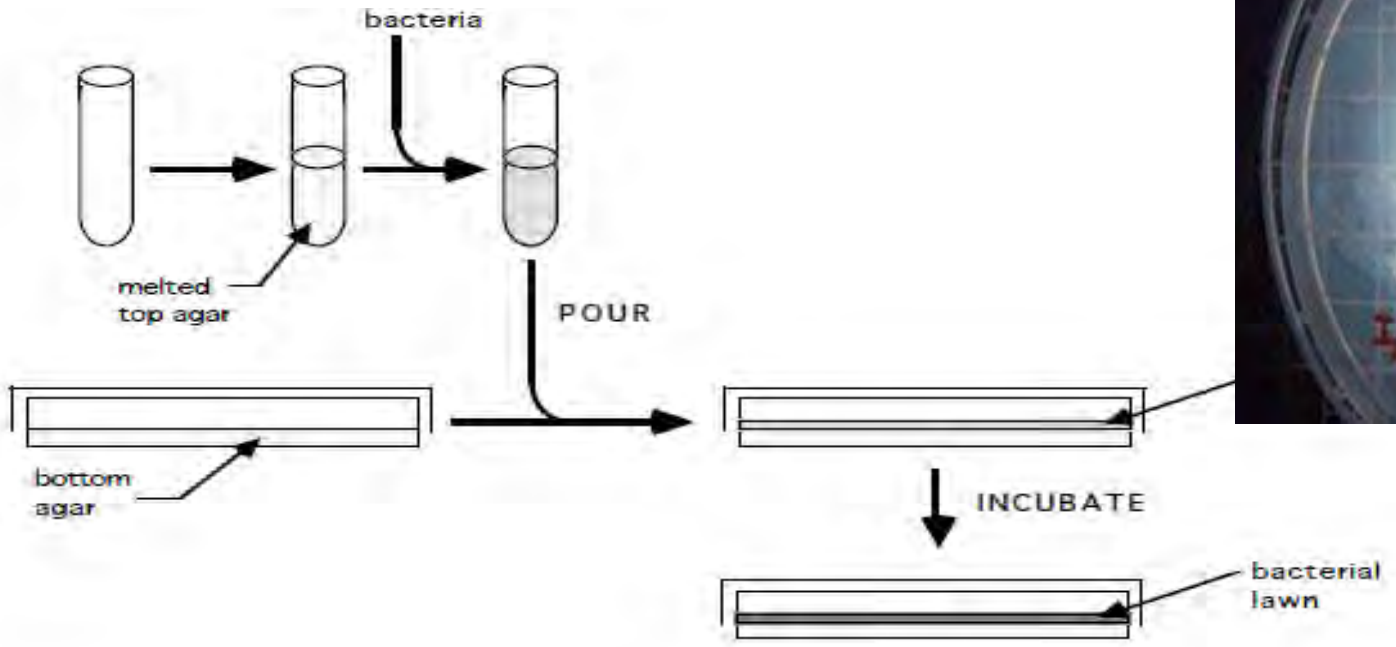
=*Bacillus* + *Vibrios* to *L.vannamei* juveniles, growth and survival, protection for *V.harveyi* and WSSV (stimulation of immune system, increased phagocytosis and antibacterial activity); Balcazar, 2003

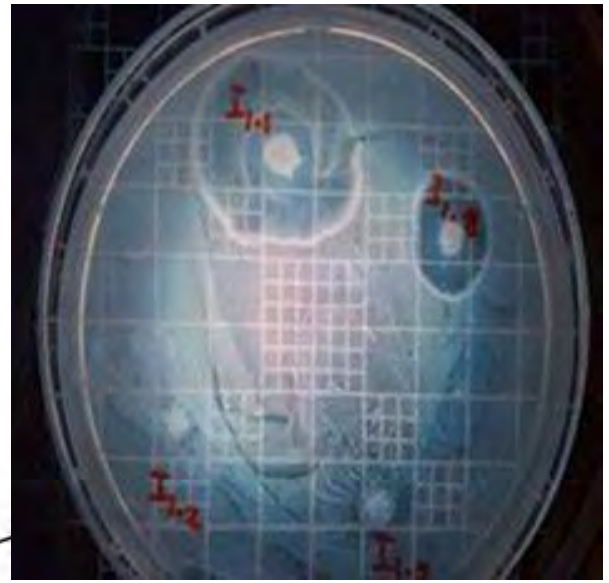
= LAB *Lactobacillus rhamnosus* (ATCC 53103) at ~10^5 cfu/g feed to rainbow trout, stimulated respiratory burst; Nikoskelainen et al., 2003

Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

= Double layer method / Overlay Method



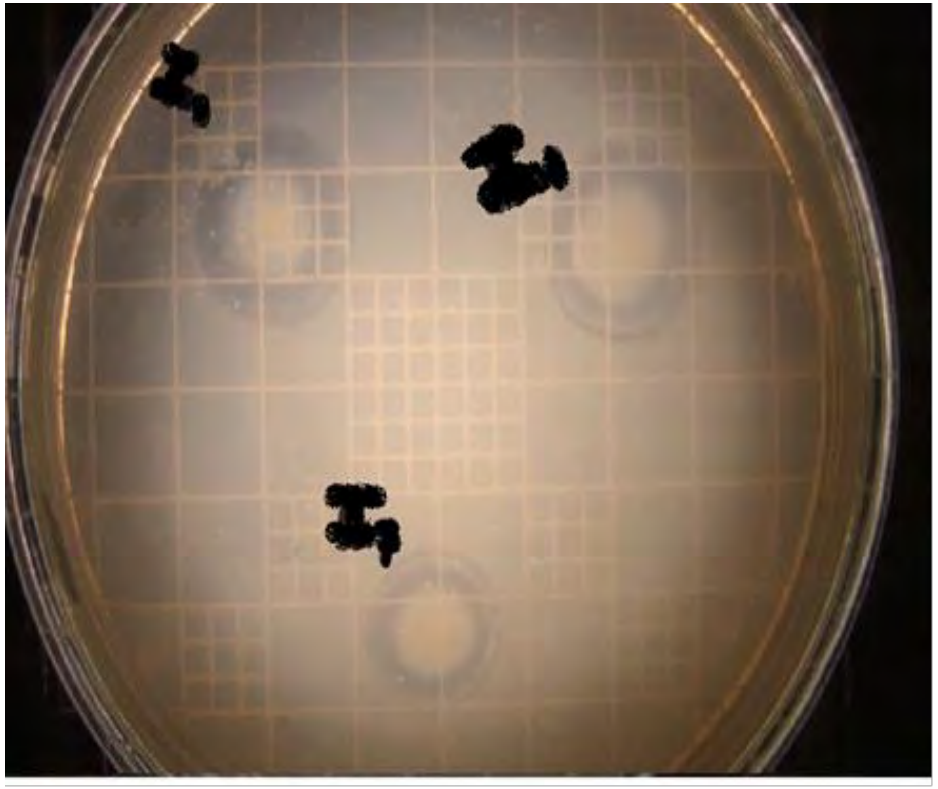


"*Discuss the limitation and Problems*"

Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

= Double layer method / Overlay Method (Example)

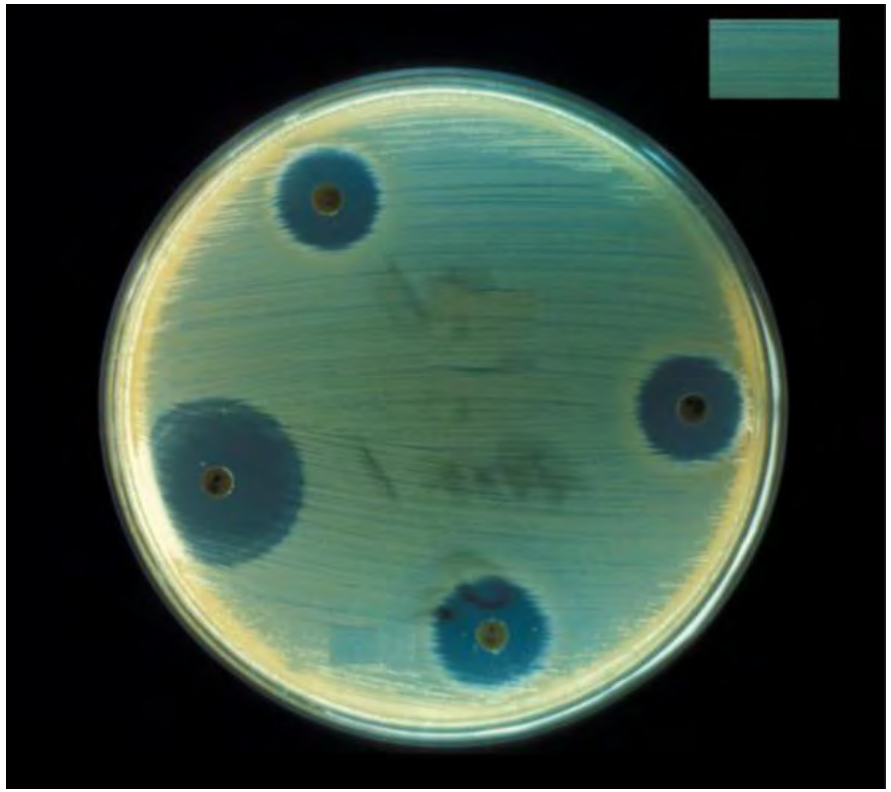


Anti-*Vibrio harveyi* (PN-9801) activity of Isolate I1 (*Streptococcus porcinus*) isolated from marine nematode gut (pond).

Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

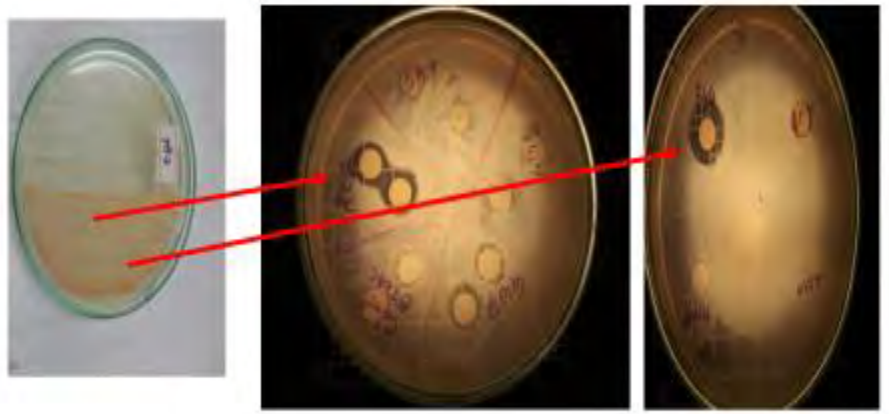
= Well Diffusion Method

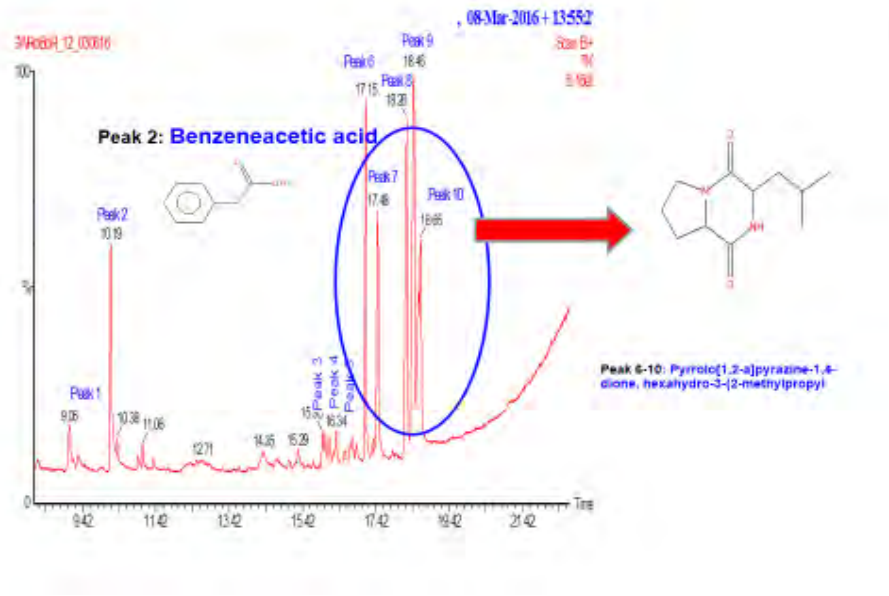


Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

= Disc Diffusion Method

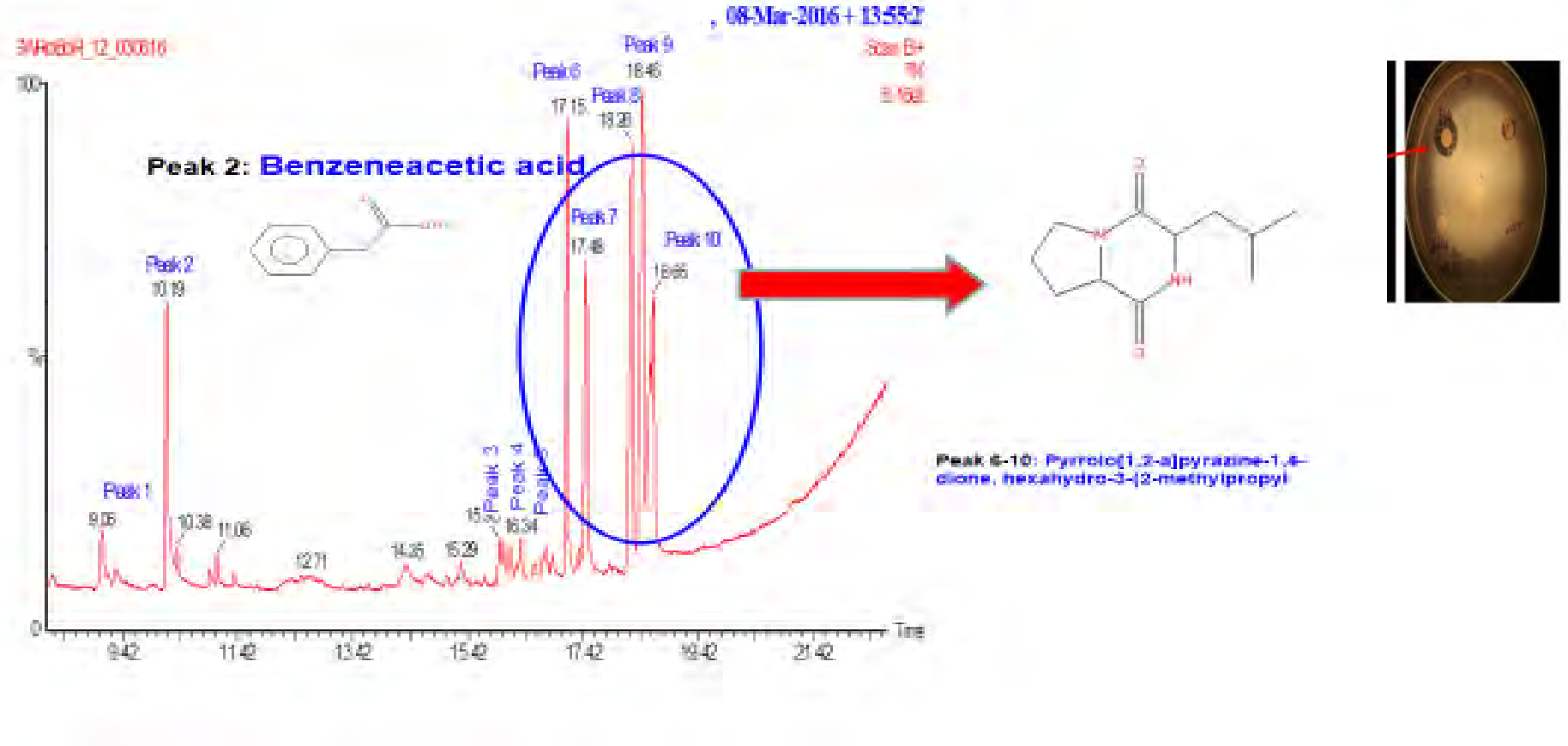




1. Organic solvent extraction
2. Thin layer chromatography
3. Mass spectrophotometry

Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*). *Halobacillus sp*. UPV hatchery canal solate isolate



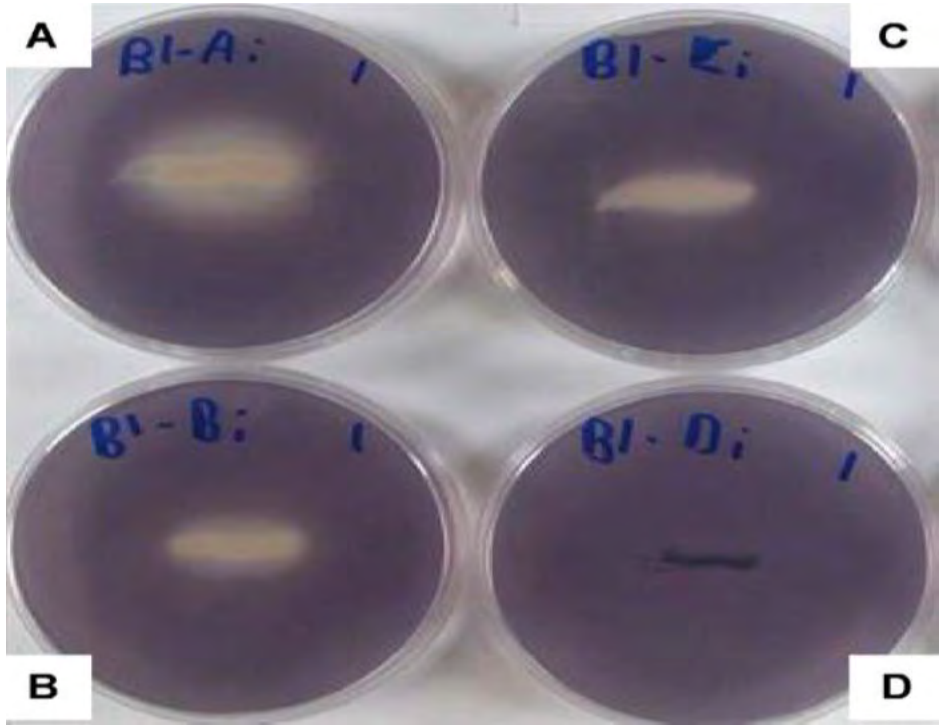
Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

Tester Bacterial Strain
Chromobacterium violaceum (Violacein)

= Quorum Sensing Inhibition

1. Organic solvent extraction or
2. Filtered culture supernatant

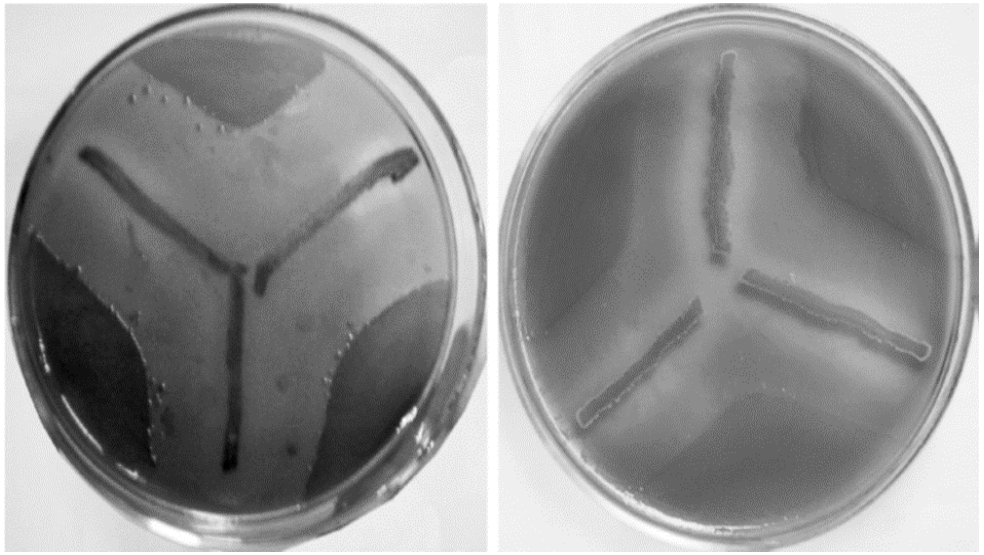


Methods of Screening & Isolation

1. Secretion of Inhibitory/antibacterial compounds (*in vitro*)

= Cross Streaking method

Streptomycetes against V, harveyi



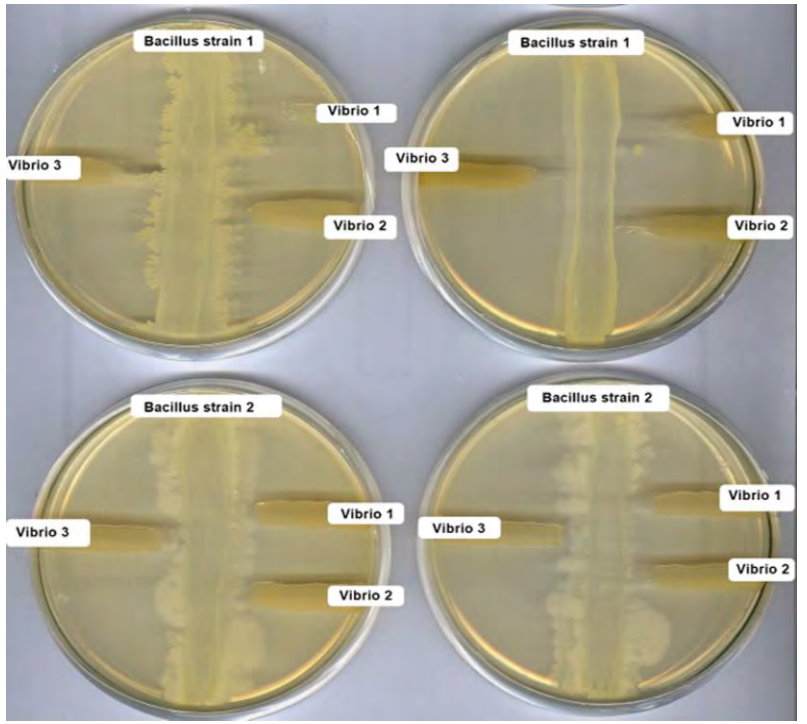


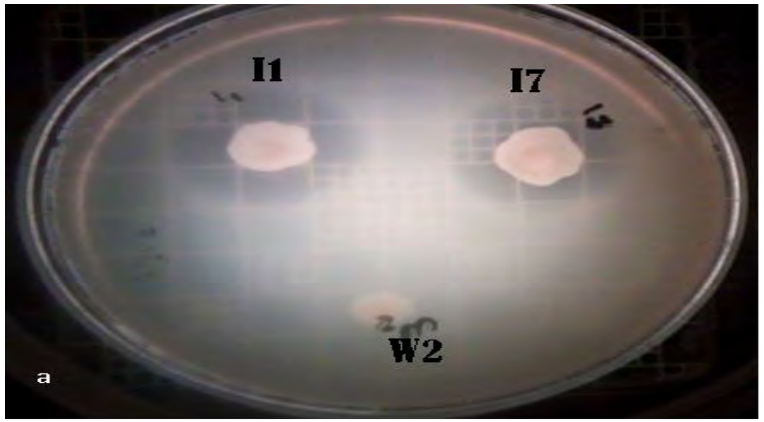
Figure 1. Evaluation of the inhibitory activity of two di!erent Bacillus strains against three pathogenicVibrio strains, by the cross-streak method. De camp et al, 2008.

Methods of Screening & Isolation

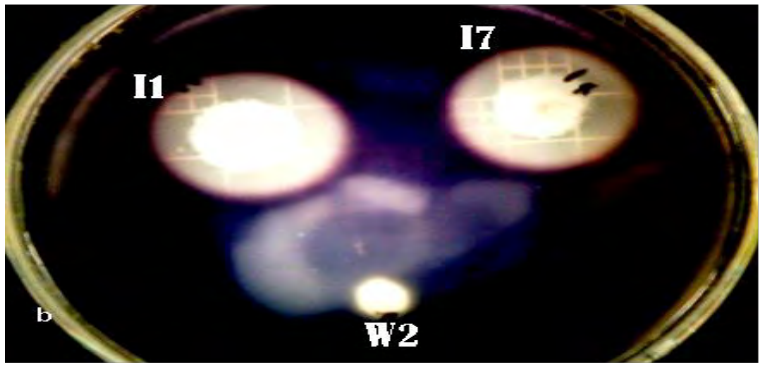
1. Secretion of Enzymes to Digest Nutrients (*in vitro*)

= Solid Plate Enzyme Assay method

Protease Activity in LB-skim milk agar



Amylase activity in LB-soluble starch.



Introduction

- Shrimp aquaculture Important source of revenue.
- Philippine Shrimp a Billion Peso Industry
- A source of Employment and a significant contributor to the Economy
- Disease is a major threat to the industry.

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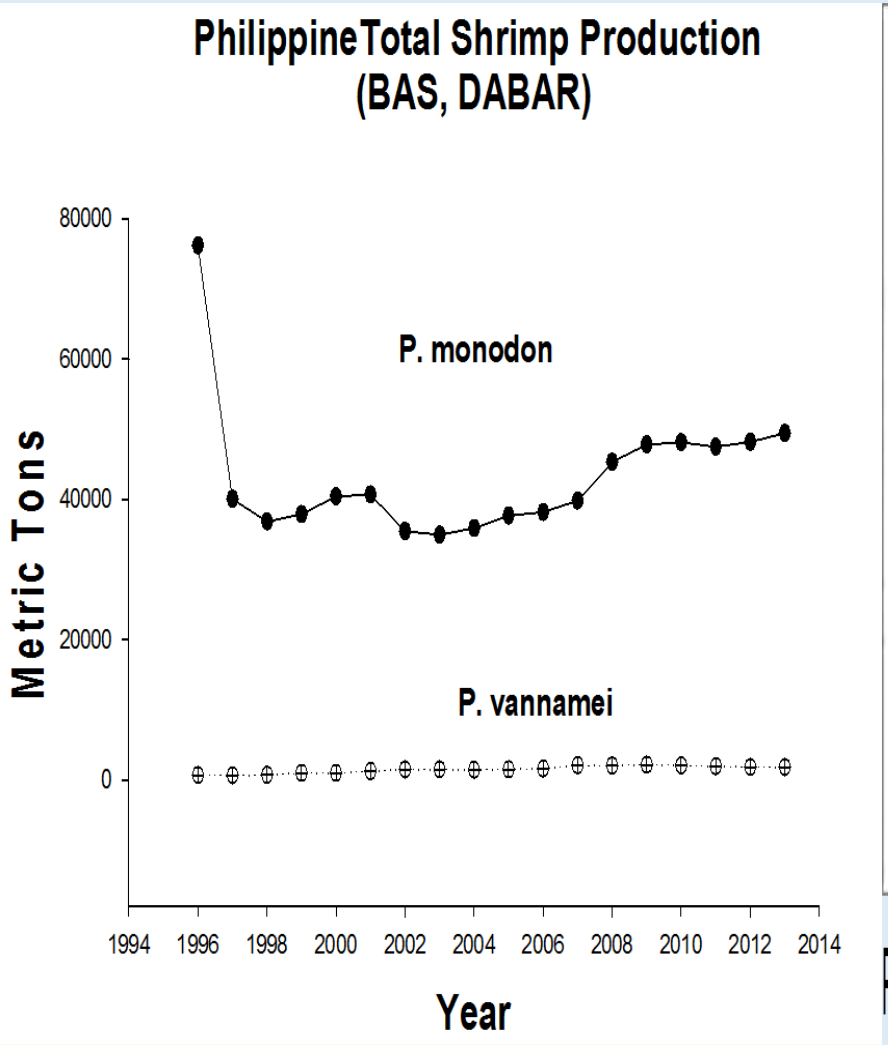
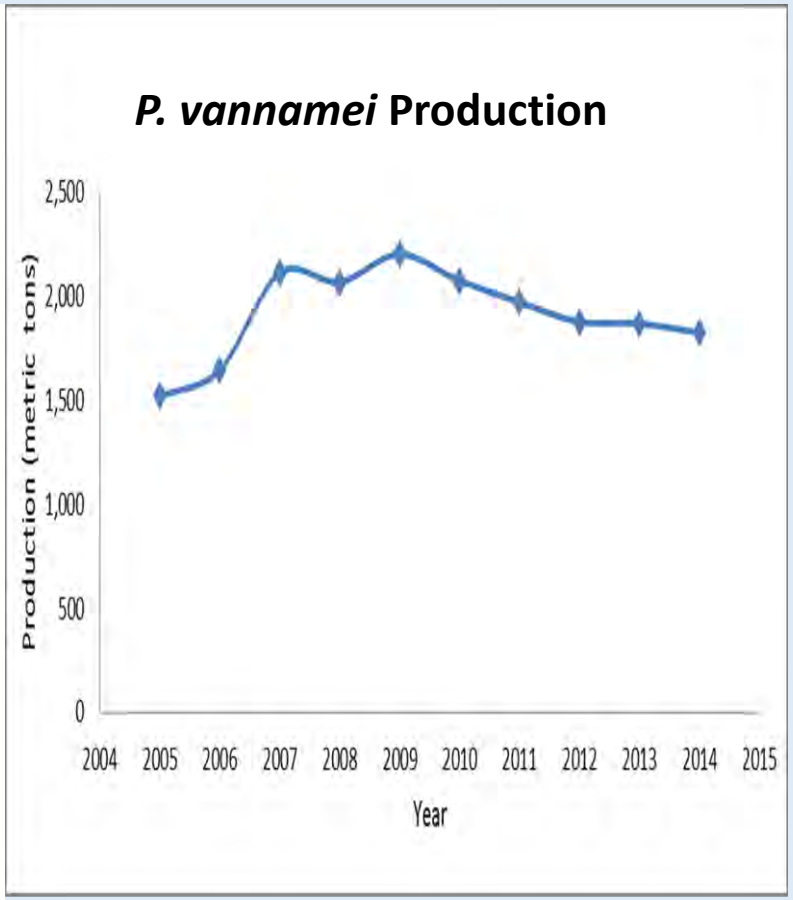
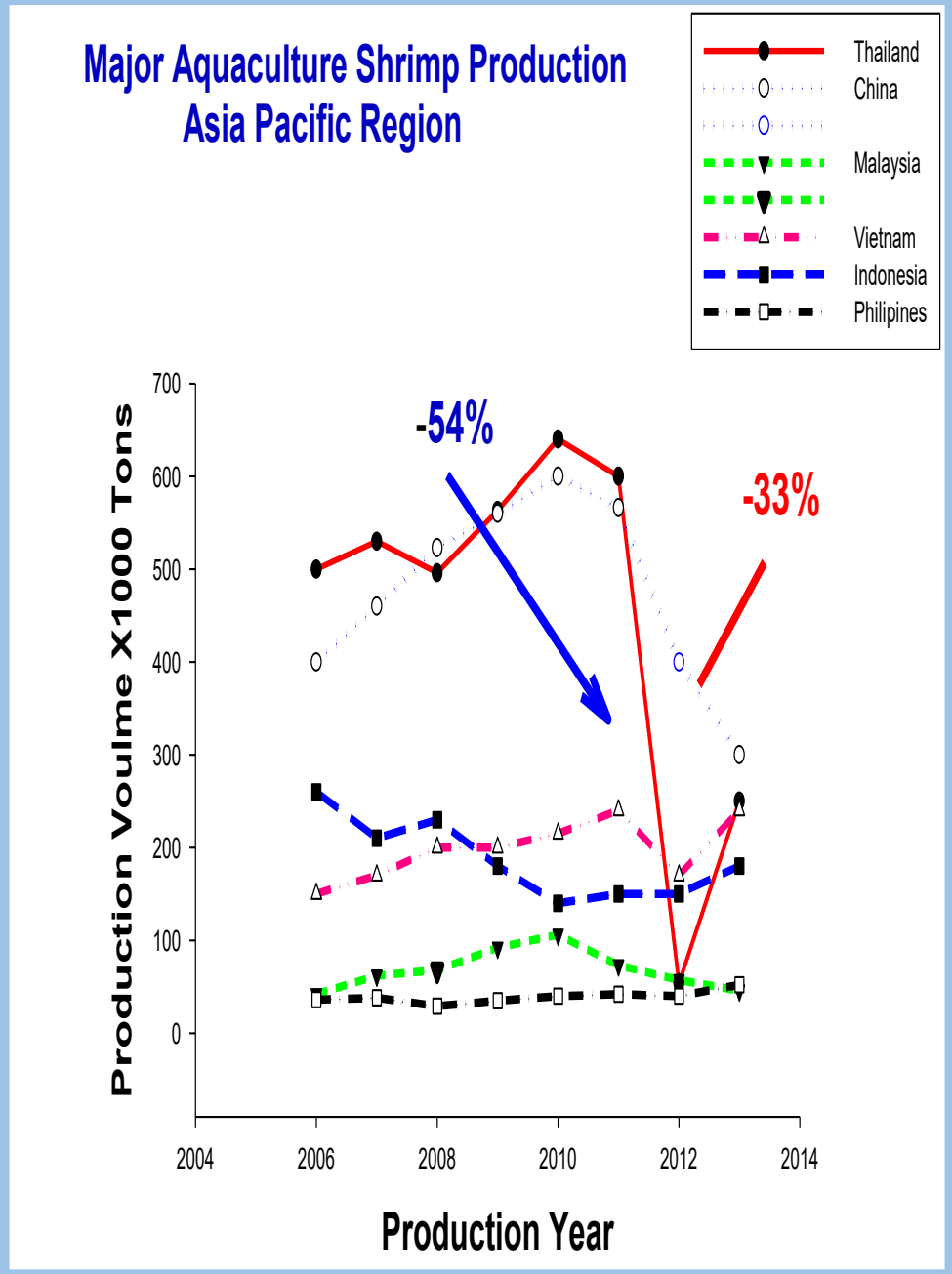
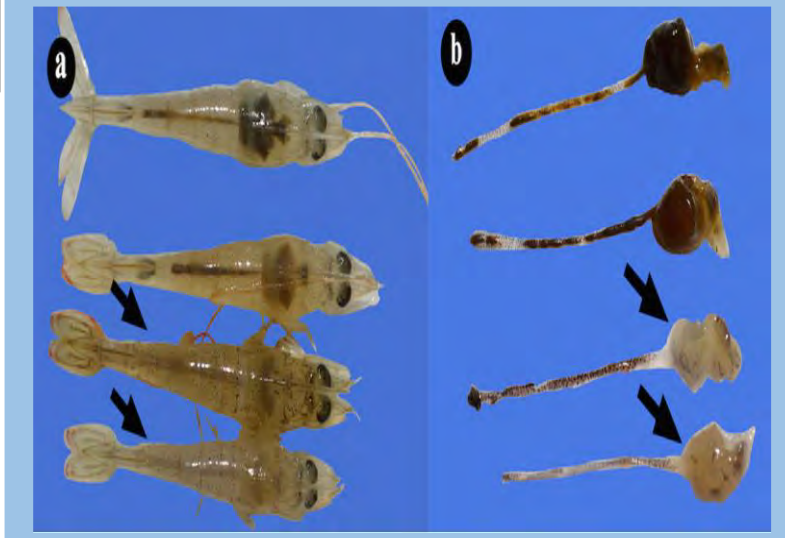




Philippine Aquaculture Production of *P. vannamei* (2004-2014) (BAS, 2015)

1. Disease is a constant threat to the industry.
2. Currently a new disease is threatening the shrimp industry in Asia-pacific region,
3. Caused by a strain of Vibrio parahaemolyticus with a toxin producing plasmid.
4. Known as Early Mortality Syndrome(EMS)or Acute Hepatopancreatic Necrosis Disease (AHPND).
5. Highly pathogenic and caused sever production losses in the region.

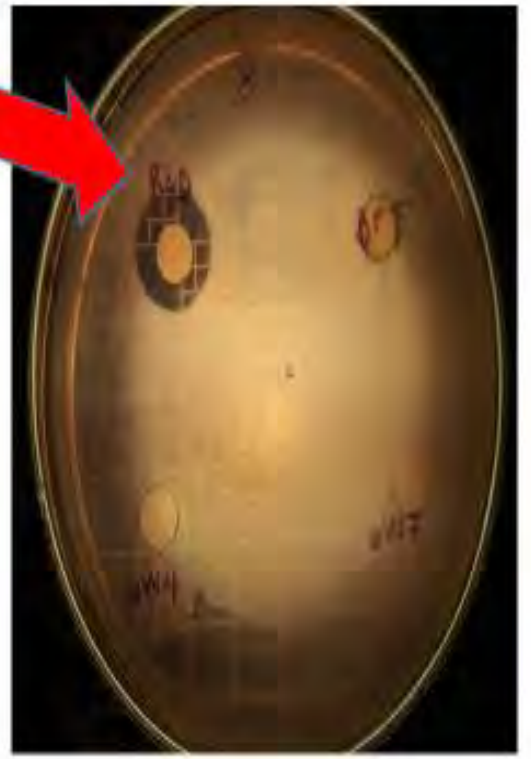




Tilapia Green water, a Solution to EMS Problem

1. At present No probiotics has concrete solution has been identified for the control of EMS/APHND *V. parahaemolyticus* in shrimp culture
2. Problems caused by bacteria, can also be controlled by bacteria.
3. Bacterial diversity in culture could be manipulated by the application of Tilapia green water.
4. Bacteria associated with TGW may eliminate *parahaemolyticus* by exclusion, competition and direct killing.

1.* *Vibrio parahaemolyticus* Inhibitory Activity of Tilapia green water

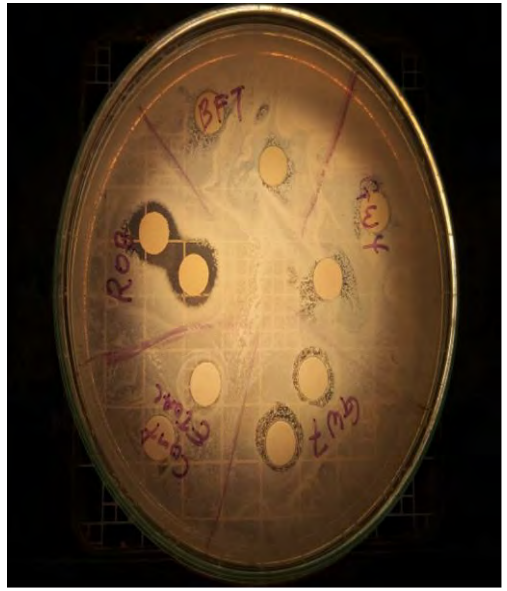


Green WATER				
		REPLICATES		
Solvent	concentration (µg/mL)	1	2	3
	0	0	0	0
n-hexane	1000	I	0	I
	2500	I	0	I
chloroform	0	0	0	
	1000	I	0	0
	2500	I	I	0
ethyl acetate	0	I	I	I
	1000	I	I	I
	2500	I	I	I
	0	0	0	0
Butanol	1000	0	0	0
	2500	0	0	0

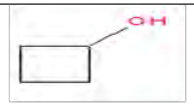
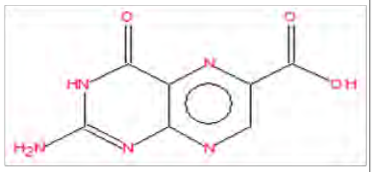
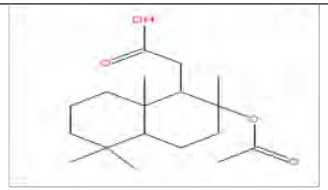
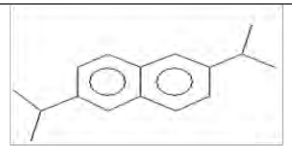
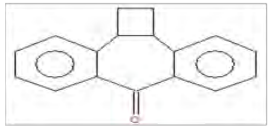
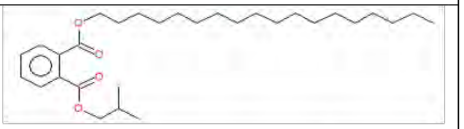
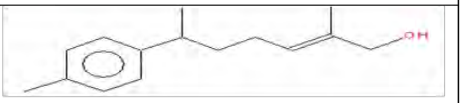
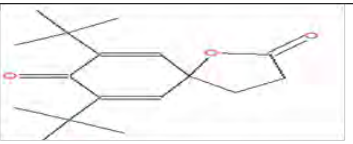
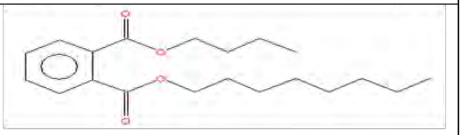
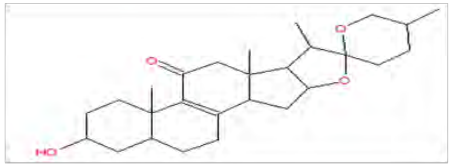
0 NO INHIBITION

I WITH INHIBITION

EDIA USING LB



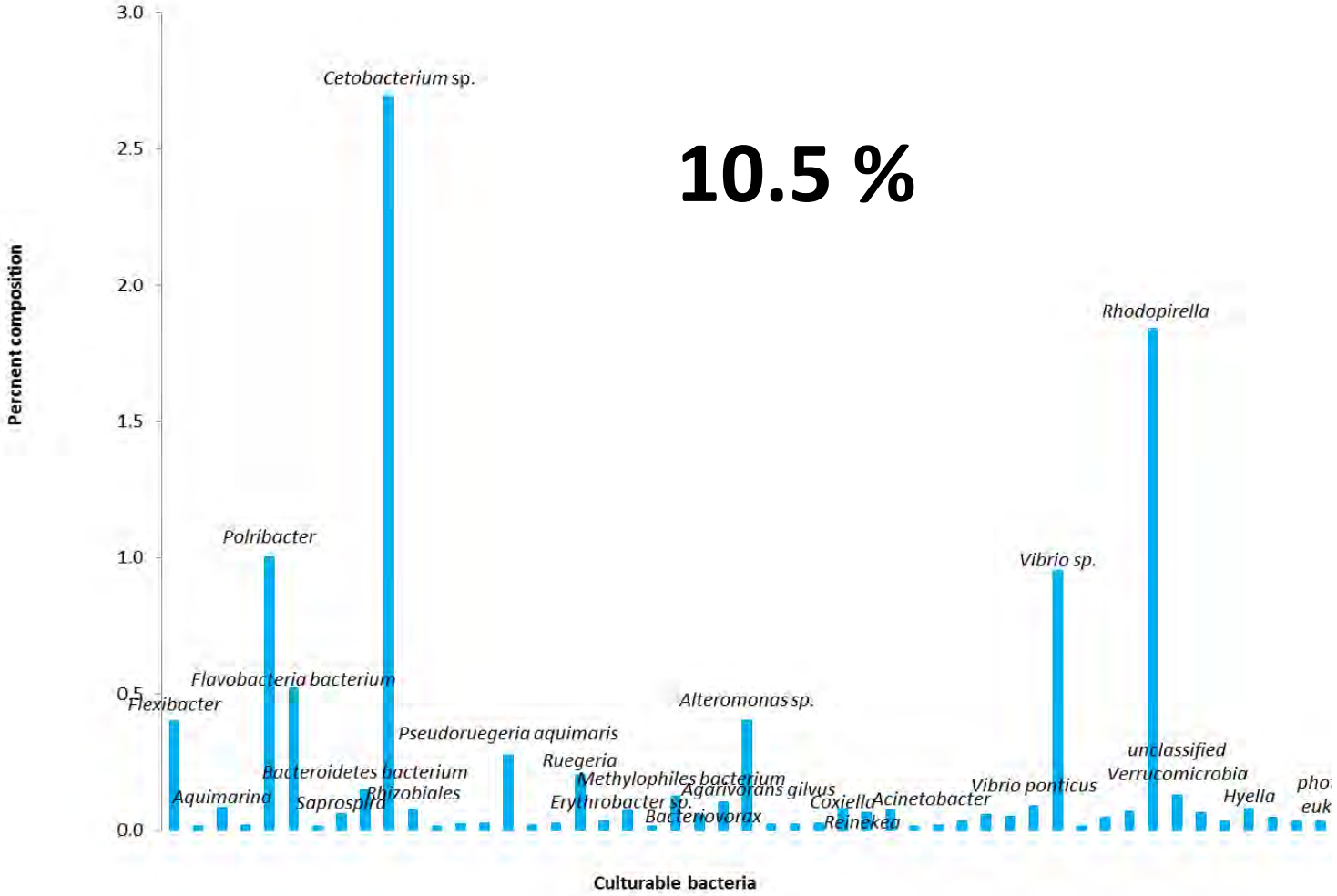
11 Antibacterial compounds elucidated

Peak Number	Compound Name	Molecular Mass & Formula	Structure
1	Nonanal	$C_9H_{18}O$ 142	
2	Cyclobutanol	C_4H_8O 72	
3	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$ 207	
4	Acetic acid, 2-(2-acetoxy-2,5,5,8a-tetramethyldecalin-1-yl)-	$C_{18}H_{30}O_4$ 310	
5	2,6-Diisopropylnaphthalene	$C_{16}H_{20}$ 212	
6	Cyclobuta[a]dibenzo[c,f]cycloheptadiene, 7-oxo-	$C_{17}H_{14}O$ 234	
7	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_4$ 474	
8	6-(p-Tolyl)-2-methyl-2-heptenol	$C_{15}H_{22}O$ 218	
9	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$ 276	
10	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$ 334	
11	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-	$C_{27}H_{40}O_4$ 428	

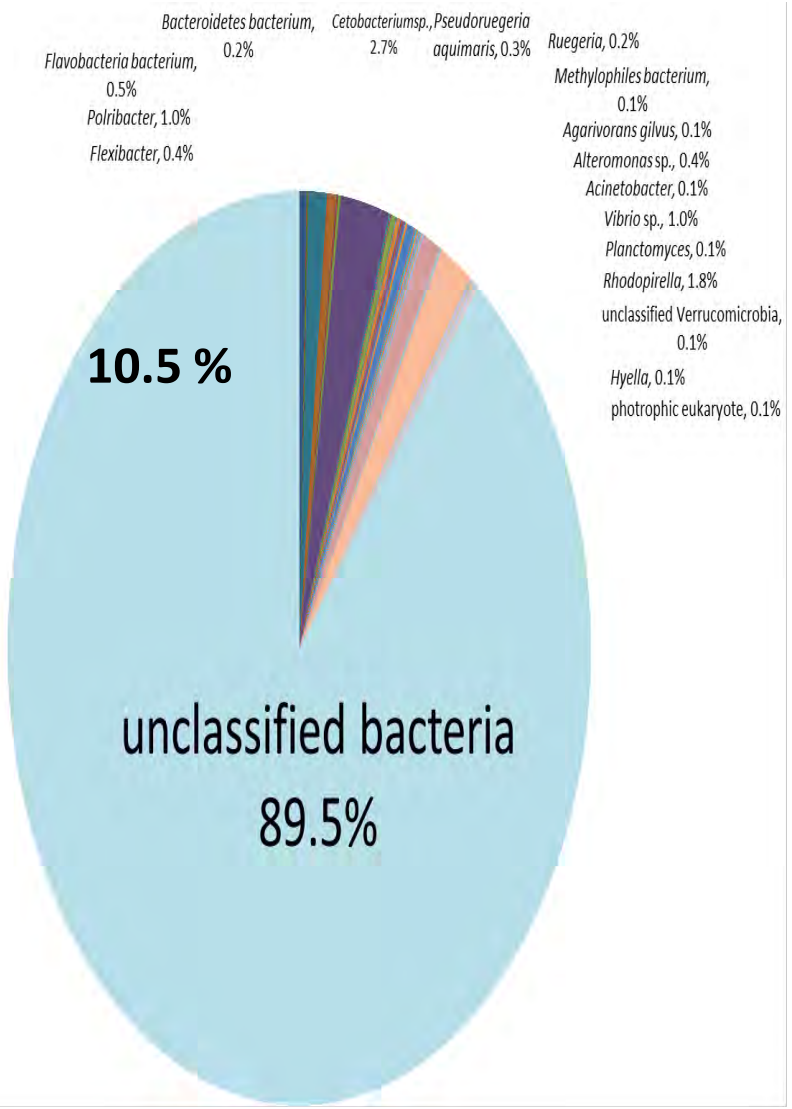
Tilapia Density Inhibitory to Vibrio parahaemolyticus

TREATMENT	
Culture period	60 days
1	Control
2	175g Tilapia
3	350g Tilapia
4	700g Tilapia
Shrimp	50 pcs/tank
Tanks	3X (CRD)

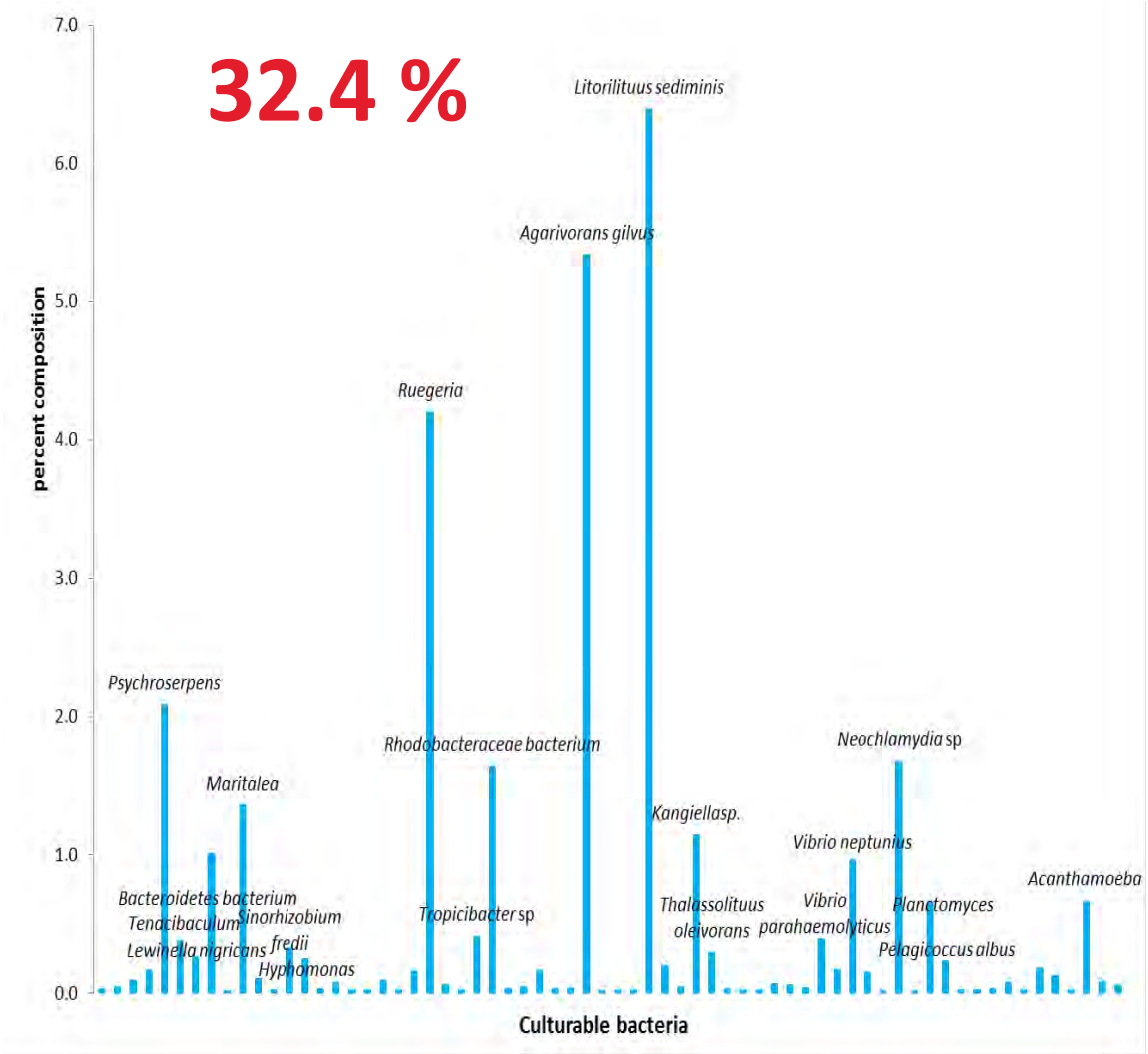
Bacterial Species Profile of Tilapia Green water (10^3 cells.ml^{-1}) Analyzed By DNA-Seq (16S DNA)

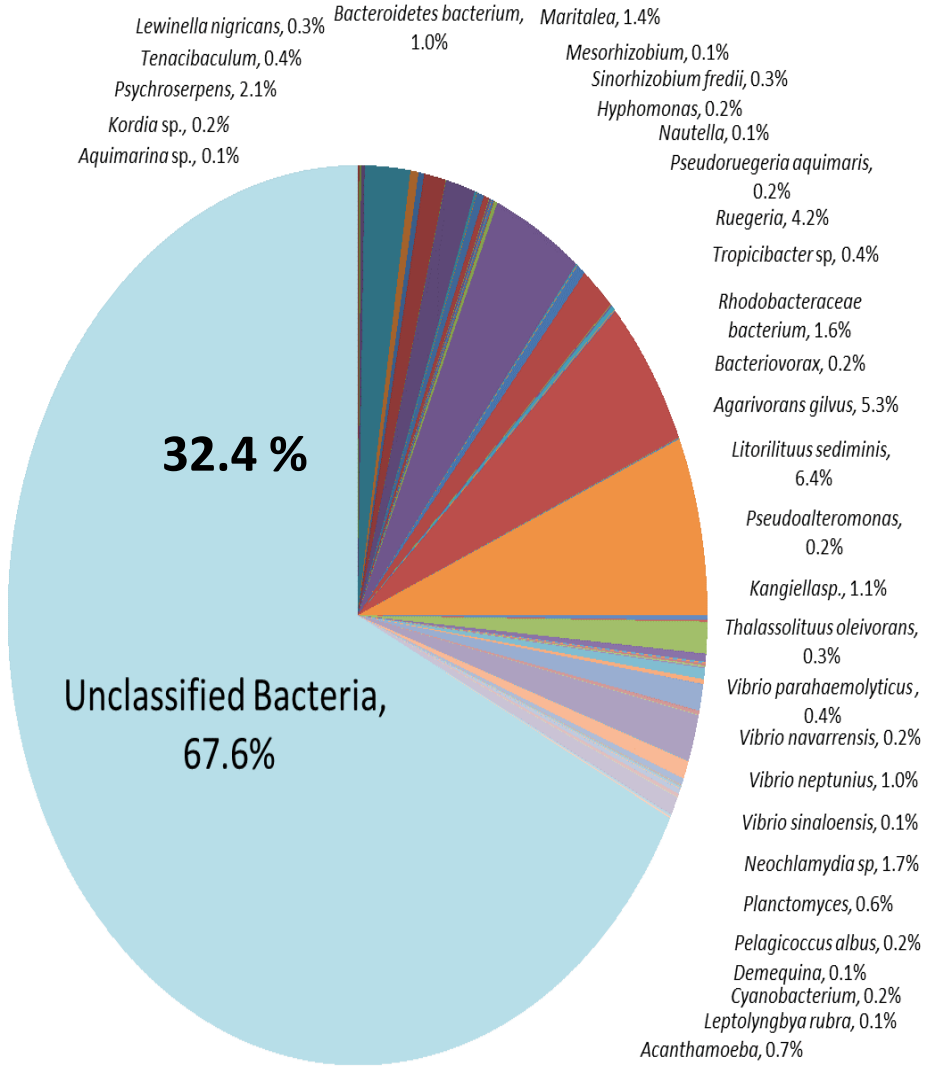


10.5 %



Bacterial Species Profile of Mature Biofloc (Stage5) Analyzed By DNA-Seq (16S DNA)



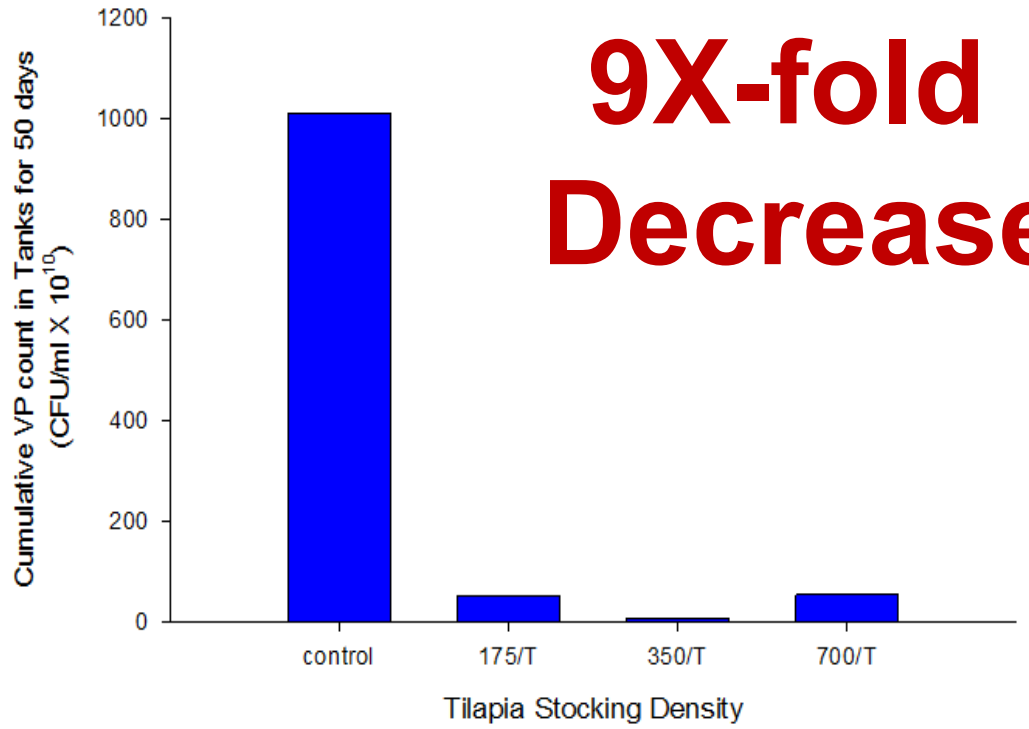


Tilapia Density Inhibitory to Vibrio parahaemolyticus

Growth performance of *Penaeus vannamei* in in tilapia green water culture with different tilapia densities.

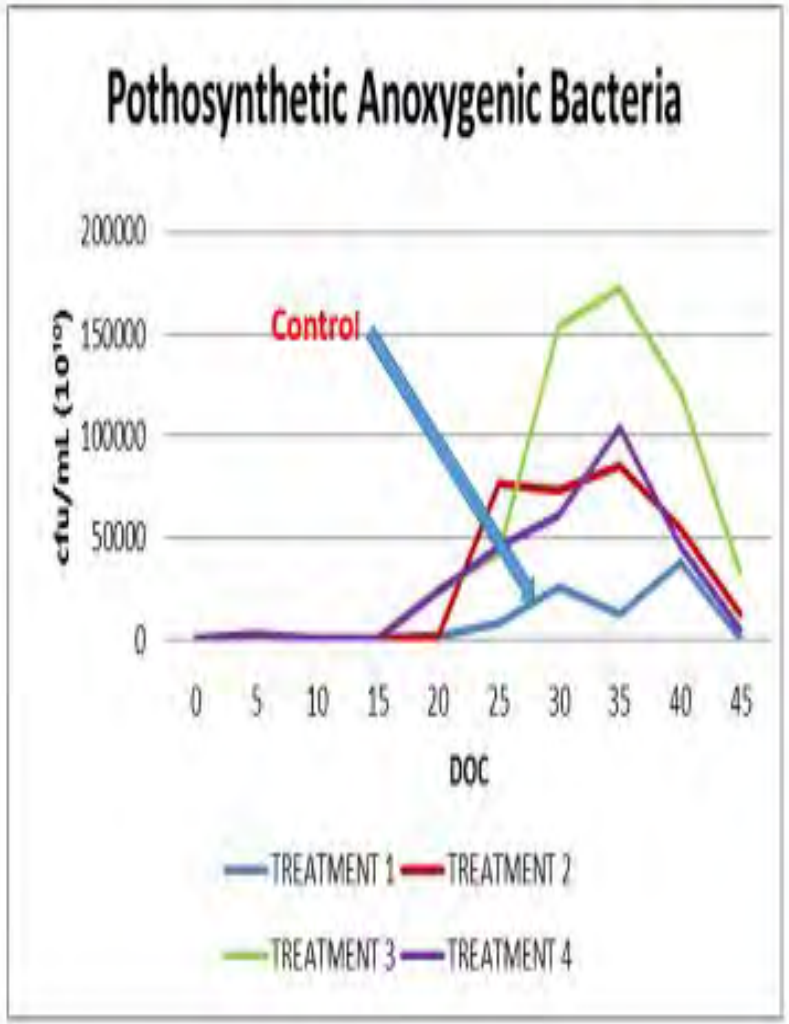
TREATMENT	% Weight gain	% Survival
control	84.41 ±1.03	89.05 ±5.64
175gTilapia/Ton	83.6± 6.52	93.2 ±6.01
350gTilapia/Ton	85.35± 0.42	94.97 ±4.71
700gtilapia/Ton	85.76 ±3.25	93.2 ±1.43

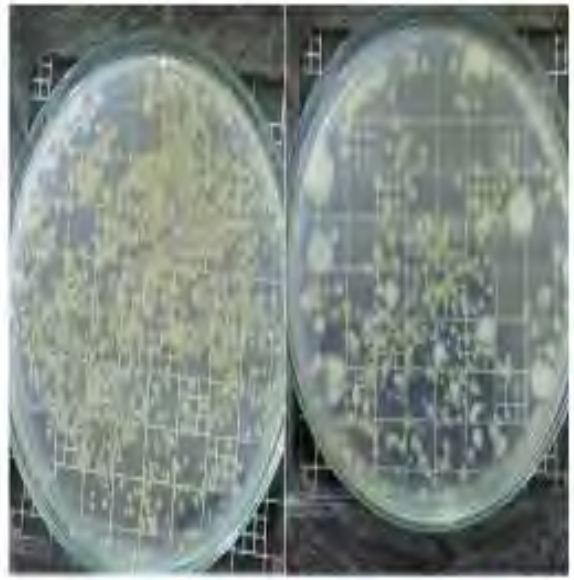
Cumulative *Vibrio parahaemolyticus* counts in culture tanks for 60 days



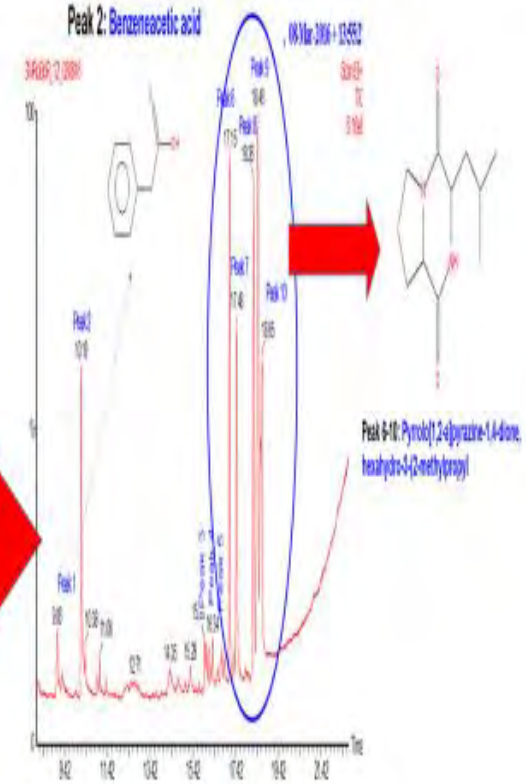
9X-fold Decrease

2.* *Tilapia Density Inhibitory to Vibrio parahaemolyticus*

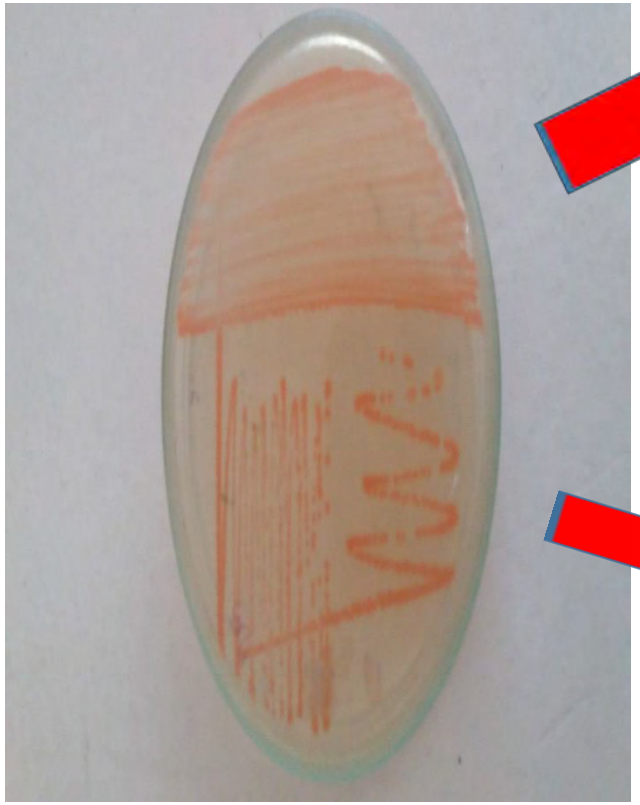


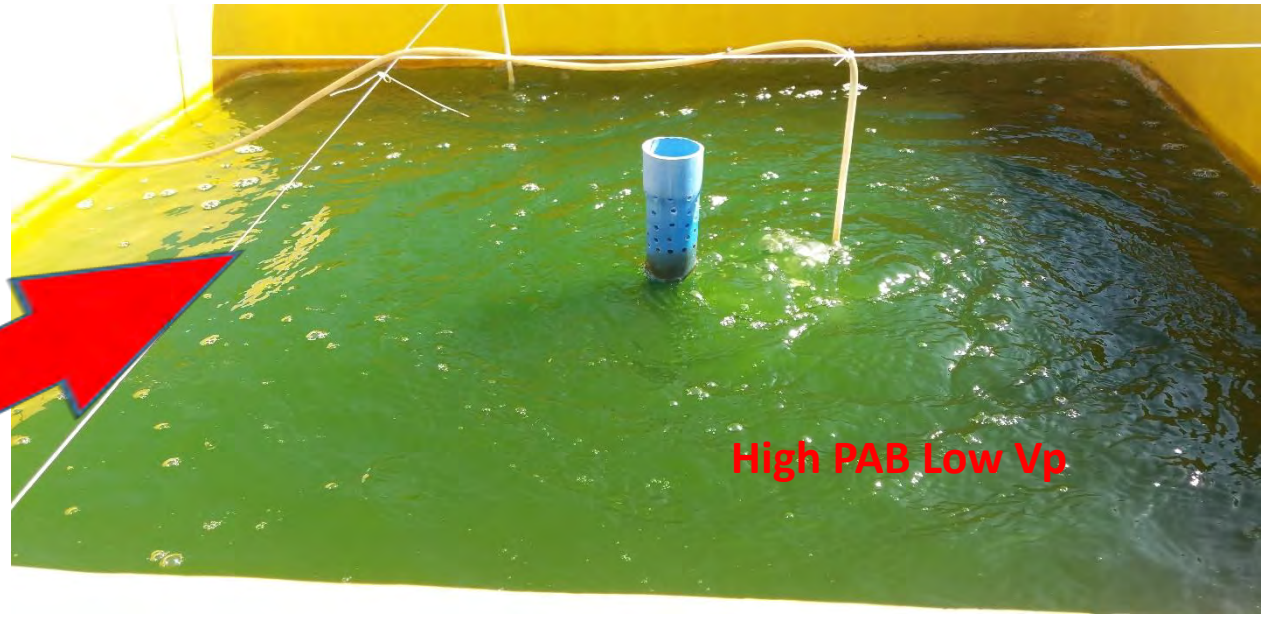


TREATMENT	
Culture period	50 days
1	Control
2	175g Tilapia
3	350g Tilapia
4	700g Tilapia
Shrimp	50 pcs/tank
Tanks	3X (CRD)



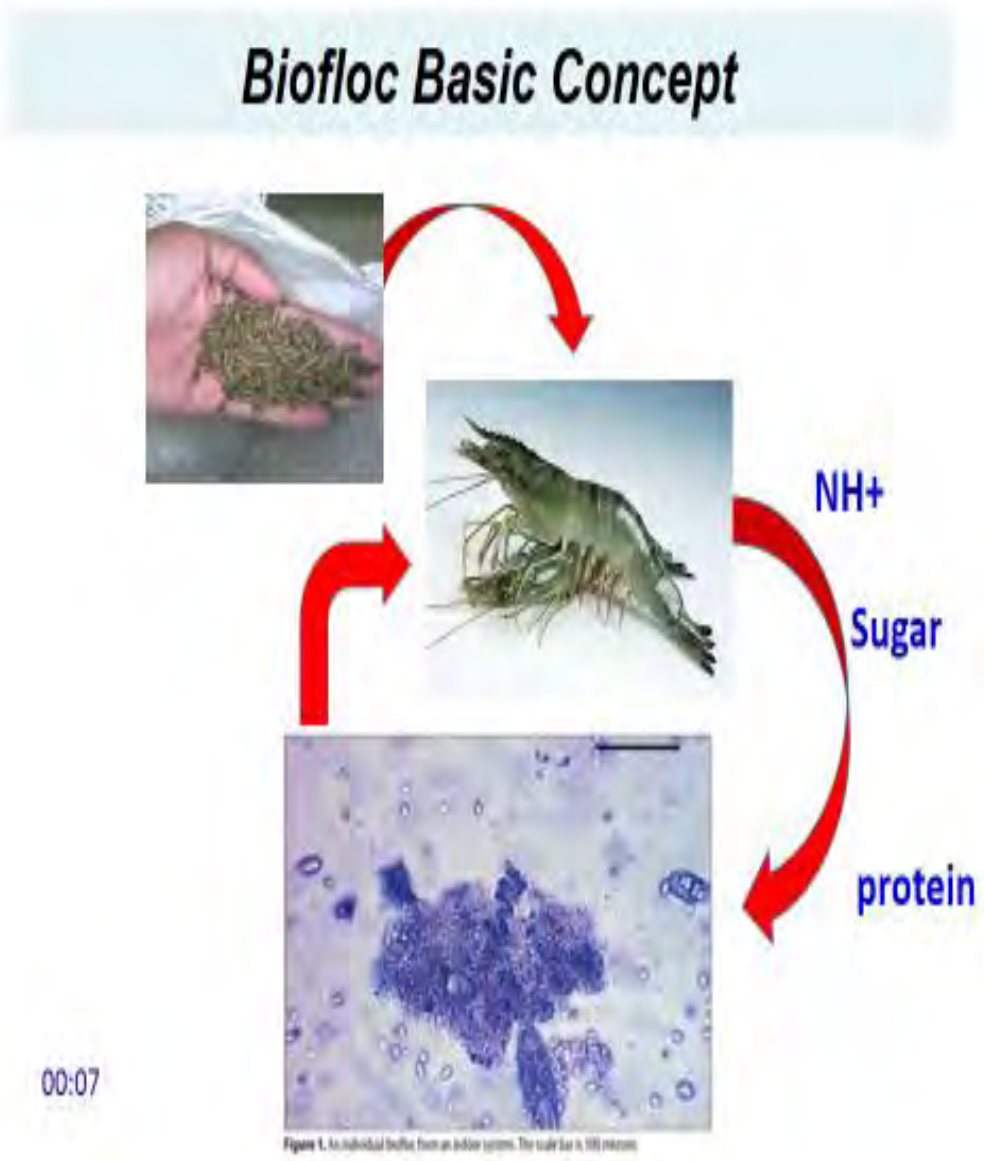
Green Algae = PAB / Vp





Correlation Table of Factors in Tilapia Green Water Affecting the Growth of V. parahaemolyticus in the Culture System

	Tilapia Stocking Density	Nanno-chloropsis Cell Density	Chorella Cell Density	Tetraselmis Cell Density	Diatoms Cell Density	Vibrio parahaemolyticus Cell counts	Total Ammonia	PAB Cell Counts
Nanno-chloropsis Cell Density	Cf = 0.425 p = 0.168							
Chorella Cell Density	Cf = 0.76 p = 0.004	Cf = 0.21 p = 0.52						
Tetraselmis Cell Density	Cf= -0.008 p = 0.979	Cf = -0.55 p = 0.06	Cf = 0.52 p = 0.086					
Diatoms Cell Density	Cf = -0.74 p = 0.006	Cf = -0.84 p = 0.0005	Cf = -0.44 p = 0.151	Cf = 0.491 p = 0.105				
Vibrio Parahae-molyticus Cell counts	Cf = -0.66 p = 0.01	Cf = -0.78 p = 0.003	Cf = -0.496 p = 0.101	Cf = 0.436 p = 0.156	Cf = 0.92 p = 0.000			
Total Ammonia	Cf = 0.93 p = 0.0000	Cf = 0.61 p = 0.03	Cf = 0.643 p = 0.024	Cf = -0.265 p = 0.405	Cf = -0.91 p = 0.000	Cf = -0.85 p = 0.000		
PAB Cell Counts	Cf = 0.465 p = 0.128	Cf = 0.74 p = 0.006	Cf = 0.402 p = 0.196	Cf = -0.326 p = 0.300	Cf = -0.85 p = 0.000	Cf = -0.80 p = 0.001	Cf = 0.71 p = 0.009	
Total Green Algae Cell Density	Cf = 0.59 p = 0.04	Cf = 0.55 p = 0.065	Cf = 0.731 p = 0.007	Cf = 0.144 p = 0.656	Cf = -0.51 p = 0.086	Cf = -0.59 p = 0.044	Cf = 0.55 p = 0.064	Cf = 0.40 p = 0.194



Experimental Biofloc Rearing Ponds at UPV

2B. *Inhibitory activity of Biofloc Stages on Vibrio parahaemolyticus*

STAGES

FLOC Development stages (vol) in pond

Stage 1 : Floc found but cannot measured (subjective)

Stage 2 : Floc found in small quantity, < 1.0 ml/litre

Stage 3 : Floc found abundance, 1.0 – 5.0 ml/litre

Stage 4 : Floc found abundance, 5.1 – 10.0 ml/litre

Stage 5 : Floc found abundance, > 10.1 ml/litre

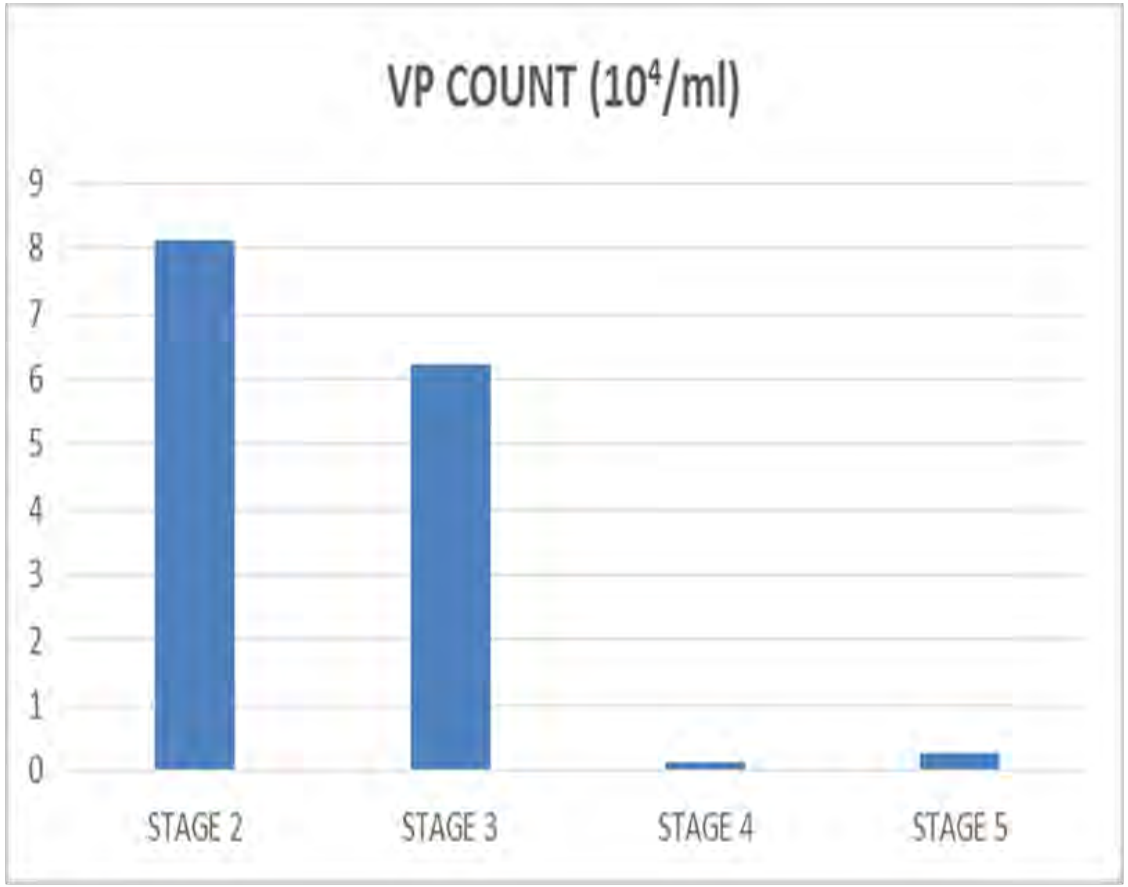


Figure 16 B. *Vibrio parahaemolyticus* cell counts in the culture water of *P. vannamei* reared at Different Biofloc Stages.

3.*Comparison of Tilapia Green water and biofloc culture system in Suppressing Vp bacteria in culture system.

TREATMENT	
Culture period	60 days
1	Control
2	Tilapia green water system
3	Biofloc culture system
Shrimp	200 pcs/ Ton tank
Tanks	3X (CRD)

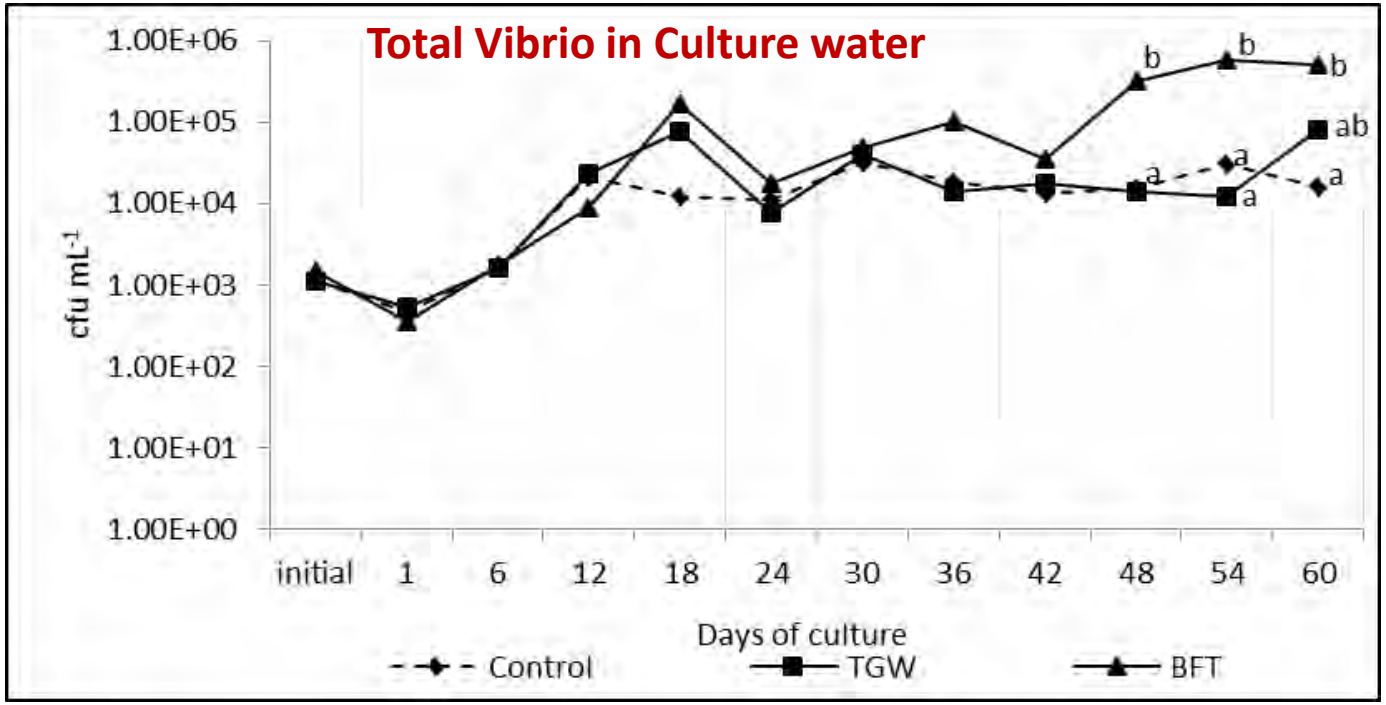


Figure 1. Total culturable *Vibrio* count in the water samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$). **R. Cadiz**

3.*Comparison of Tilapia Green water and biofloc culture system in Suppressing Vp bacteria in culture system.

TREATMENT	
Culture period	60 days
1	Control
2	Tilapia green water system
3	Biofloc culture system
Shrimp	200 pcs/ Ton tank
Tanks	3X (CRD)

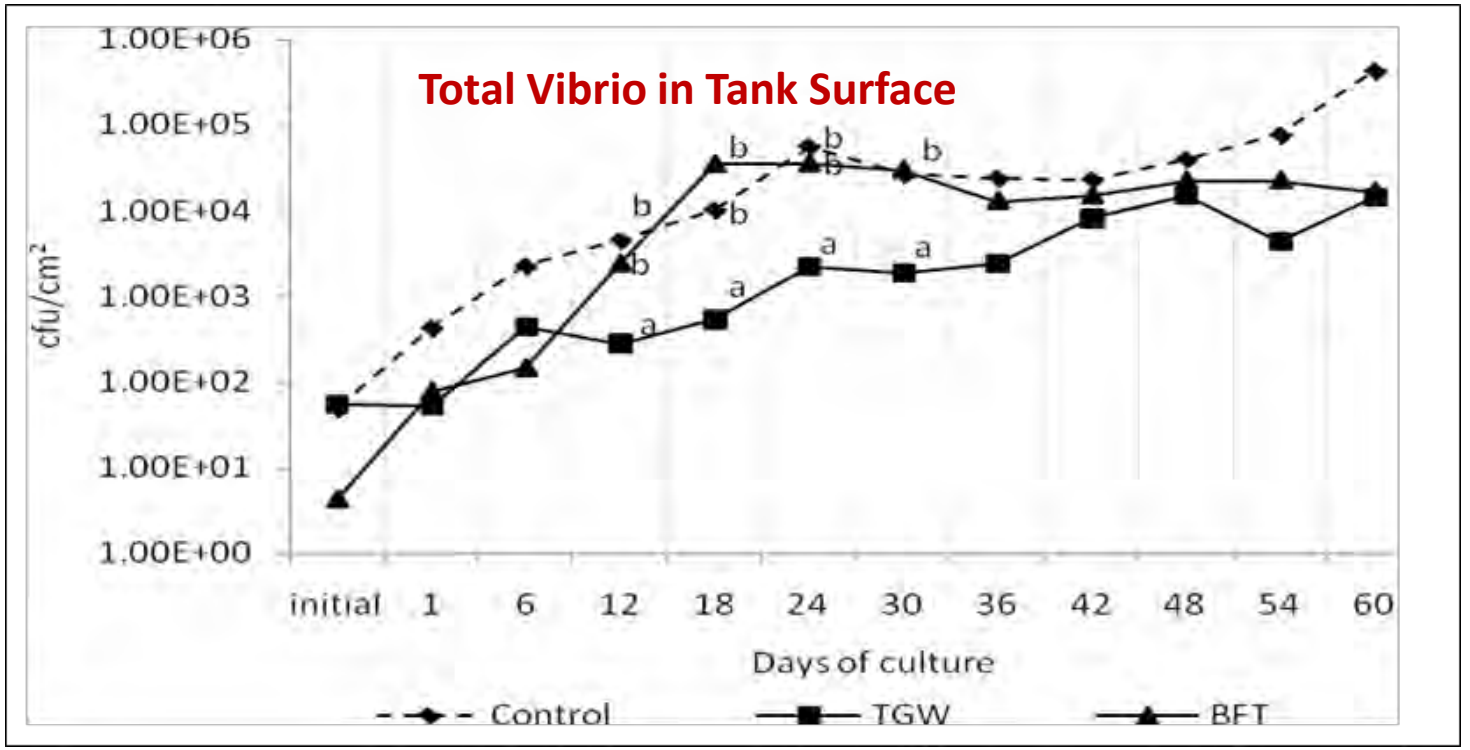


Figure 2. Total culturable *Vibrio* count in the surface samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$). ***(R.Cadiz)***

3.*Comparison of Tilapia Green water and biofloc culture system in Suppressing Vp bacteria in culture system.

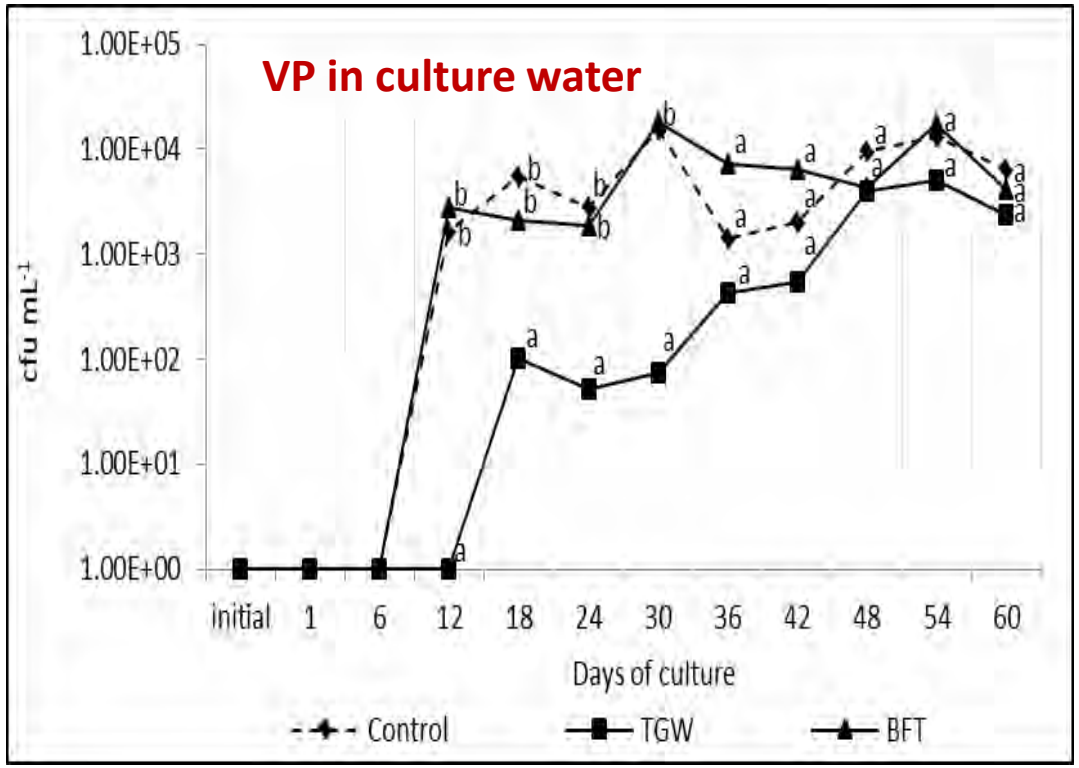


Figure 3. *Vibrio parahaemolyticus* count in the water samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$)

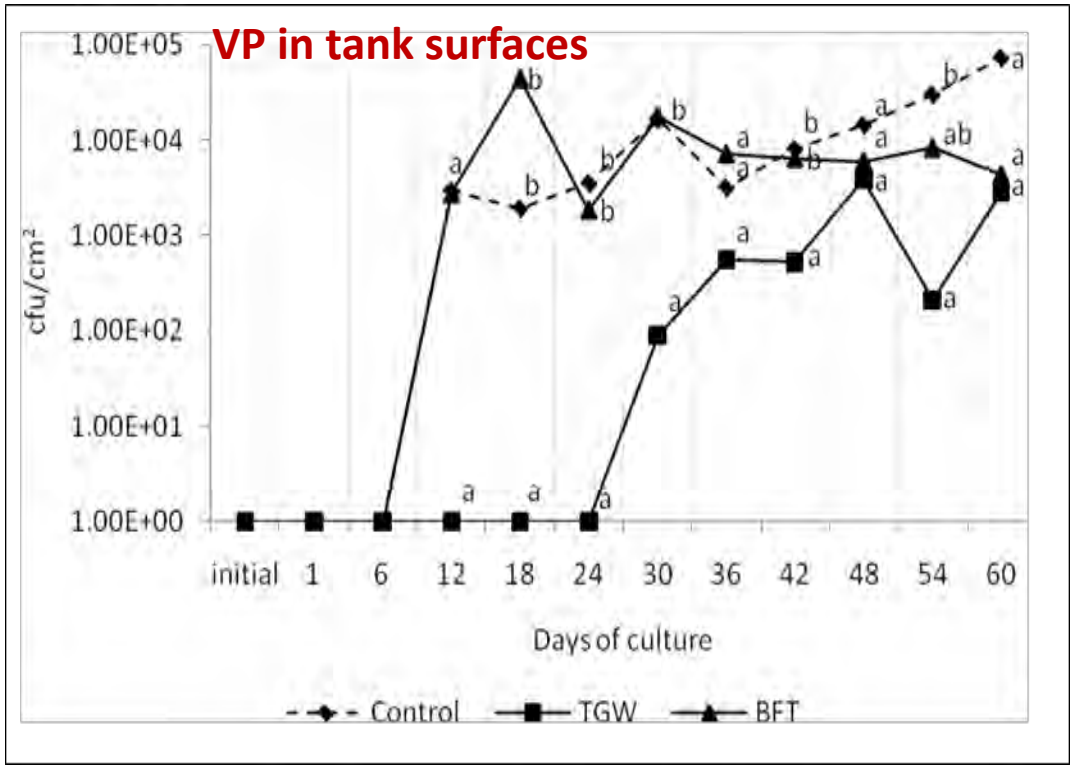
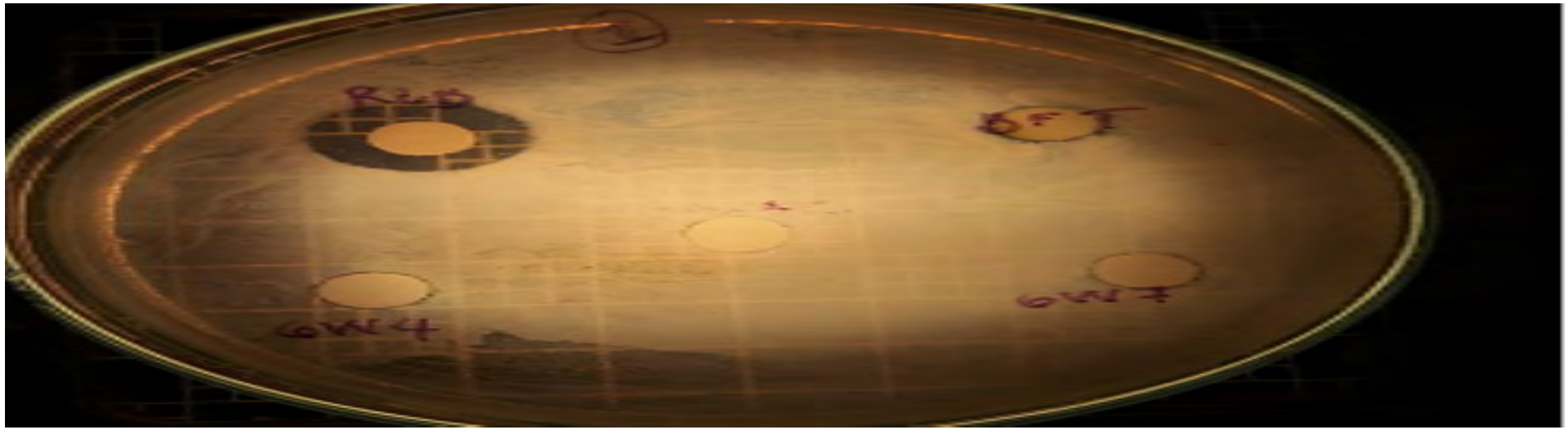


Figure 4. *Vibrio parahaemolyticus* count in the surface samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$).

(R.Cadiz)

Microbial Isolates with strong antibiotic activity against Vibrio parahaemolyticus

47 active microbial isolates from Tilapia green water



List of Probiotic Bacterial Isolates (active against *V. parahaemolyticus*) from Tilapia Green water and Biofloc System

CODES		SOURCE	CHARACTERISTICS
1.	SH3.2	Shrimp Hepatopancreas	Gram + (bacillus)
2.	GW3	Greenwater	Gram + (bacillus)
3.	GW5	Greenwater	Gram – (bacillus)
4.	SH10	Shrimp Hepatopancreas	Gram – (coccus)
5.	TM6.16	Tilapia Mucus	Gram + (bacillus)
6.	W2.2	Biofloc (Carcar, Cebu)	Gram + (coccus)
7.	I1/I7	Biofloc (Carcar, Cebu)	Gram + (coccus)
8.	TG5	Tilapia Gut	Gram + (bacillus)
9.	SH2	Shrimp Hepatopancreas	Gram + (bacillus)
10.	SH2.1	Shrimp Hepatopancreas	Gram + (bacillus)
11.	SH5	Shrimp Hepatopancreas	Gram + (bacillus)
12.	SH1	Shrimp Hepatopancreas	Gram + (bacillus)
13.	SH4	Shrimp Hepatopancreas	Gram + (bacillus)
14.	SH7	Shrimp Hepatopancreas	Gram + (bacillus)
15.	GW2	Greenwater	Gram + (bacillus)
16.	SH3.1	Shrimp Hepatopancreas	Gram – (bacillus)
17.	GW1	Greenwater	Gram + (bacillus)
18.	TG2	Tilapia gut	Gram + (coccus)
19.	GW7	Greenwater	Gram – (bacillus)
20.	GW4	Greenwater	Gram – (coccus)
21.	BF1	Biofloc (UPV Hatchery)	Gram – (bacillus)
22.	ROB	Greenwater	Gram + (bacillus)

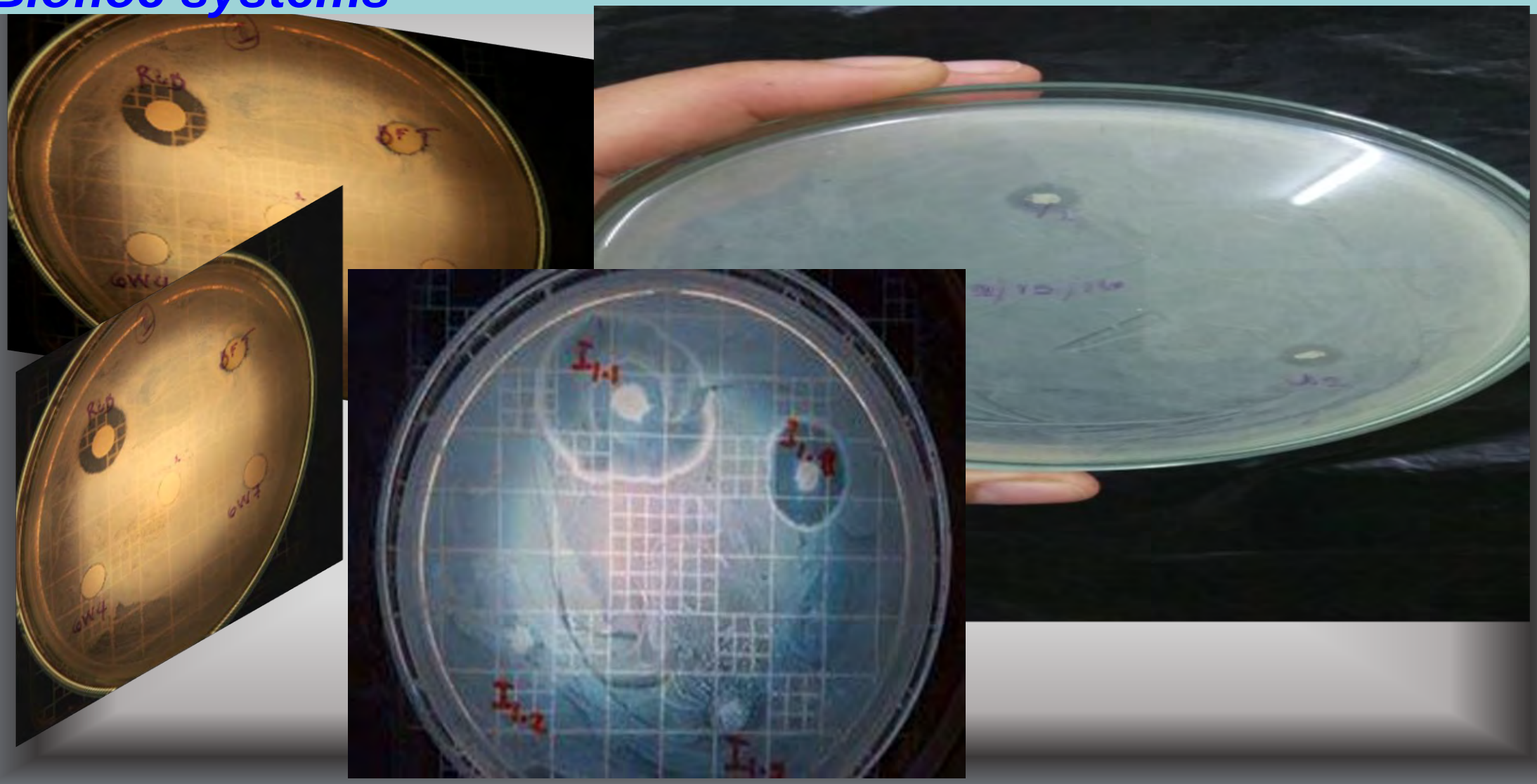
List of Probiotic Bacterial Isolates (active against *V. parahaemolyticus*) from Tilapia Green water and Biofloc System

23.	OB	Greenwater	Gram – (bacillus)
24.	TM6	Tilapia Mucus	Gram + (coccus)
25.	**GW8**	**Greenwater**	**Gram + (bacillus)**
26.	**GW6**	**Greenwater**	**Gram + (bacillus)**
27.	**SH9**	**Shrimp Hepatopancreas**	**Gram + (bacillus)**
28.	**GW1**	**Greenwater**	**Gram + (bacillus)**
29.	**SH6**	**Shrimp Hepatopancreas**	**Gram + (bacillus)**
30.	**YB**	**Greenwater**	**Gram + (coccus)**
31.	**TM5.3**	**Tilapia Mucus**	**Gram + (coccus)**
32.	**TM5.4**	**Tilapia Mucus**	**Gram + (coccus)**
33.	**Tm6.2**	**Tilapia Mucus**	**Gram + (coccus)**
34.	**TM6.3**	**Tilapia Mucus**	**Gram + (coccus)**
35.	**TM6.6**	**Tilapia Mucus**	**Gram + (coccus)**
36.	**TM6.8**	**Tilapia Mucus**	**Gram + (coccus)**
37.	**TM6.9**	**Tilapia Mucus**	**Gram + (coccus)**
38.	**TM6.13**	**Tilapia Mucus**	**Gram + (coccus)**
39.	**TM5.3**	**Tilapia Mucus**	**Gram + (coccus)**
40.	**TM5.4**	**Tilapia Mucus**	**Gram + (coccus)**
41.	**TM 5.7**	**Tilapia Mucus**	**Gram + (coccus)**
42.	**TF 5.6**	**Tilapia Feces**	**Gram + (coccus)**
43.	**TF5.13**	**Tilapia Feces**	**Gram + (coccus)**
44.	**TF5.14**	**Tilapia Feces**	**Gram + (coccus)**
45.	**GU1**	**Greenwater**	**Whitish green yeast**
46.	**GU2**	**Greenwater**	**Fungi**
47.	**GU3**	**Greenwater**	**Fungi**

Identified Probiotics Isolates

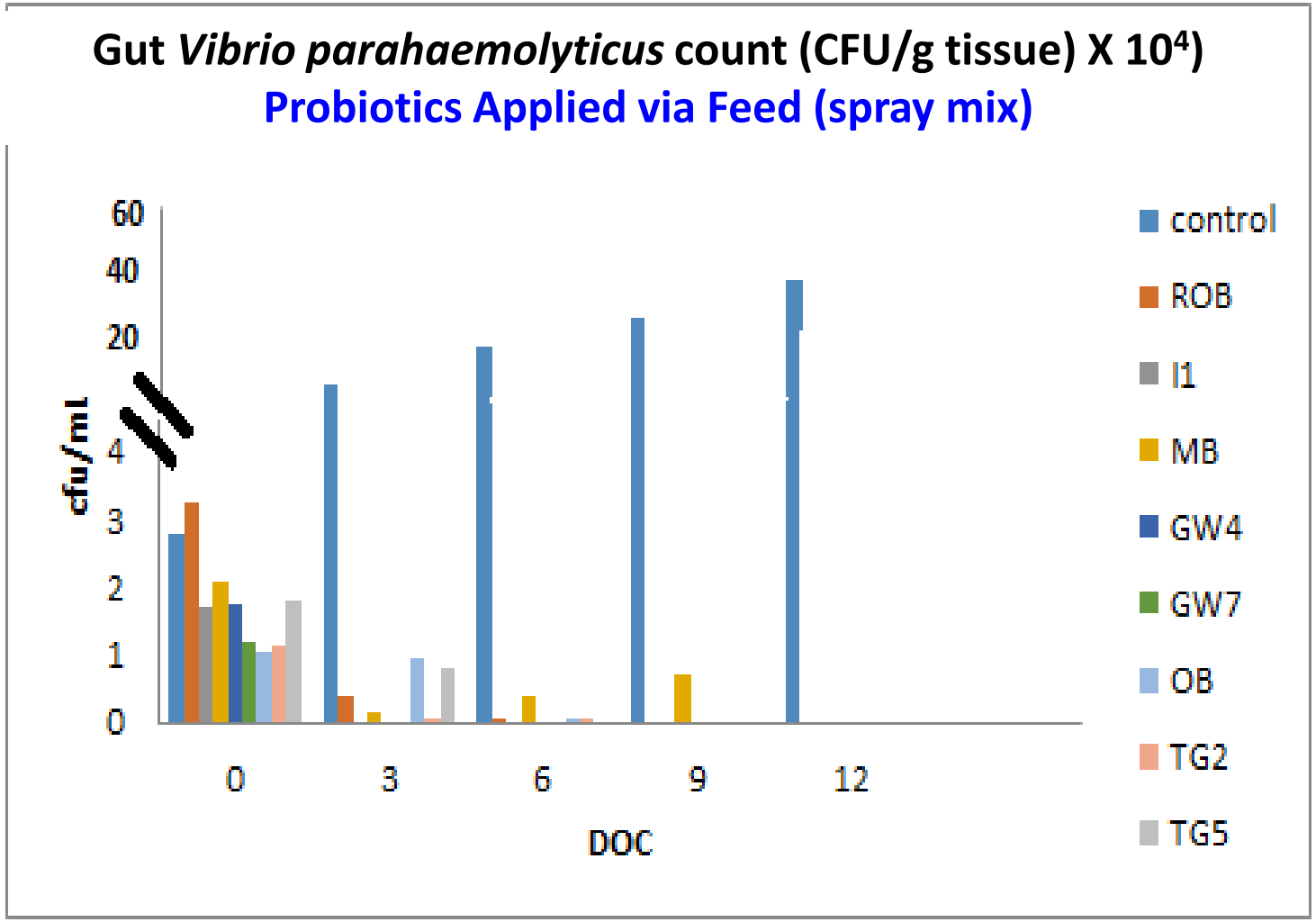
CODE	Source	Gram (+/-)	Identified by/date of identification	Bacterial Name
TG2	Tilapia Gut	+ (coccus)	NSRI 7/18/16	*Staphylococcus aureus*
TG5	Tilapia Gut	+ (bacillus)	NSRI 7/18/16	*Bacillus cibi*
GW7	Greenwater	- (bacillus)	NSRI 7/18/16	*Brevundimonas diminuta*
ROB	Greenwater	+ (bacillus)	NSRI 7/18/16	*Halobacillus trueperi*
OB	Greenwater	- (bacillus)	SEAFDEC 12/6/16	*Branhamella spp.*
TM6.16	Tilapia Mucus	+ (bacillus)	SEAFDEC 12/6/16	*Bacillus sp.*
GW4	Greenwater	- (bacillus)	SEAFDEC 12/6/16	*Vibrio coralliilyticus*
BF1	Biofloc	- (bacillus)	UPV 2/28/17	*Pseudomonas luteola*
I1/I7	Shrimp gut	+ (coccus)	UPV 1/03/16	*Streptococcus porcinus*
W2	Biofloc water	+ (coccus)	UPV 1/03/16	*Micrococcus luteus*

Probiotic Activity Testing: Isolates from Tilapia Greenwater and Biofloc systems

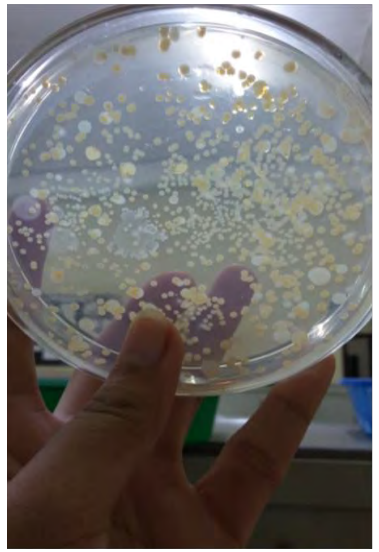


Research Question 7: Could the Probiotics Eliminate Vp that colonized shrimp Gut?

Summary: Shrimps were fed diets with Vp for 3 days. Then shrimp were fed with Probiotics supplemented diets. Samples taken from shrimp gut for Vp quantification. Five individual shrimps per treatment were analyzed.



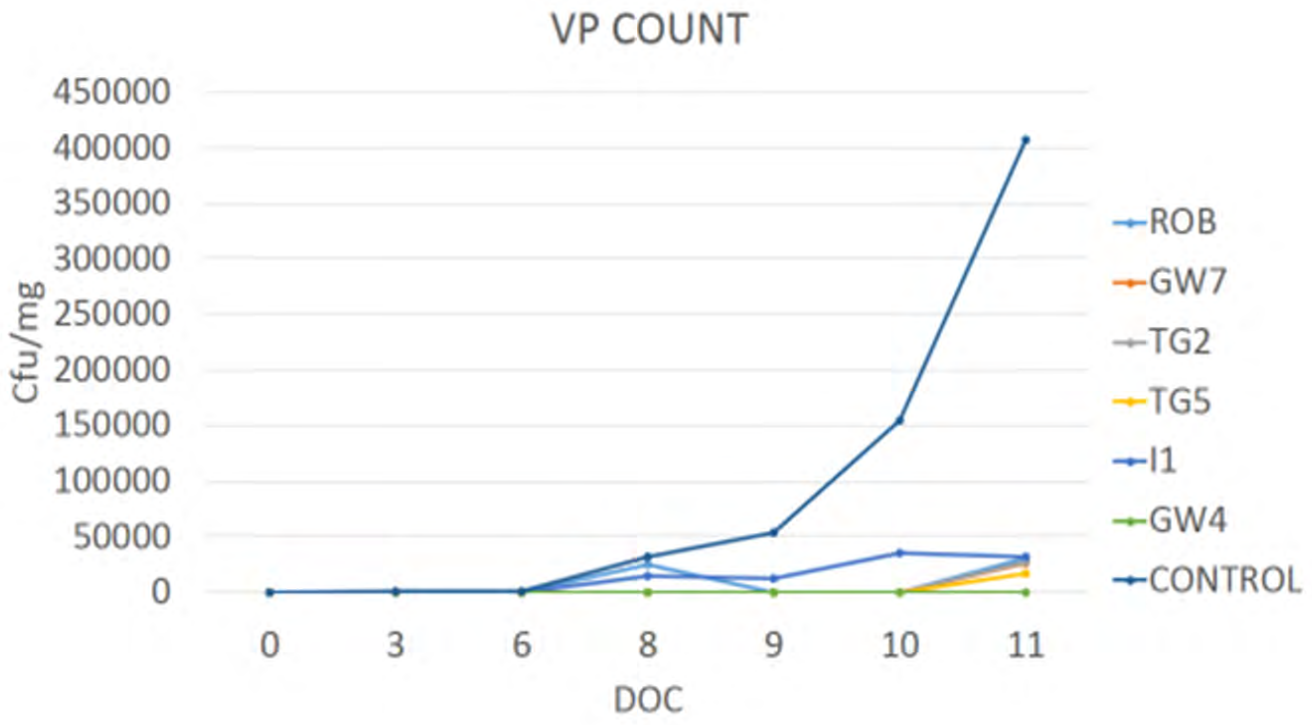
Eliminated on the 6th Day



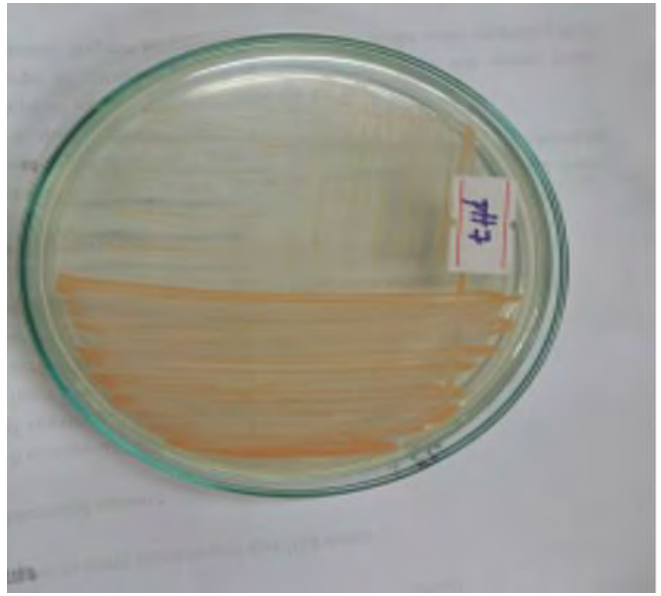
Research Question : Could the Probiotics Prevent the Colonization of VP to shrimp Gut?

Summary: Shrimps were fed probiotics for 7 days. Then shrimps were fed for 3 days with VP supplemented diets. Samples taken from shrimp gut for VP quantification. Five individual shrimps per treatment were analyzed.

Probiotics Inhibits Gut colonization of Vp



Hallobacillus trueperi

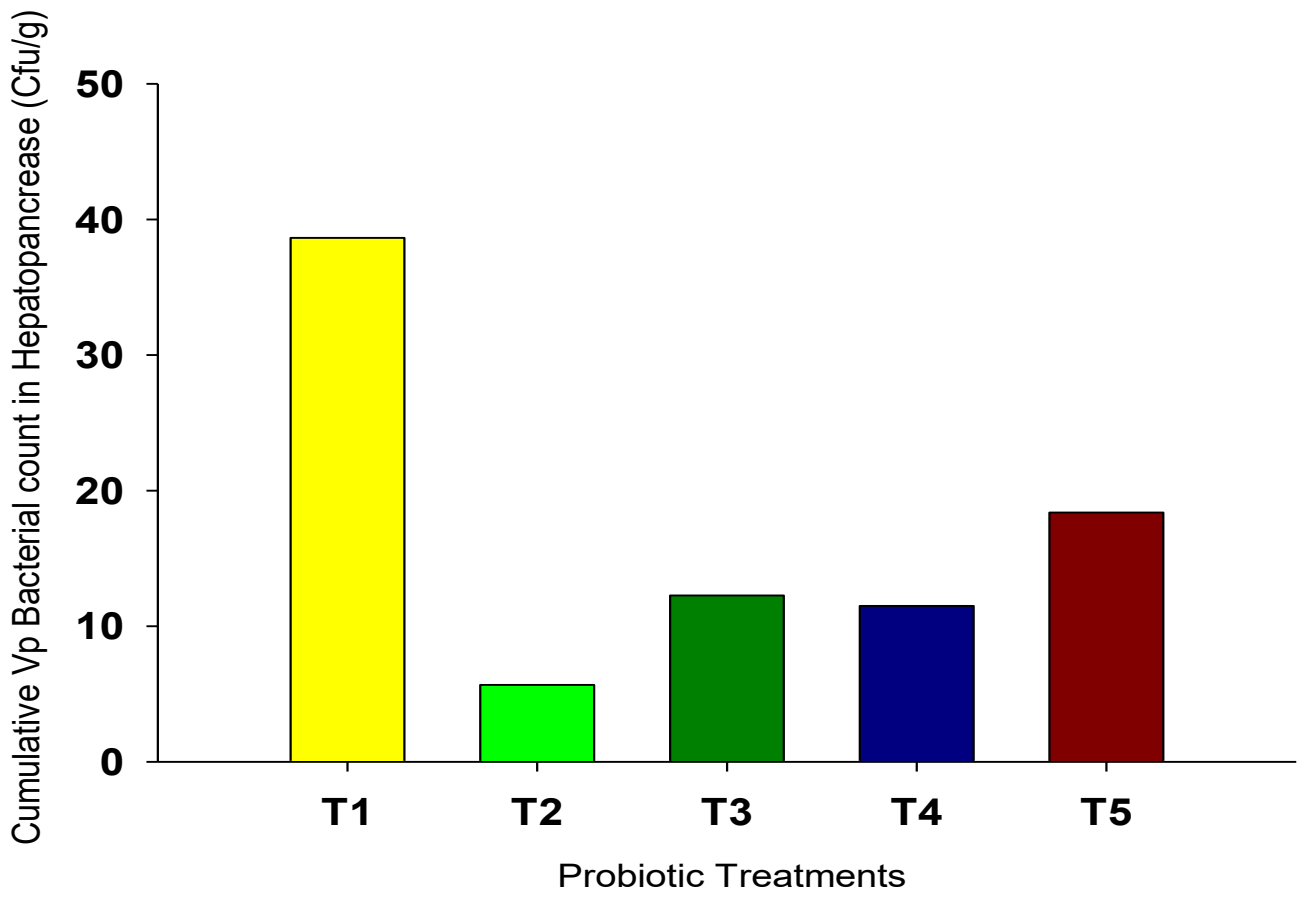


Probiotics Tank Trial (*P. vannamei* culture,45 Days)

Growth Performance of *Penaeus vannamei* exposed to different probiotics for 45 days of culture.

TREATMENT	% WEIGHT GAIN	% SURVIVAL
1-Control	309.81±24.41	86±1.0
2-I1	436.4±98.71	75.5±22.5
3-DB	369.63±16.86	74.5±0.5
4-TG5	380.58±66.01	80±1.0
5-GW7	318.13±8.99	78±3.0

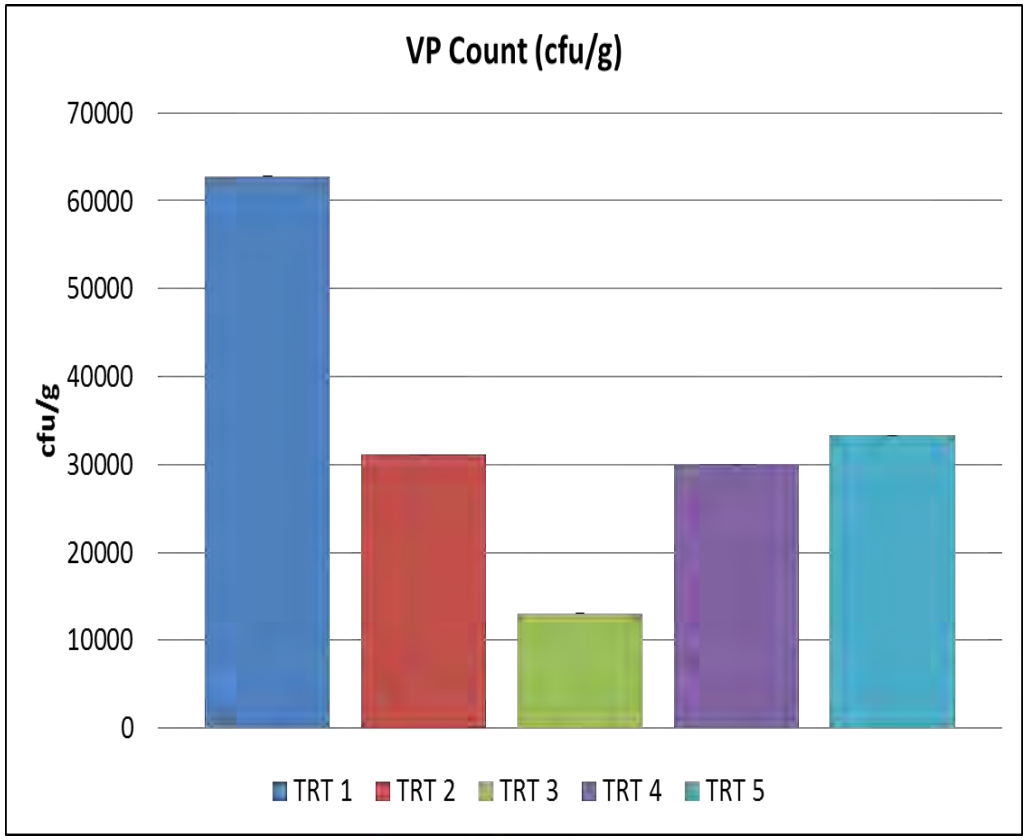
Cumulative Vibrio parahaemolyticus Count in Shrimp Gut (stomach) (CFU/g) X 10^4



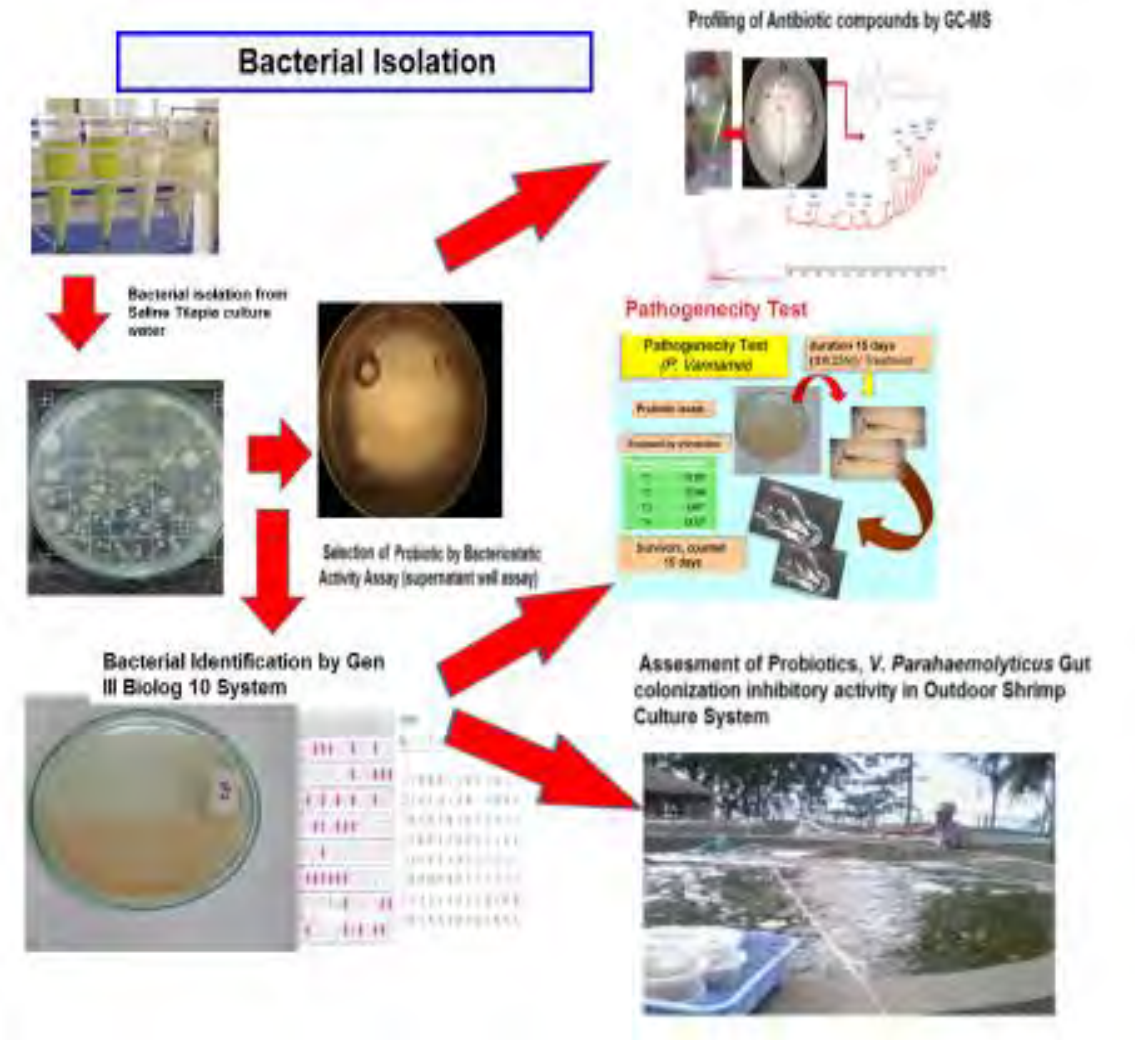
Vibrio parahaemolyticus Count in Shrimp Gut (Vp gut colonization Test)

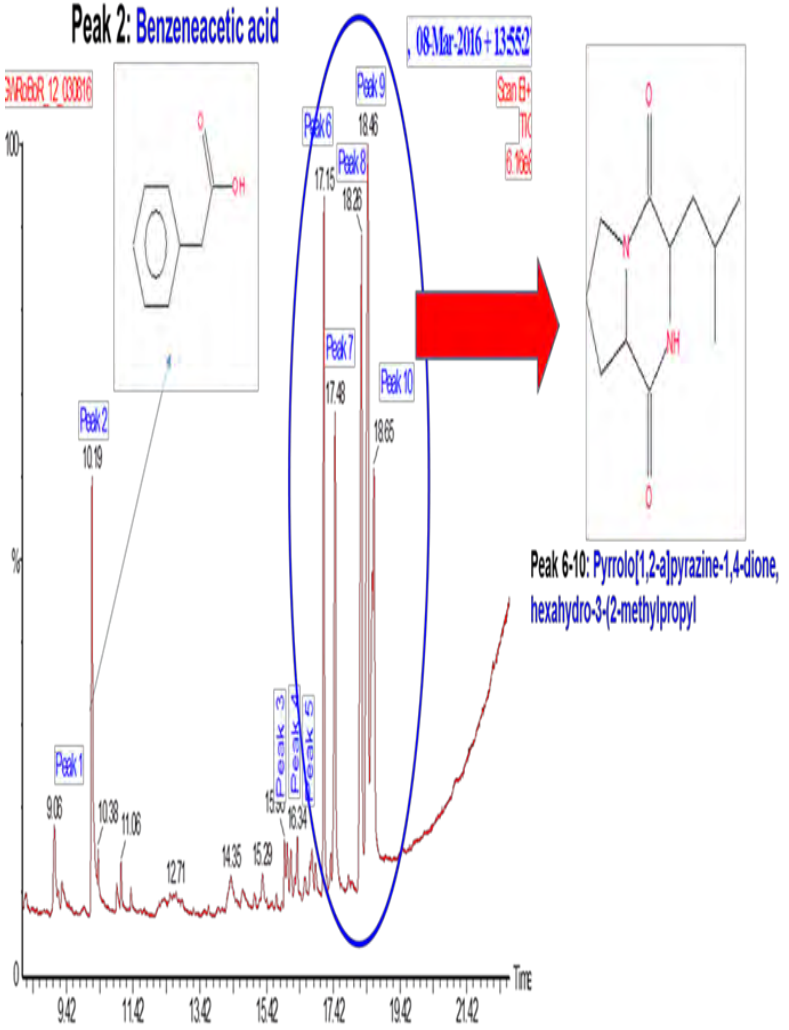
Vibrio parahaemolyticus Colonization CHALLENGE

Shrimps after 45 days of culture with probiotics were given feeds coated with *Vibrio parahaemolyticus* for two days and gut were analyzed for VP. This is to asses if the probiotics can prevent VP gut colonization.

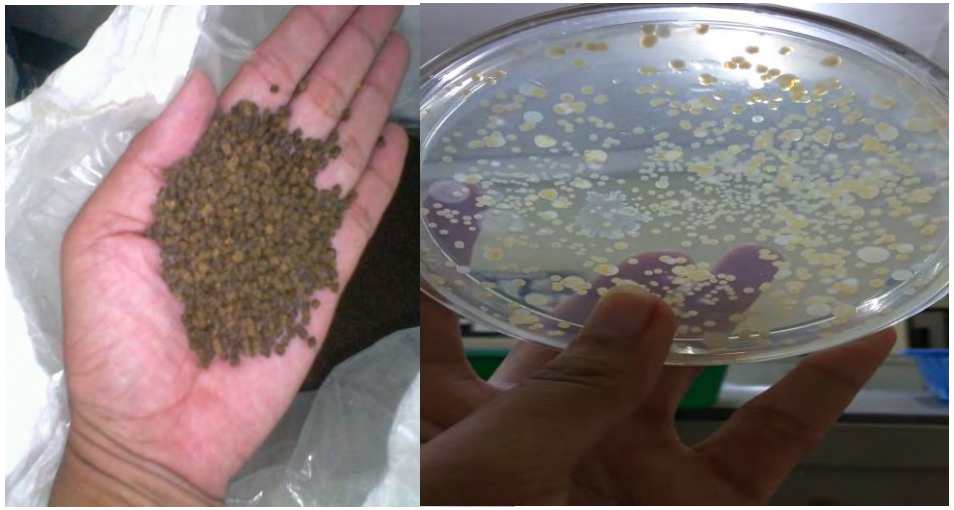


Hallobacillus trueperi



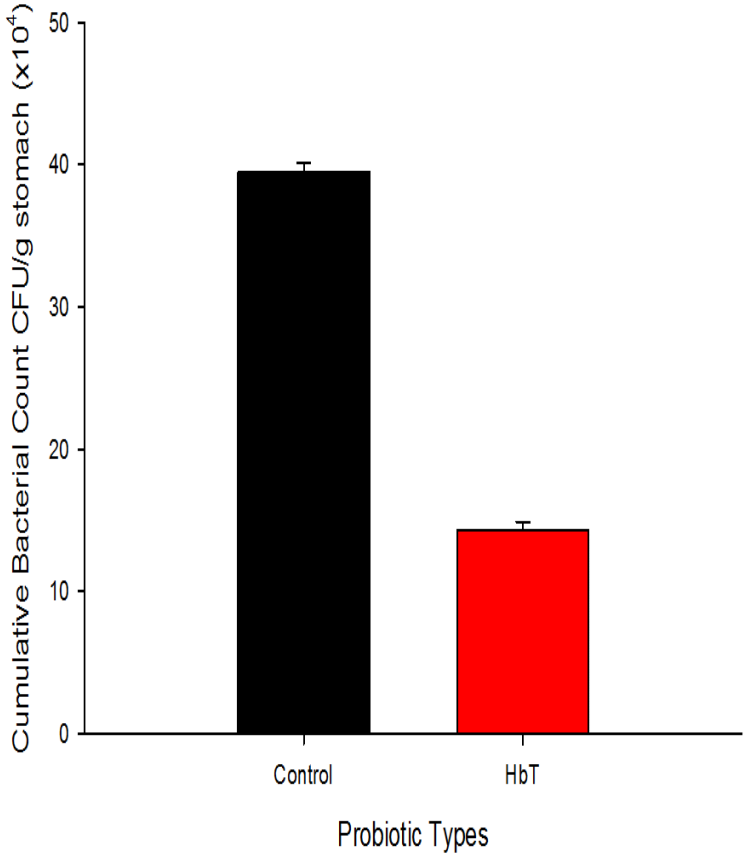


Results



Influence on Growth & Survival Feed Added (60 Days)		
Probiotic Code	% WEIGHT GAIN	% SURVIVAL
Control	309.81±24.41	86±10
HbT	369.63±16.86	74.5±5
	Not statistically different (T-test)	

Cumulative *Vibrio parahaemolyticus* Count in Shrimp Stomach (CFU/g) X10^4/60 days culture



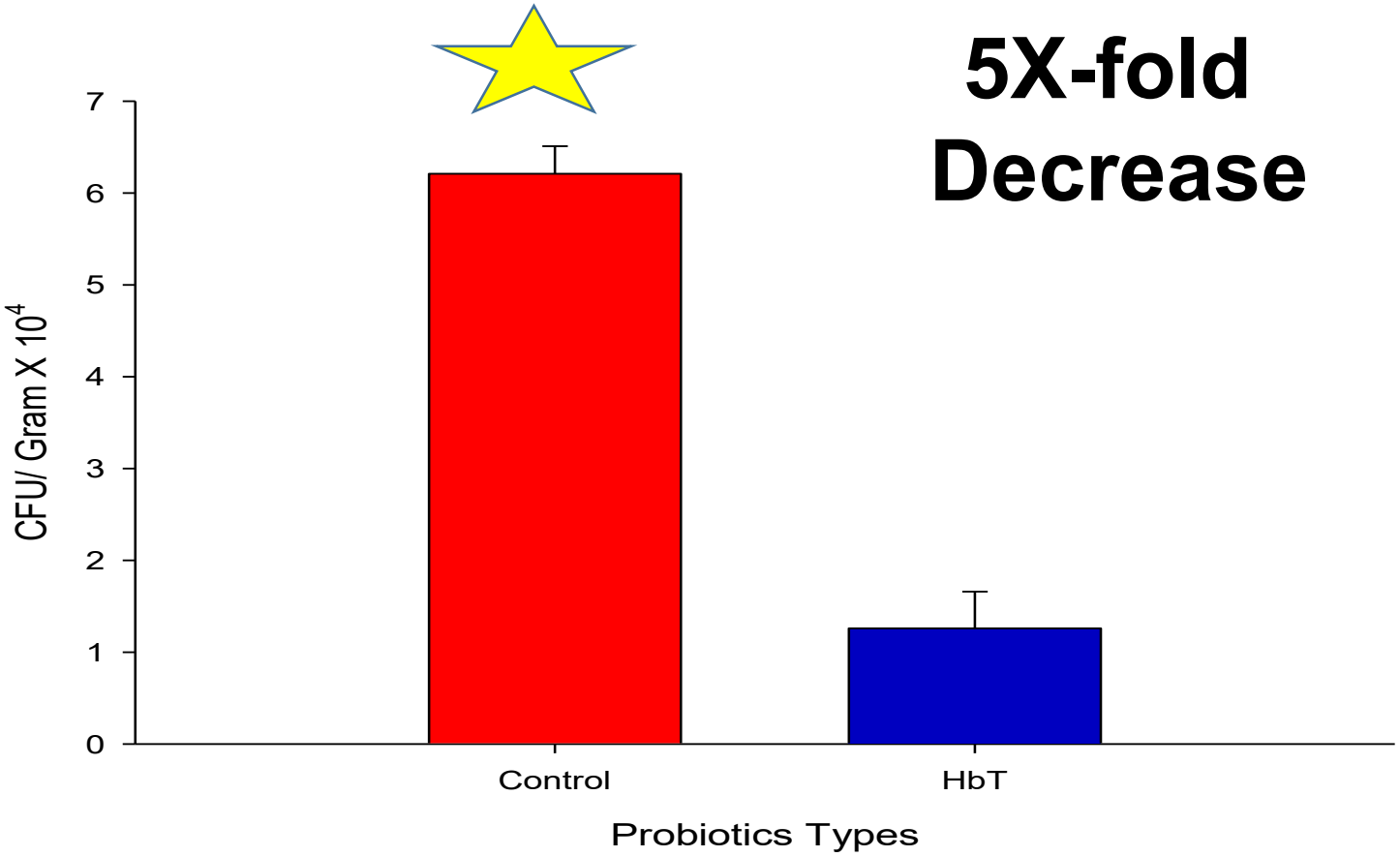
Vibrio parahaemolyticus Shrimp Gut
(Stomach Colonization Test)

Vibrio parahaemolyticus Colonization CHALLENGE

Shrimps after 60 days of culture with probiotics were given feeds coated with *Vibrio parahaemolyticus* for two days and gut were analyzed for VP. This is to asses if the probiotics can prevent VP gut colonization. N=5

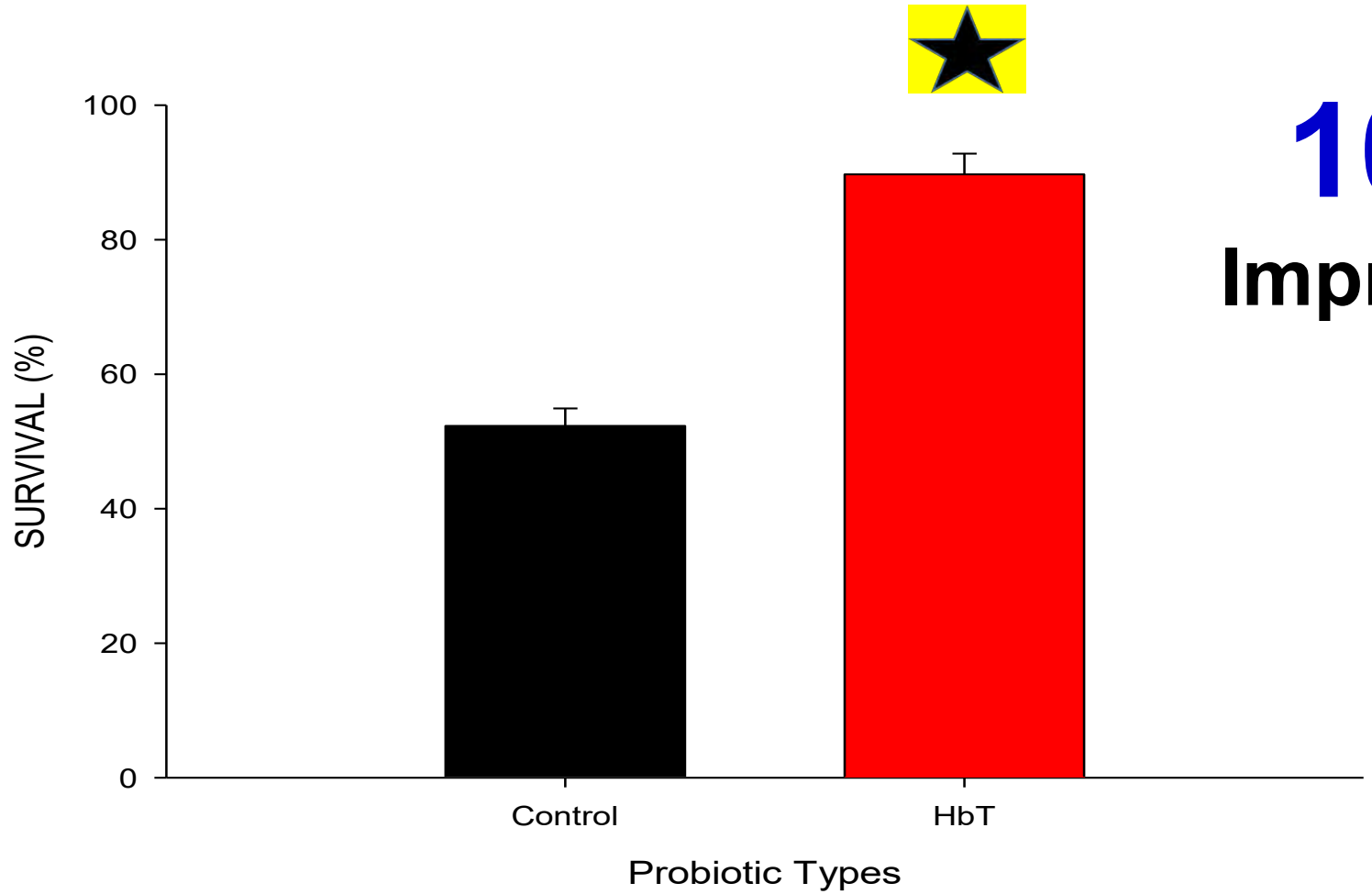
1x10^8 CFU *Vp*/gram Feed

Stomach *Vibrio parahaemolyticus* content



Shrimp Survival after Exposure to Pathogenic *Vibrio parahaemolyticus* Through Feed delivery

$1x10^8$ CFU *Vp*/gram Feed

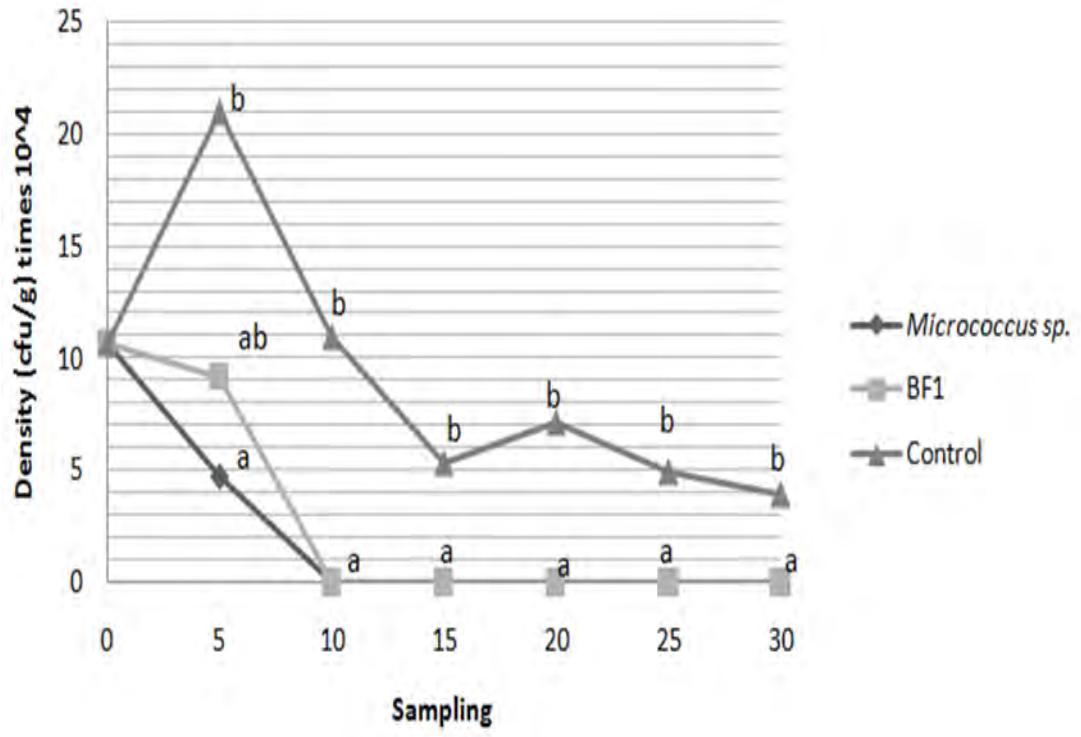


100 %

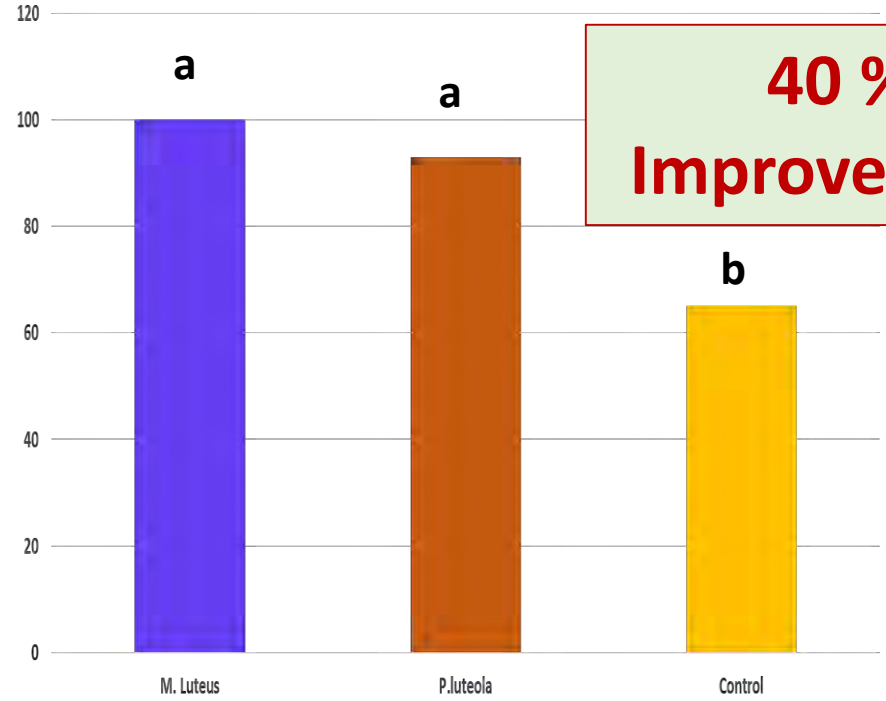
Improvement

Pseudomonas luteola & Micrococcus luteus

Summary: ***P. monodon*** **juveniles were fed diets supplemented with probiotics (10^{10}CFU.g^{-1} diet) for 30 days. The Vp count in the gut were monitored and the animals were exposed to Vp infection challenge test after the feeding trial.**



Survival after infection challenge with Vp



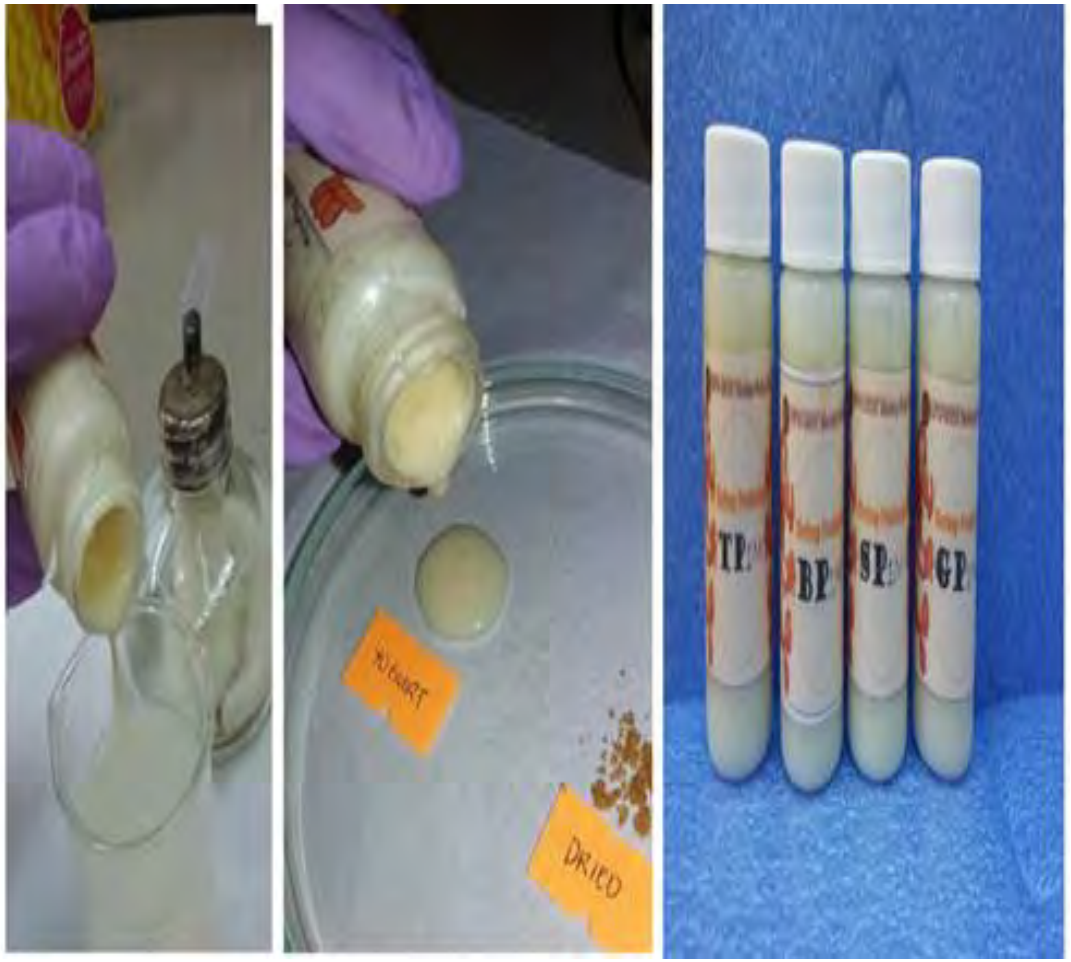
40 % Improvement

Figure 5. *V. parahaemolyticus* count in the gut of *P. monodon* during the feeding trial. Values in the same sampling period with different labels are significantly different at P<0.05.

Temario

Probiotics Field Test : Actual Pond Production Trials

Pond Trials
Carcar Prawn Farm
Carcar, Cebu



Emulsion Type Probiotics (Biofloc) Mixed Cocci

Penaeus vannamei

TREATMENT	POND	Total Larvae stocked (PL) (100PL/m2)	POND AREA
P8 Probiotics	5	340,400	3,400 sq. m
Emulsion Type	12	391,200	3,912 sq. m
	6	314,900	3,149 sq. m
control	11	370,000	3,700 sq. m

With P8 probiotics

3g emulsion type probio/ 1kg feeds

Everyday or every 3 days interval application until harvest after stocking

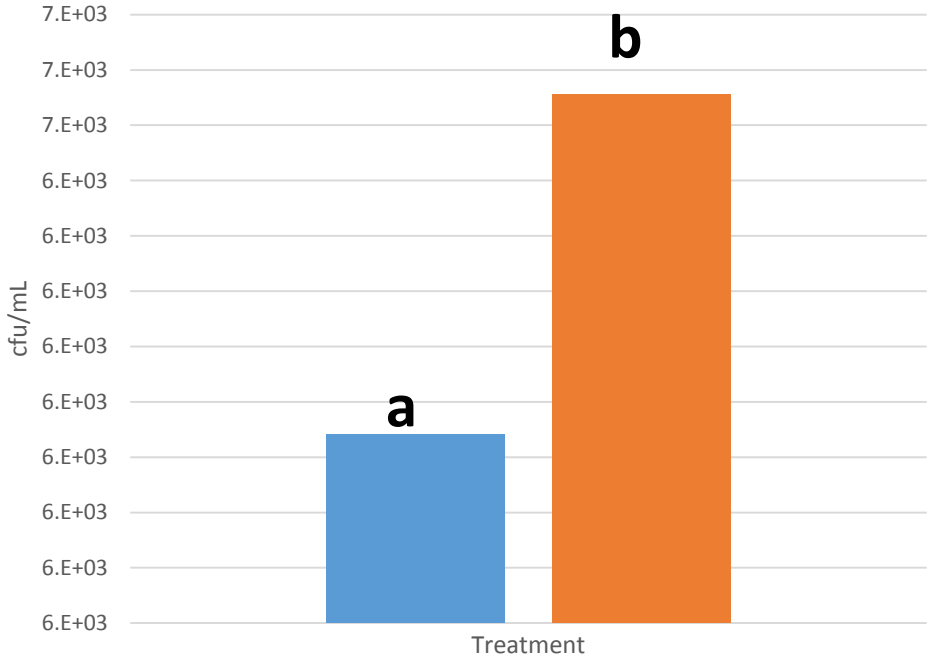
Control

BZT aqua/ 1kg feeds

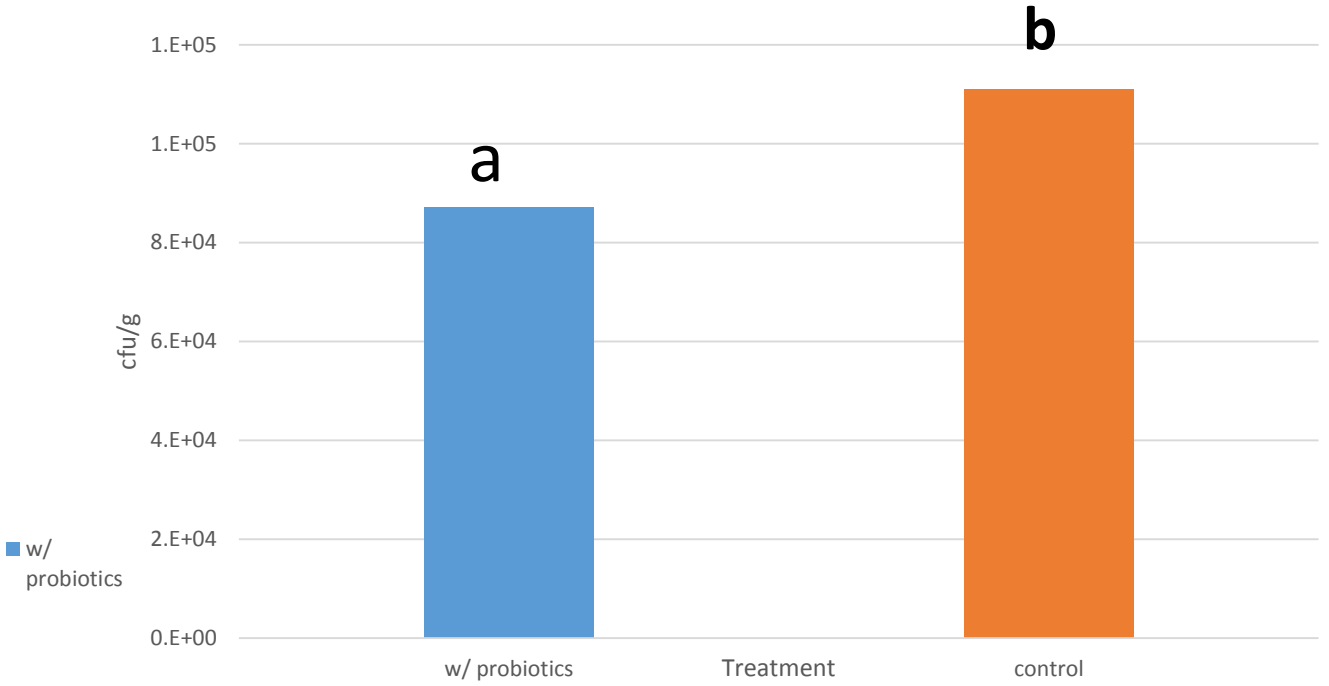
Everyday or every 3 days interval application until harvest

Cumulative Total Vibrio Count

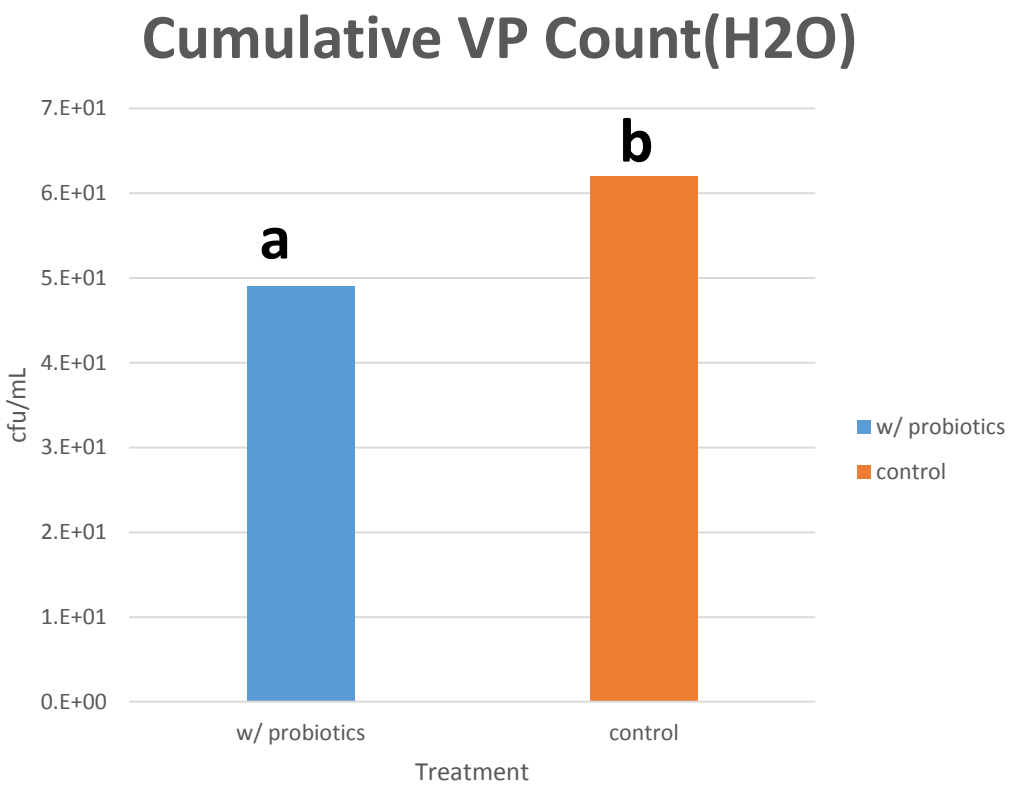
Cumulative Vibrio Count(H2O)

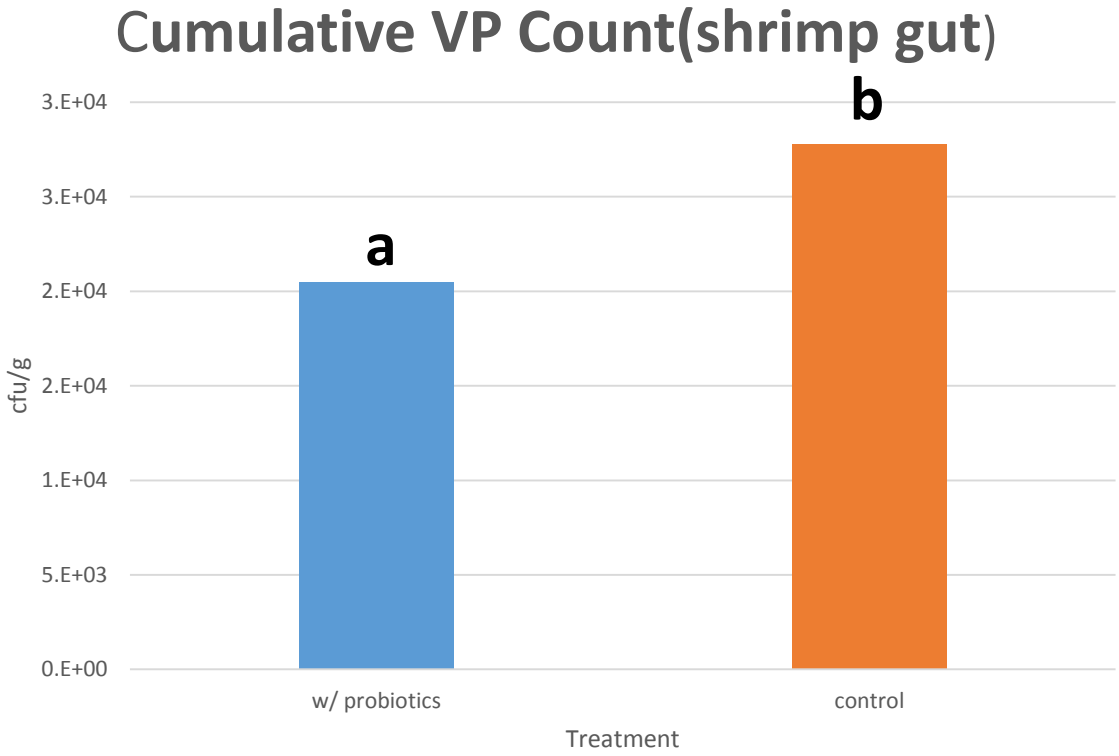


Cumulative Vibrio Count(Shrimp Gut)



Cumulative VP Count





Indices		
	Conrol (BZT)	Treated (P8_Probio)
Harvest Weight	31±3	26±5
Survival	80±0	80±0
FCR	1.8±0.02	1.6±0.2
Total Harvest Biomass (Kg)	10,689±1,797	9,113±1,934
Culture Period (Days)	90	90

RDEX Kawas Prawn Farm

Kawas, Alabel, Sarangani Province

TREATMENT	POND Number	Total PL Stocking Density (@100/m2)	POND AREA
AquaPro_Y(UP V-DOST) Probiotics	10	500,000	5,370 sq m
Commercia Probioticsl BZT Aqua Control	11	500,000	5,100 sq m

Penaeus vannamei

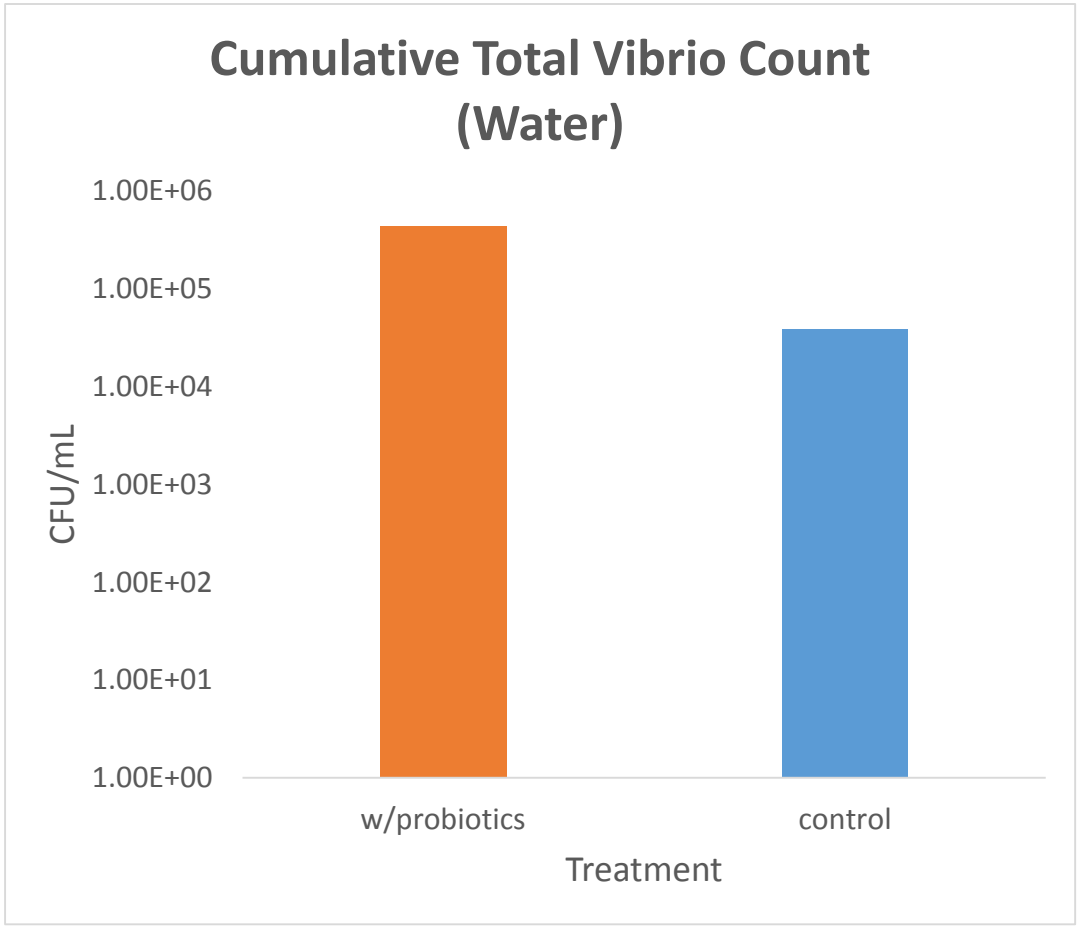
AquaPro-Y

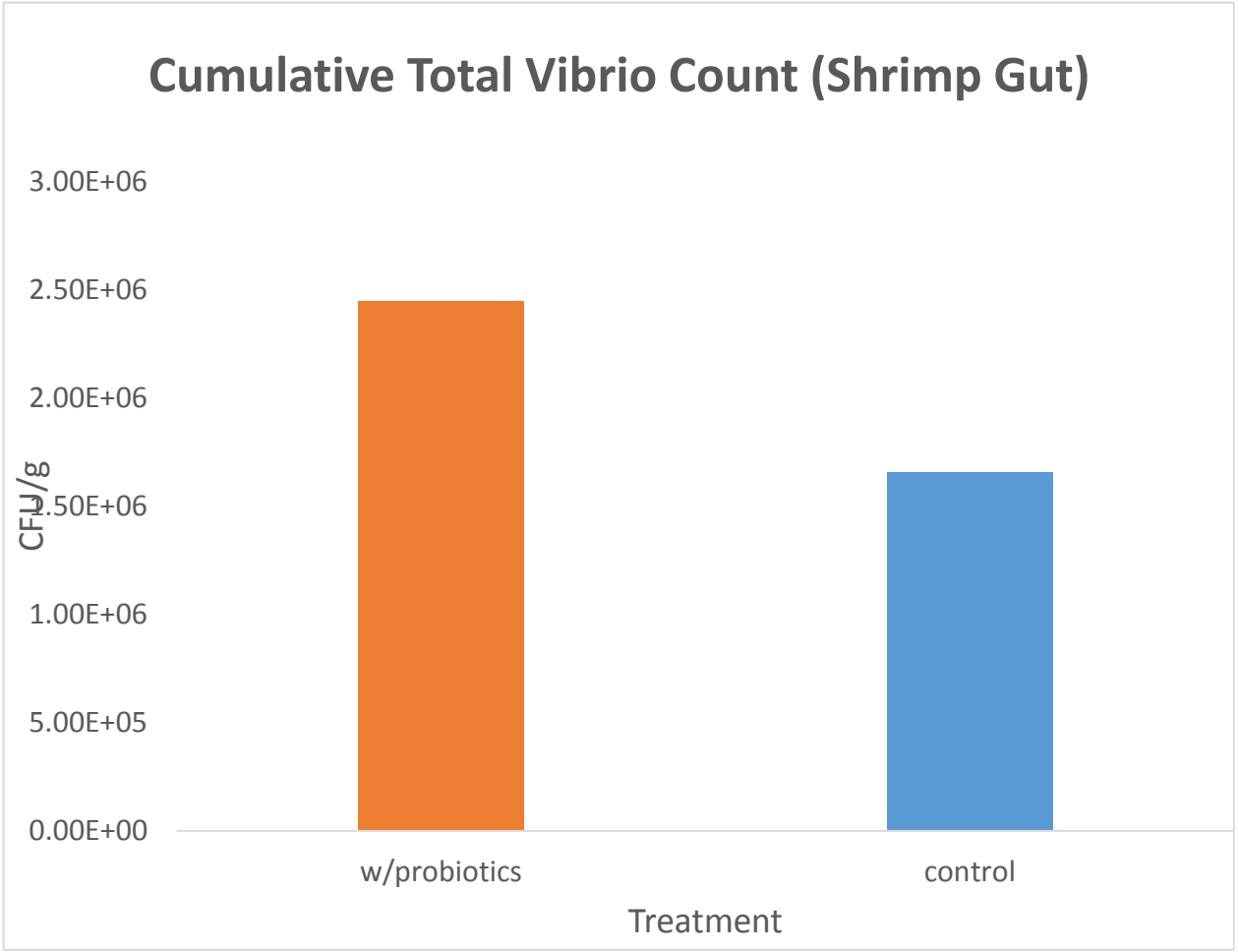
- 3g probio/kg feeds
- Every 3 days interval application after stocking

Control

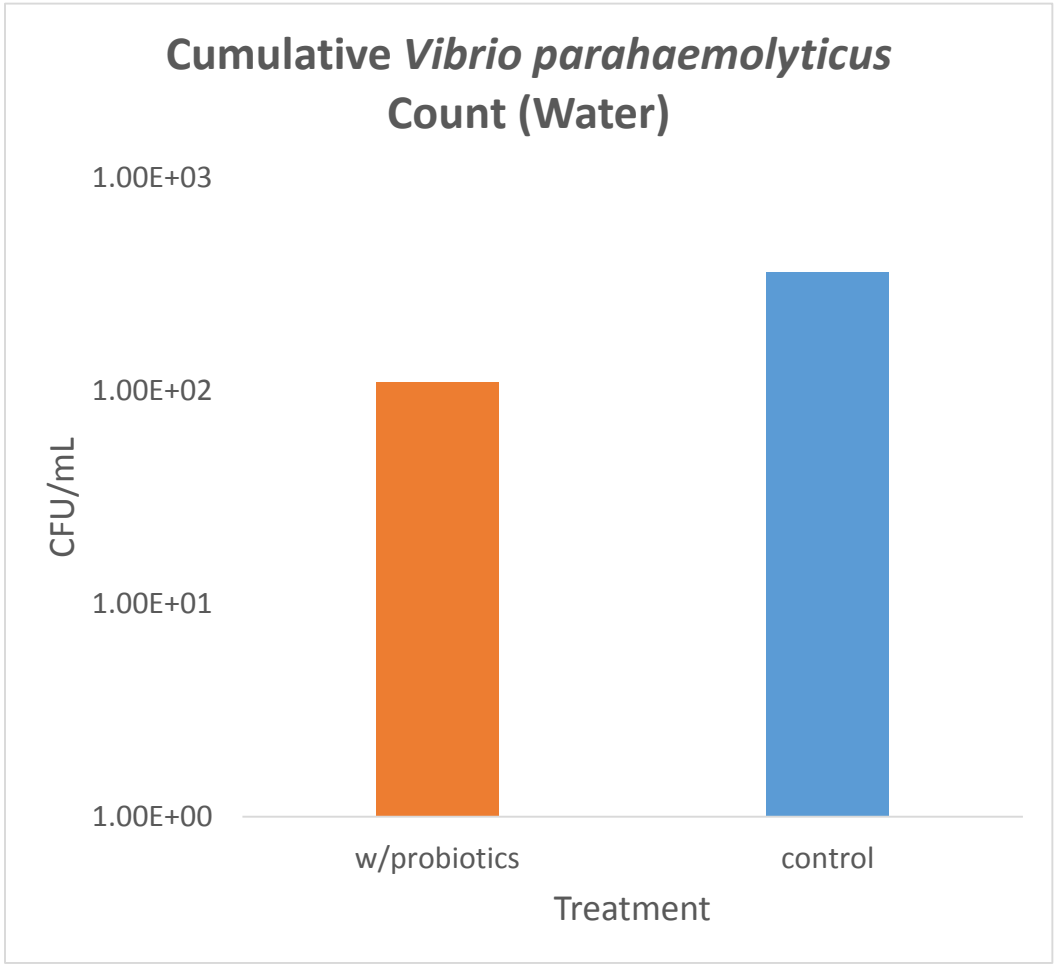
- BZT aqua/kg feeds
- Every 3 days interval after stocking

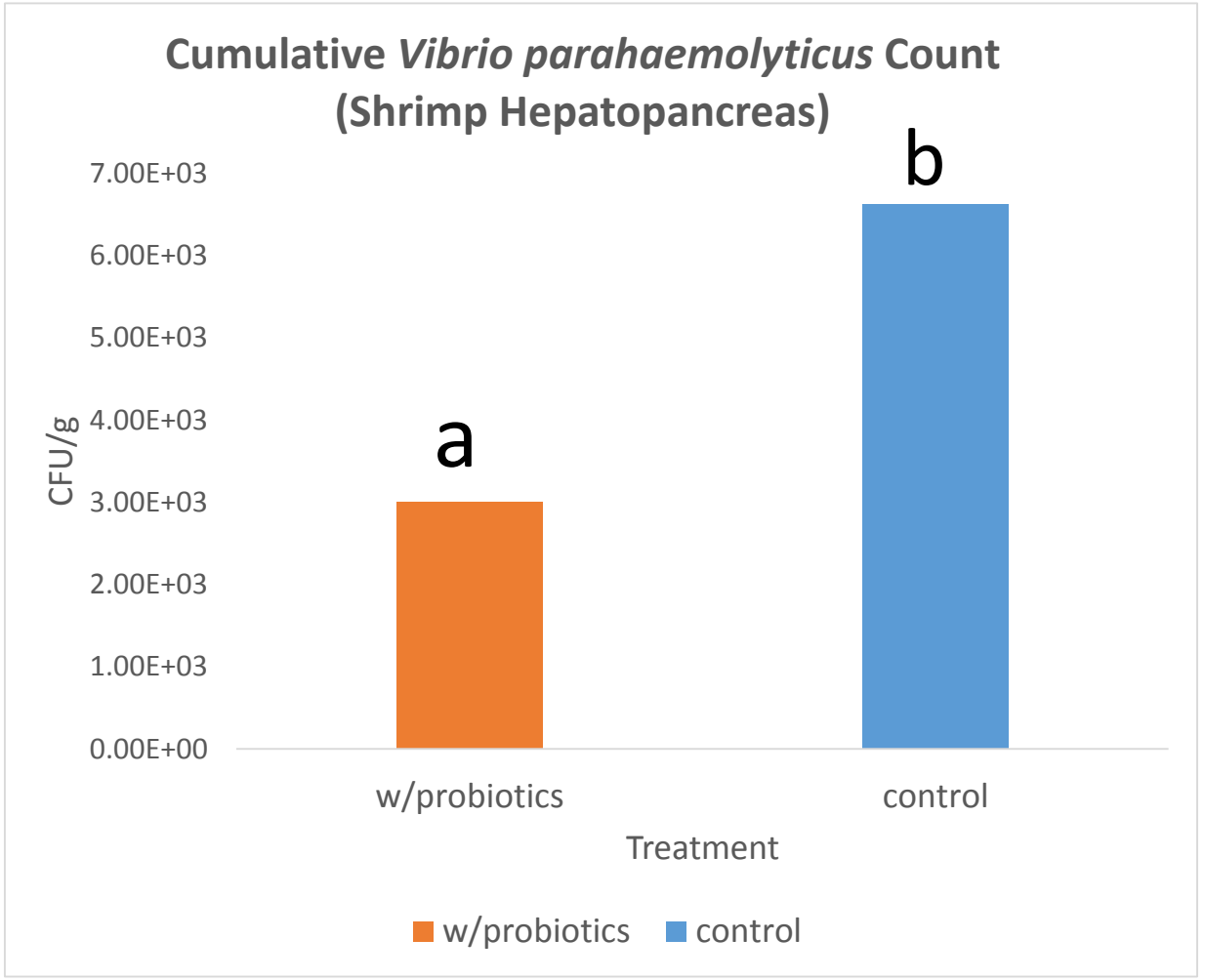
Cumulative Total Vibrio Count





Cumulative Total *Vibrio parahaemolyticus* Count





	(AquaPro-mY) Probiotics	Control (BZT®AQUA)
ABW	13.87g	14.98g
Percent(%) Weight Gain	593.5%	649.0%
FCR	1.55	1.58
Survival Rate	75.82%	77.57%
Total Harvest Biomass	5,260 kg	5,810 kg

Take Home Lessons

Application of Tilapia green water, Biofloc and Probiotics bacterial isolates in shrimp aquaculture inhibits pathogenic Vibrio population and lessens the risks of EMS/APHND occurrence in the culture. This technique is a practical approach to prevent EMS occurrence in cultured shrimp.

Thank You Very Much