ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(4): 173-178

www.fisheriesjournal.com
Received: 22-05-2016
Accepted: 23-06-2016

Chandroshakar Biswas
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh

Md. Mer Mosharraf Hossain
Assistant Professor, Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology, Jessore-7408, Bangladesh

Md. Hasan-Uj-Jaman
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh.

Hironmoy Sovon Roy
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh.

Md. Eftakher Alam
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh.

Sanjoy Banerjee Bappa
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh.

Aisha Khatun
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore-7400, Bangladesh

Correspondence
Md. Mer Mosharraf Hossain
Assistant Professor, Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology, Jessore-7408, Bangladesh

Dietary probiotics enhance the immunity of Thai pangas (*Pangasius hypophthalmus*) against *Pseudomonas fluorescens*

Chandroshakar Biswas, Md. Mer Mosharraf Hossain, Md. Hasan-Uj-Jaman, Hironmoy Sovon Roy, Md. Eftakher Alam, Sanjoy Banerjee Bappa and Aisha Khatun

DOI: https://doi.org/10.22271/fish.2016.v4.i4c.835

Abstract
The use of probiotics in aquaculture is now gaining a wide acceptance. This study was conducted to evaluate the commercially available probiotic Bioprob (PIC-BIO company, Tokyo, Japan) on the efficacy of its use on mean immune parameters and *Pseudomonas fluorescens* disease challenge in Thai pangas (*Pangasius hypophthalmus*) under laboratory conditions. The fishes were fed with five different experimental pellet diets enriched with 0 g, 0.5 g, 1 g, 1.5 g and 2 g Bioprob probiotic per kg feed that enhanced the immune potentiality investigated on weeks 1, 2 and 4. The phagocytic and bactericidal activity significantly increased in *P. hypophthalmus* fed with 2 g Bioprob probiotic per kg feed against *P. fluorescens* on weeks 2 and 4. After the feeding trial, the fishes were injected intraperiotonaly (IP) with 0.2 ml of 1.8 $\times 10^{-7}$ CFU ml^{-1} pathogenic *P. fluorescens*. The cumulative mortality was low in T_5 (2 g kg^{-1}) whereas high in T_2 (0.5 g kg^{-1}) as 68% compared to control (0 g kg^{-1}) as 84% against pathogen. Therefore, dietary probiotics with 2 g kg^{-1} feed were found to enhance the disease resistance of *P. hypophthalmus* against *P. fluorescens*.

Keywords: Probiotics, *Pangasius hypophthalmus*, *Pseudomonas fluorescens*, Immune responses, Phagocytic activity and Challenge test

1. Introduction
Pungus is a genus of medium-large to very large shark catfishes native to freshwater in South and Southeast Asia [1]. Under natural conditions, they are primarily benthic omnivores that derive nutrition from bacteria, detritus, vegetative material, macro algae, zooplankton, crustaceans and some fishes [2, 3]. These microorganisms are responsible for ulcer type diseases including ulcerative syndrome, bacteria haemorrhagic septicaemia, tail and fin rot, and bacteria gill rot and dropsy [4]. *Pseudomonas*, also known as ulcerous disease, is a common fish disease. It is an infectious disease caused by the water bacteria *Pseudomonas fluorescens* [5, 6, 7]. Disease can be sometimes fatal. Prevention and control of diseases have led to a substantial increase in the use of veterinary medicines in the recent years. However, the utility of antimicrobial agents and antibiotics as a remedial measure has been questioned [8]. The use of antibiotics as disease controllers and growth promoters is currently restricted or forbidden in many countries and a growing concern about the high consumption of antibiotics in aquaculture has initiated a definite need in which both consumer and manufacturer are looking for the alternative health management strategy, which can be accomplished by microbial intervention [9]. Many countries have banned the aquacultural products due to the presence of antibiotics residual. For this reason probiotics have been developed for use in aquaculture [10]. Dietary probiotics can be used as living cells but some studies have also shown their benefits when supplied as heat-inactivated cells (also known as heat-killed cells), formalin-killed (FKC), freeze-dried, dead cells or cell-free supernatant (CFS) [11]. Probiotics is used as fish diet and contribute the benefits to increase length and weight of organisms, bacterial control diseases, nutrients source, essential enzymes for better food digestion, elimination of organic matter and increase of immune response against pathogen organisms, reducing diseases risks and use of chemical drugs who pollute water habitat [12, 13]. The common probiotics used in aquaculture include a wide taxa range, since lactic bacteria (*Lactobacillus, Lactococcus, Bifidobacterium, Pediococcus, Carnobacterium*); bacilli bacteria (*Bacillus, Paenibacillus, Brevibacillus*) and different genus like *Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Roseobacter, Aeromonas, Nitrosomonas, Nitrobacter* and *Vibrio*; and yeast

like *Debaryomyces*, *Saccharomyces* [14]. The study highlights the role of probiotics in helping the fishes to fight against the different physical, chemical and biological stress. These can be reduced by the intervention in terms of bioremediation, vaccinnation, immunostimulants and probiotics when these are needed of the day [9]. The aim of this study is to know the disease resistance of thai pangas (*P. hypophthalmus*) against *P. fluorescens* using Bioprob (PIC-BIO, INC, Tokyo, Japan), as a the dietary probiotics.

2. Materials and Methods

2.1 Bioprob (dietary probiotic)

Bioprob (dietary probiotic) was purchased from Chancara Bazar, Jessore at Syfulla Krishi and Motsho Biponi. Bioprob was manufactured PIC-BIO Company, Tokyo, Japan. The specification and composition of bacterial strains of Bioprob was shown in Table 1.

Table 1: Specification and composition of bacterial strains of Bioprob.

Specification		Composition
Beta-xylanase	350 U / g min	*Bacillus subtillis, B. Licheniformes, Trichoderma viride, Nitrosomona europaea, Nitrobacter winogradskyi, Aspergillus oryzae, Rhodococcus, Rhodospirillum ubrum, Cyanobacteria, Pseudomonas denitrificans, Pseudomonas oxalacticus*
Total Bacillus count	2×10^9 CFU/ g min	
Moisture	4.0%	
Crude protein	16.8%	
Crude ash	56.7%	
Crude fat	3.0%	
Crude fiber	4.8%	

2.2 Fish and husbandry

Pangasius hypophthalmus, healthy Thai Pungus (n= 375 pieces) were collected from Kopothaksha hatchery at Chanchra, Jessore and fishes were immediately examined to find out their health status and acclimatized, transferred into in the quarantine tank (100 L) with recirculation aerated water for three days the laboratory of the Dept. of Fisheries and Marine Bioscience (FMB), in Jessore University of Science and Technology (JUST), Jessore on June 2014. The fishes were divided into five equal groups (T_1, T_2, T_3, T_4 and T_5) each with two replicate containing 25 fishes per replicate. Continuous aeration was provided to maintain dissolved oxygen level at 7.5±0.5 mgl^{-1} and one-third of the aquarium water was exchanged daily by siphoning the waste materials were removed. During the experimental period water temperature, pH and TDS (total dissolved solid) were 22±0.8 ^{0}C, 5.94±0.21 and 4.34±0.29 mgl^{-1} respectively. Fishes were provided with normal basal feed (Table.2) at the rate of 5% of their body weight twice a day at 09:00 and 17:00 hour for 3 days but at the first day of their arrival no feed was provided.

Fig 1: Map showing the study area

All fish groups were fed on the diets at the rate of 3% from the body weight during 6 weeks of the experiment. On the week of 2, 4 and 6 three fish per group were randomly collected from each group, used for blood collection for specific and nonspecific immunological assays. The experimental fish were challenged with a virulent strain of *Pseudomonas fluorescens* at 3×10^{-7} CFU mL^{-1} by injected intraperiotonaly with 25 µl PBS for analyzing cumulative mortality.

Table 2: Composition of basal diet.

Ingredients	Content (%)
Fish meal	50
Rice bran	24
Corn	15
White middling	2.5
Vitamin mixture	2
Mineral mixture	2
Soya oil	1.8
Gelatin	1.5
CMC (Carboxymethyl cellulose) Na	1.2

Table 3: Proximate composition of the experimental diet.

Ingredients	Content (%)
Crude protein	38
Crude fat	9
Moisture	7
Ash	14

2.3 Experimental diet preparation
The experimental diet was prepared by mixing with locally available Mega feed which proximately contains protein: 34%, crude fiber: 6%, crude ash: 18%, moisture: 11%, lipid: 6%, fat: 3% showing in table 3 (Source: Spectra fish feed Com. Ltd.). At first Mega feed was grinded by a grinder and mixed with Bioprob porobiotics powder. All the ingredients were mixed thoroughly by adding water and pelletized by hand and then sun dried. Five different experimental pellet diets were prepared which contained five different mixture of Bioprob probiotic such as 0 g (control), 0.5 g, 1 g, 1.5 g and 2 g per kg feed. The pellet feed was stored in a cool dry place until use.

2.4 Blood analysis (specific immune response)
Blood was drawn from the caudal peduncle region using sterile 2 mL syringes rinsed first with 2.7% EDTA solution as an anticoagulants from five groups separately and collected blood was kept in 1 mL eppendorf tube randomly selected and allowed to clot for 45 min in an inclined position at room temperature, followed by 30 min incubation at 4 ^{0}C and then centrifuged at 3000 rpm for 10 min at 4 ^{0}C. Serum was collected for each group three culture plates. Bacterial stock solution was serial diluted for 10 times and 10^{-3}, 10^{-4} and 10^{-5} concentration were selected for further usage. Then 25 µl volume from each diluted solution was mixed with 25 µl volume separated serum of five different groups of fishes then spread on the different culture plates and finally all plates were placed in an incubator at 37 ^{0}C for 24 hours. After 24 hours all plates were observed.

2.5 Immune response assay
The phagocytic activity was quantified according to the method of [15].

2.6 Phagocytic activity
For this assay 25 µl blood cell suspension of thai pangus and 25µl bacterial solution in PBS was previously fixed with glutarldehyde was placed on a coverslip. After 30 minute coverslip was carefully washed with PBS (Phosphate Buffer Solution) then air dried and fixed with methanol and after that stained with giemsa. The engulfed fish blood cell (phagocytic rate) was determined by using photographic microscope (Axiocam Erc 5s with Axio Vision driver Carl Zeiss, Germany).

2.7 Challenge study
Pseudomonas fluorescens was obtained from Central Biological Laboratory at Jessore University of Science and Technology, Bangladesh. *P. fluorescens* was grown on nutrient broth for 24 h at 30 ^{0}C the culture broth was centrifuged at 3000 RPM (Rotation Per Minute) for 10 min. The supernatant was discarded and the pellets were resuspended in sterile phosphate buffer saline (PBS, pH 7.4). The final bacterial concentration was adjusted to 10^{-7} CFU ml^{-1} by serial dilution. By the end of the feeding experiment, the fish of the experimental group and the control group was injected intraperiotonaly (IP) with pathogenic *P. fluorescens* 0.2 ml of 1.8×10^{-7} CFU (Colony Forming Unit) ml^{-1}. All groups were kept under daily observation for 7 days for any abnormal clinical signs with recording the daily mortality rate. The freshly dead fish were subjected to bacterial reisolation for confirmation. Mortality percentage was calculated by using the following formula:

$$\text{Percentage of mortality} = \frac{\text{No.of dead fish after challenge}}{\text{Total No.of injected fish}} \times 100$$

$$\text{Relative Percent Survival (RPS)} = 1 - \frac{\%\ \text{Mortality in treated group}}{\%\ \text{Mortality in control group}} \times 100$$

2.8 Statistical analysis
The mean and standard error were calculated for each variable. The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at (P<0.05) using SPSS software statistical program (SPSS).

3. Results and Discussion
The current study aimed to find out the disease resistance power of *P. hypophthalmus* against *P. fluorescens* by using dietary probiotics. The effect of probiotics on the immune systems in aquatic animal has not been established though it has been used as an important product for boosting the defense mechanisms. Probiotics as live microorganisms can be good alternatives to chemotherapy [16 1718] since they have stimulation effect of non-specific host defences mechanisms, increases the disease resistance and growth promotion [12].

3.1 Serum bactericidal activity (Specific immunity)
Immune response level significantly increased with diets on week 2 and 4. Immune response level significantly increased with diets on week 2 and 4.

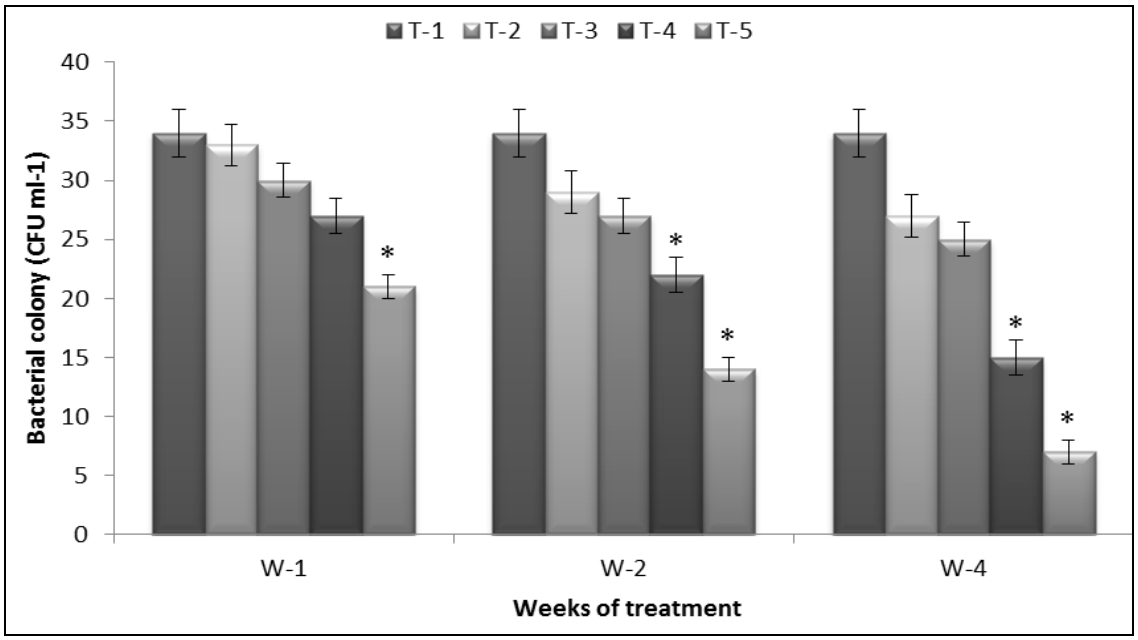

Fig 2: Bacterial activity of serum of thai pangas fed dietary probiotics against *P. fluorescens.* [* indicates relatively significant (*P*<0.05)]

[19] Who observed that *Bacillus* sp. provided disease protection to shrimp by triggering both cellular and humoral immune defenses? Immune response level did not significantly change in control (Fig. 2.). [20] Have stated that probiotic use can enhance the immune response of tilapia and improve disease resistance. The implementation and application of probiotics in diets for aquatic animals is suggested as a prevention measure of diseases [21] and increase of immune response to allow better survival when illness were shown [22, 23].

3.2 Phagocytic activity (Non-specific immunity)

Phagocytic activity did not significantly enhance with T_2 (0.5g kg-1)**,** T_3 (1g kg-1), T_4 (1.5g kg-1), T_5 (2g kg^{-1} feed) diet on first week against *P. fluorescens*.

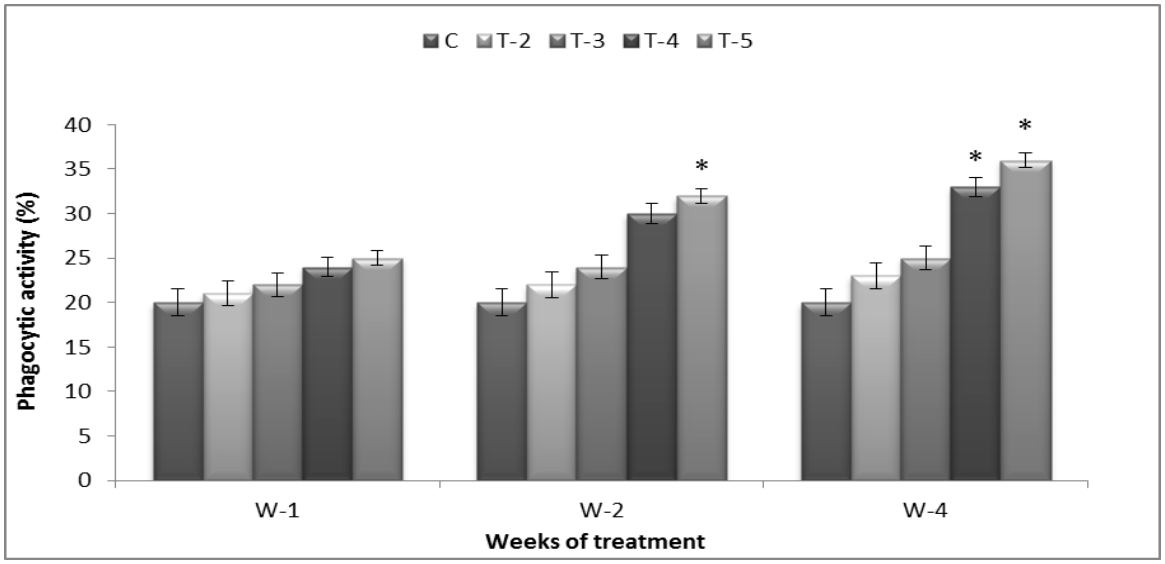

Fig 3: Phagocytic activity (%) of thai pangas fed dietary probiotics against *P. fluorescens.* [* indicates relatively significant (*P*<0.05)]

However with T_4 (1.5g kg-1) and T_5 (2g kg^{-1} feed) doses the activity significantly increased on week 2 and 4 but T_2 (0.5g kg-1) and T_3 (1g kg-1) doses are not showed same result as compared with T_1-control (84%) (Fig**.** 3.). Nile tilapia was reported that the probiotics-treatment stimulated the non-specific immune parameters, resulting in the enhancement of fish resistance against *Edwardsiella tarda* infection showed same results. Similar results were previously recorded by [24] who studied the non-specific immune system of Nile tilapia and reported that the probiotics-treatment stimulated the non-specific immune parameters against *Edwardsiella tarda* infection. The stimulation of the nonspecific immune system showed the same results which has been observed from [25, 26, 27] there investigations. This finding is supported by [28] results which are indicated that the survival of larvae of sea bass fed 1.1% live yeast was significantly higher than the control.

3.3 Disease resistance (Challenge test)

The cumulative mortality was lowest (Fig. 4.) 16% at T_5 (2g kg^{-1} feed) compared with T_1-control (84%) and other treatments, which were 32%, 52% and 68% in case of T_4 (1.5g kg^{-1}), T_3 (1g kg^{-1}), T_2 (0.5g kg^{-1}). In this study challenge with dietary probiotics (Bioprob) against *P. fluorescens* of thai pangas (*P. hypophthalmus)* have showed 84% survivability and 81% Relative Percent Survival (RPS) at T_5 for 30 days which was higher than other treatments (Table 4).

Table 4: Treatment challenge of dietary probiotics against *P. fluorescens* in Thai pangus.

Treatment	Challenge Dose (cfu) ml^{-1}	Total fish	No. of infected fish	No. of dead fish	Mortality (%)	Survivality (%)	RPS (%)
T_1-control	1.8×10^{-7}	25	23	21	84	16	--
T_2 (0.5g kg^{-1})	1.8×10^{-7}	25	21	17	68	32	19
T_3 (1g kg^{-1})	1.8×10^{-7}	25	19	13	52	48	38
T_4 (1.5g kg^{-1})	1.8×10^{-7}	25	14	8	32	68	62
T_5 (2g kg^{-1})	1.8×10^{-7}	25	9	4	16	84	81

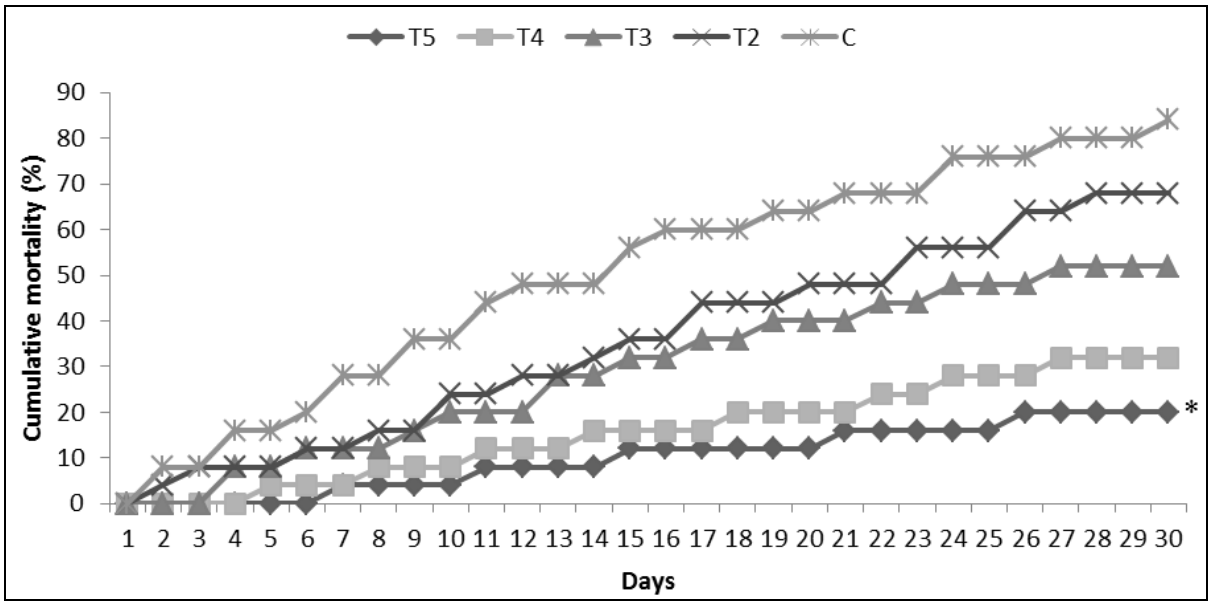

Fig 4: The cumulative mortality of thai pangas fed with different doses of dietary probiotics against *P. fluorescens.* [* indicates relatively significant (P<0.05)]

Lower mortality was observed in Atlantic salmon vaccinated against *Aeromonas salmonicida* [29, 30], in turbot vaccinated against *Enterococcus sp.* [31], in yellowtail challenged with *Enterococcus seriolicida* [32], and in swordtails, rosy barbs and black tetras challenged with *Aeromonas hydrophila* or *Pseudomonas fluorescens* [33] fed beta-glucans. In this study challenge with dietary probiotics (Bioprob) against *P. fluorescens* of thai pangas (*P. hypophthalmus)* have showed 84% survivability and 81% RPS at T_5 for 30 days which was higher than other treatments which was similar with the effectiveness of probiotics has also been demonstrated to be as a result of enhanced immunity [34, 35].

4. Acknowledgement

The authors would like to thank Professor Dr. Md. Anisur Rahman, Chairman, Department of Fisheries & Marine Bioscience and Md. Iqbal Kabir Zahid, Chairman, Department of Microbiology, Jessore University of Science and Technology, Jessore, Bangladesh for permeating the research team to carry out the experimental work in the laboratory.

5. References

1. Kulabtong S. Biology and culture techniques of bocourti catfish, *Pangasius bocourti* Sauvage, 1880 in Thailand. Veridian E-Journal. 2012; 5(3):411-419.
2. Kunlapapuk S, Thapanand-Chaidee T. Daily ration of Bocourti's catfish (*Pangasius bocourti* Sauvage, 1880) in Mekong River, Nongkhai Province. Journal of fishery technology research. 2009; 3:6.
3. Sirikul C, Prarom V. Culture and nursing of bocourti catfish, *Pangasius bocourti* Sauvage, 1880 larva. Technical paper, Chiangrai Inland fishery Station, Department of Fisheries, Thailand 1995, 23.
4. Cao H, Baldini RL, Rahme LG. Common mechanisms for pathogens of plants and animals. Annual Review Phytopathology. 2001; 39:259-284.
5. Mermod N, Harayama S, Timmis KN. New route to bacterial production of indigo. Biological Technology. 1986; 4:321-324.
6. Ramos JL, Wasserfallen A, Rose K, Timmis KN. Redesigning metabolic routes: Manipulation of TOL plasmid pathway for catabolism of alkylbenzoates. Science. 1987; 235:593-596.
7. Cases I, Lorenzo V. Expression systems and physiological control of promoter activity in bacteria. Current Opinion on Microbiology. 1998; 1:303-310.
8. Balcazar JL, Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Muzquiz JL. The role of probiotics in aquaculture. Veterinary Microbiology. 2006; 114:173-186.
9. Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S, Sugita H. The Viability of Probiotic Bacteria as a Factor Influencing the Immune Response in Rainbow Trout Oncorhynchus mykiss. Aquaculture. 2005; 243:241-254.
10. Brunt J, Austin B. Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of Fish

Diseases. 2005; 28:693-701.
11. Cordero H, Esteban MA, Cuesta A. Use of Probiotic Bacteria against Bacterial and Viral Infections in Shellfish and Fish Aquaculture. Journal of Invertebrate Pathology. 2011; 96:239-266.
12. Irianto A, Austin B. Use of Probiotics to Control Furunculosis in Rainbow Trout (*Oncorhynchus mykiss*, Walbaum). Journal of Fish Diseases. 2002; 25:333-342.
13. Burr G, Hume M, Neill WH, Gatlin DM. Effects of prebiotics on nutrient digestibility of soybean-meal-based diets by red drum (*Sciaenops ocellatus*). Aquaculture Research. 2008; 39:1680-1686.
14. Nikoskelainen S, Ouwehand AC, Salminen S, Bylund G. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillu rhamnosus*. Aquaculture. 2001; 198:229-236.
15. Cobaud PA, Warblewski T. Colorimeteric determination of lactic acid dehydrogenase of body fluids. American Journal of Pathology. 1958; 10:234-236.
16. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*. 2008; 274:1-14.
17. El-Rhman AMA, Khattab YA, Shalaby AM. *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, Oreochromis niloticus. Fish Shellfish Immunology. 2009; 27:175-180.
18. Giri SS, Sen SS, Sukumaran V. Effects of dietary supplementation of potential probiotic *Pseudomonas aeruginosa* VSG-2 on the innate immunity and disease resistance of tropical freshwater fish, Labeo rohita. Fish and shellfish immunology. 2012; 32:1135-1140.
19. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasveta P. Probiotics in Aquaculture: a Case Study of Probiotics for Larvae of the Black Tiger Shrimp (*Penaeus monodon*). In Flegel TW (Ed) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok. 1998; 1:1-5.
20. Merrifield DL, Harper GM, Rawling MD, Mustafa S. The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology. 2010; 109:851-862.
21. Lara-Flores M, Olivera-Castillo L, Olvera-Novoa MA. Effect of the inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus*), and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). International Journal of Fish Aquaculture. 2010; 2:93-101.
22. Aboagye D. Evaluation of the commercially-available probiotic Lymnozyme as an effective control of bacterial infections in channel catfish. MS Thesis. Department of Fisheries and Allied Aquacultures, Auburn University, Alabama, 2008.
23. Mesalhy ASM, Yousef AGA, Ghareeb AAA, Mohamed MF. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish and Shellfish Immunology. 2008; 25:128-136.
24. Taoka Y, Maeda H, Jo J, Kim SM, Park SI, Yoshikawa T, *et al*. Use of Live and Dead Probiotic Cells in Tilapia Oreochromis niloticus. Fisheries Science. 2006; 72:755-766.
25. Kulkarni AD, Fanslow WC, Rudolph FB, Van Buren CT. Effect of dietary nucleotides on response to bacterial infections. Journal of Parental Enteric Nutrition. 1986; 10:169-171.
26. Secombes CJ, Fletcher TC. The role of phagocytes in protective mechanism of fish. Annual Review of Fish Diseases. 1992; 2:53-71.
27. Carver JD. Dietary nucleotides: cellular immune, intestinal and hepatic system effects. Journal of Nutrition. 1994; 124(1):144-148.
28. Tovar-Ramirez D, Zambonino J, Cahu C, Gatesoupe FJ, Vazquez J. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture. 2004; 234:415-427.
29. Nikl L, Evelyn TPT, Albright LJ. Trials with an orally and immersion-administered beta-1, 3 glucan as an immunoprophylactic against *Aeromonas salmonicida* in juvenile chinook salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms. 1993; 17:191-196.
30. Rorstad G, Aasjord PM, Robertsen B. Adjuvant effect of a yeast glucan in vaccines against furunculosis in Atlantic salmon (*Salmo salar* L.). Fish & Shellfish Immunology. 1993; 3:179-190.
31. Toranzo AE, Devesa S, Romalde JL, Lamas J, Riaza A, Leiro J, *et al*. Efficacy of intraperitoneal and immersion vaccination against *Enterococcus sp*. infection in turbot. *Aquaculture*. 1995; 134:17-27.
32. Iman MK, Abbas WT, Awaad ES, Authman MMN, El-Shafei K, Sharaf OM, *et al*. Evaluation of *Lactobacillus plantarum* as a probiotic in aquaculture: Emphasis on growth performance and innate immunity. Journal of Applied Sciences Research. 2013; 9(1):572-582.
33. Tuernau D, Schmidt H, Kuerzinger H, Boehm KH. Potency testing of beta-glucan immunostimulating effect in food for ornamental fish. Bulletin of European Association of Fish Pathology. 2000; 20:143-147.
34. Johnson-Henry KC, Donato KA, Shen-Tu G, Gordanpour M, Sherman PM. *Lactobacillus rhamnosus* strain GG prevents enterohemorrhagic *Escherichia coli* induced changes in epithelial barrier fuction. Journal of Infection and Immunity. 2008; 76:1340-1348.
35. Welker TL, Lim C. Use of probiotics in diets of tilapia. Journal of Aquaculture Research and Development. 2011, 8.