A Seminar Paper

On

Role of *Lactobacillus* as a Probiotic in Fish

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ABSTRACT

Proper performance of fish in morphological, physiological and immunological aspects is necessary for fish production. But several inhibitors like disease, pathogen, and adverse environment can overpower these performances. At present, antibiotics regarding preventing these inhibitors have been seen becoming favorable to those inhibitors. So, *Lactobacillus*, an important probiotic group of bacteria can be an alternative to these antibiotics regarding proper performance of fish in mentioned aspects. *Lactobacillus* have been seen used in different experiments, mainly as a supplement in feed at various concentration. These experiments show positive results like improved growth with minimum cost, improvement in reproduction, haematology, immunology, disease resistance as well as better proximate composition in selective fishes. So many more experiments should be conducted in commercially important fishes for better growth and health of fishes which will certainly increase fish production.

Key Words: Probiotic, *Lactobacillus*, Antibiotics, Fish.

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[1]A seminar paper for the course BTL 598, winter'17.

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Chapter 1

INTRODUCTION

Lactobacillus bacteria are a major part of the lactic acid bacteria (LAB) group which convert sugars to lactic acid. In humans, they constitute a significant component of the microbiota at a number of body sites, such as the digestive system, urinary system, and genital system. *Lactobacillus* forms biofilms in the gut microbiota to allow them to persist during harsh environmental conditions and maintain ample populations (Salas-Jara*et al.*, 2016).*Lactobacillus* exhibits a mutualistic relationship with the host body to protect the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients (Martin *et. al.*, 2013).*Lactobacillus* is the most common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being.

The Greek word probiotic means "for life", was introduced by Parker (Parker, 1974). According to him, probiotics are "Organisms and substance, which contribute to intestinal and microbial balance". A broader definition of the term probiotic is defined as a live microbial adjunct which has a beneficial effect on the host by modifying the host – associated microbial community, by ensuring improved utilization of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment (Verschuere*et al.*, 2000). According to the currently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (Yadav*et al.*, 2016).

Now a days, Probiotics are commonly used as therapeutic, prophylactic and growth supplements for fish (Pandiyan*et al.*, 2013). Probiotics are used in aquaculture to improve growth performance (Silva *et al.*, 2013), nutrition *(*Zhou *et al.*, 2009), to decrease diseases (Irianto*et al.*, 2002) and to develop immune system (Nayak*et al.*, 2007). Apparently, Lactic Acid Bacteria have been broadly used as probiotic strains which are usually present in the intestine of healthy fishes such as the *Lactobacilli* and *Bifidobacteria* (Pandiyan*et al*., 2013).

In case of disease occurrence, antibiotics are being used as traditional strategy for the prevention and control of diseases and fish growth. However, the development of antibiotic resistant pathogens were recognized but there is a huge risk of transmission of resistance bacteria from aquatic environment to human (Pandiyan*et al*., 2013). As per them, using antibiotic is harmful for aquatic animals because it kills beneficial microbiota in gastrointestinal system of animals and it also accumulates in fish products to be unsafe for human consumption. Chemotherapeutic treatment may cause the development of resistant bacteria (Aoki *et al*, 1985). Also the yield residues in fish can introduce potential hazard to public health and there is a high possibility of leaving antibiotic residues both in the fish and in the environment (FAO/WHO/OIE, 2006).

Considering these factors, the improvements of non-antibiotic agents are more suitable for health management in this culture. Dietary supplement such as probiotic provides nonspecific disease protection and also acts as growth promoting factors (Denev, 2008).

As a main group of probiotic, Lactic Acid Bacteria are being used as a probiotic where *Lactobacillus*is widely used probiotic for fish. It is used in fish nutrition to improve growth, survivability, feed efficiency, and also prevention intestinal disorders and neutralize of anti-nutritional factors present in the feedstuffs (Suzer *et al.*, 2008). They are also applied to increase microbial monitoring, growth and feed efficiency (Panigrahi *et al.*, 2005).

In the current review, the main focus is on *Lactobacillus* that are being used as probiotic for the immense welfare of fish and fish production.

Objectives of the Study

i. To review the potential roles of *Lactobacillus* as a probiotic.

ii. To review the scientific experiment performed for establishing the beneficial roles of *Lactobacillus* as a probiotic in fish physiology and morphology.

Chapter 2

MATERIALS AND METHODS

This seminar paper is a review paper. All the information have been collected from the secondary sources. During the preparation of the review paper, I collected various information from various relevant books, journals, proceedings, reports, and internet. Apart from those, my major professor as well as course instructors also provided much valuable suggestions for the preparation of this seminar paper. After collecting all the available information, I myself compiled the collected information and finally prepared this seminar paper.

Chapter 3

REVIEW OF FINDINGS

The details on the assigned topic so far extracted and reviewed are discussed below under different sub-headings.

3.1 *Lactobacillus*

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria (Makarova *et al.*, 2006).The genus *Lactobacillus* currently contains over 180 species and encompasses a wide variety of organisms (Vancanneyt *et al.*, 2005). The genus is polyphyletic, with the genus *Pediococcus* dividing the *L. casei* group, and the species *L. acidophilus*, *L. salivarius*, and *L. reuteri* being representatives of three distinct subclades. The genus *Paralactobacillus* falls within the *L. salivarius* group. More recently, the *Pediococcus* species *P. dextrinicus* has been reclassified as a *Lactobacillus* species (Haakensen *et al.*, 2009).

According to metabolism, *Lactobacillus* species can be divided into three groups:

- ❖ Obligately homofermentative (group I) including:
    - *L. acidophilus, L. delbrueckii*, *L. helveticus*, *L. salivarius*
- ❖ Facultatively heterofermentative (group II) including:
    - *L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*
- ❖ Obligately heterofermentative (group III) including:
    - *L. brevis*, *L. buchneri*, *L. fermentum*, *L. reuteri*.

3.2 Probiotics

Probiotics can be defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989). It can also be defined as “a viable micro-organism which when ingested through the oral cavity in a sufficient quantity confer on the host a beneficial effect due to an improvement in the intestinal microbial balance” (Giorgio *et al*., 2010). A good definition for application for aquaculture is “a live, dead or component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving either disease resistance, health statues, growth performance, feed utilization, stress response or general vigor, which is achieved at least in part via improving the hosts microbial balance or the microbial balance of the ambient environment” (Merrifield *et al*., 2010c). Another proposed definition of probiotics used in aquaculture is “live microbial cultures added to feed or environment (water) to increase viability (survival) of the host” (Ringo *et al*., 2010). They are also referred to as bio-proteins containing living microbial cells that optimize the colonization and composition of the growth and gut microflora in animals and stimulate digestive processes and immunity (Dhanaraj *et al*., 2010). Probiotics may minimize the incidence of diseases or lessen the severity of outbreaks in aquaculture or fish culture. It can be used as alternative to microbial chemotherapeutics. They are primarily used as feed additives to prevent infectious intestinal diseases through the secretion of micro-toxins that inhibit the growth of other virulent micro-organism (such as *Escherichia coli* and *Salmonella*) in the intestinal lumen (Barth*et al*., 2009). A definition of probiotic to be considered appropriate for aquaculture is given in a review by Merrifield *et al*. (2010) which is “any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part at least, by improving the microbial balance of the fish”. In this context, they regard direct benefits to the host as immuno-stimulants, improved disease resistance, reduced stress response, improved gastro intestinal morphology and benefits to the fish farmer or consumer. These benefits to the fish farmer or the consumer include improved fish appetite, growth performance, feed utilization, improvement of carcass quality, flesh quality and reduced malformations. Probiotic species provide energy for indigenous bacteria to proliferate and thereby to utilize ammonia and branch chain fatty acids for the synthesis of protein (Fujieda *et al.*, 2001). The mucosal immune system has to retain the ability to respond actively to pathogens, while avoiding active potentially inflammatory responses to pathogens (Bailey, 2009).

3.3 Characteristics of a probiotic

A probiotic organism must have following characteristics (Capriles *et al.*, 2005):

i. Resistant to the acidic stomach environment, bile and pancreatic enzymes.
ii. Accession to the cells of the intestinal mucosa.
iii. Capable of colonization.
iv. Staying alive for a long period of time, during the transport, storage, so that they can colonize the host efficiently.
v. Capable of producing antimicrobial substance against the pathogenic bacteria.
vi. Absence of translocation
vii. Should be non-pathogenic and non-toxic.

3.4 Mechanisms of action of Probiotics

The mechanisms of action of bacteria used as probiotics, although not yet fully elucidated, are described as following (Balcázar *et al.*, 2006):

i. Competition for binding sites: It is also known as "competitive exclusion", where probiotics bacteria bind with the binding sites in the intestinal mucosa, forming a physical barrier, preventing the connection by pathogenic bacteria;
ii. Production of antibacterial substances: Probiotic bacteria synthesize compounds like hydrogen peroxide and bacteriocins, which have antibacterial action, mainly in rela-

tion to pathogenic bacteria. They also produce organic acids that lower the environment's pH of the gastrointestinal tract, preventing the growth of various pathogens and development of certain species of *Lactobacillus*;

iii. Competition for nutrients: The lack of nutrients available that may be used by pathogenic bacteria is a limiting factor for their maintenance;

iv. Stimulation of immune system: Some probiotics bacteria are directly linked to the stimulation of the immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon.

3.5 Administration of *Lactobacillus* as Probiotic in Fish

Administration of probiotic strains such as *Lactobacillus*to fish is generally carried out by adding viable *Lactobacillus* to the feed (Bucio *et al*., 2009). Choosing a strain will depend on the fish species, rearing conditions and desired outcome of supplementation such as immunostimulation, disease prevention, improved growth performance etc. For successful application of probiotic strains as microbial ingredients in fish, other characteristics seem to be essential, such as high viability during processing, storage and after gastro-intestinal transit (Bucio *et al*., 2009).

Another way of administering probiotic strains could be the use of rotifers as carriers or by inoculating the rearing water with live bacteria, as the sowing of the gut with harmless bacteria may prevent infection by pathogenic bacteria with optimum doses (Ringo *et al.*, 1999).

3.6 Roles of *Lactobacillus* as a probiotic in Fish

Lactobacillus can contribute a lot to the internal physiological, morphological, hematological, immunological condition of fish by being present in the fish through the administration at an optimum level. The findings of the roles of *Lactobacillus* in fish as a probiotic are being illustrated below.

3.6.1 Growth Performance

Growth performance were tabulated in Table 1 showing that the final weight, weight gain, Specific Growth Rate and Feed Conversion Ratio of *Catla catla* increased significantly when lots were fed with a diet containing rational or mixed bacteria of *Lactobacillus*. Probiotic bacteria were supplemented in basal diet to conduct 7 treatments: base diet untreated (control – C); base diet mixed probiotic bacteria test as follows: *L. delbrueckii* subsp.*lactis* (P_1), *L. rhamnosus* (P_2), *L. Bulgaricus* (P_3).

Table 1. Growth parameters of the fish treated with 3 different *Lactobacillus*probiotic bacteria

Treatments	Initial weight (g/Fish)	Final Weight (g/Fish)	Weight Gain (g/Fish))	Specific Growth Rate (%)	Feed Conversion Ratio
Control (C)	5.55	6.75	1.20	1.33	8.500
P_1	5.60	7.22	1.62	1.80	6.296
P_2	5.56	7.66	2.10	2.33	4.857
P_3	5.53	7.66	2.13	2.36	4.788
P_1+P_2	5.50	7.27	1.77	1.96	5.762
P_1+P_3	5.90	7.56	1.66	1.84	6.144
P_2+P_3	5.54	7.84	2.30	2.55	4.434

(Source: Ravi *et al.*, 2013).

In single probiotic feed experiments P_2& P_3 shows highest growth while comparing to other single probiotic treatment. Control group (C) shows decreased values while comparing to other groups. In case of mixed trial, mixture of P_2 and P_3 shows highest rate of weight gain, SGR and FCR. This findings are being represented in a graph (Figure 1).

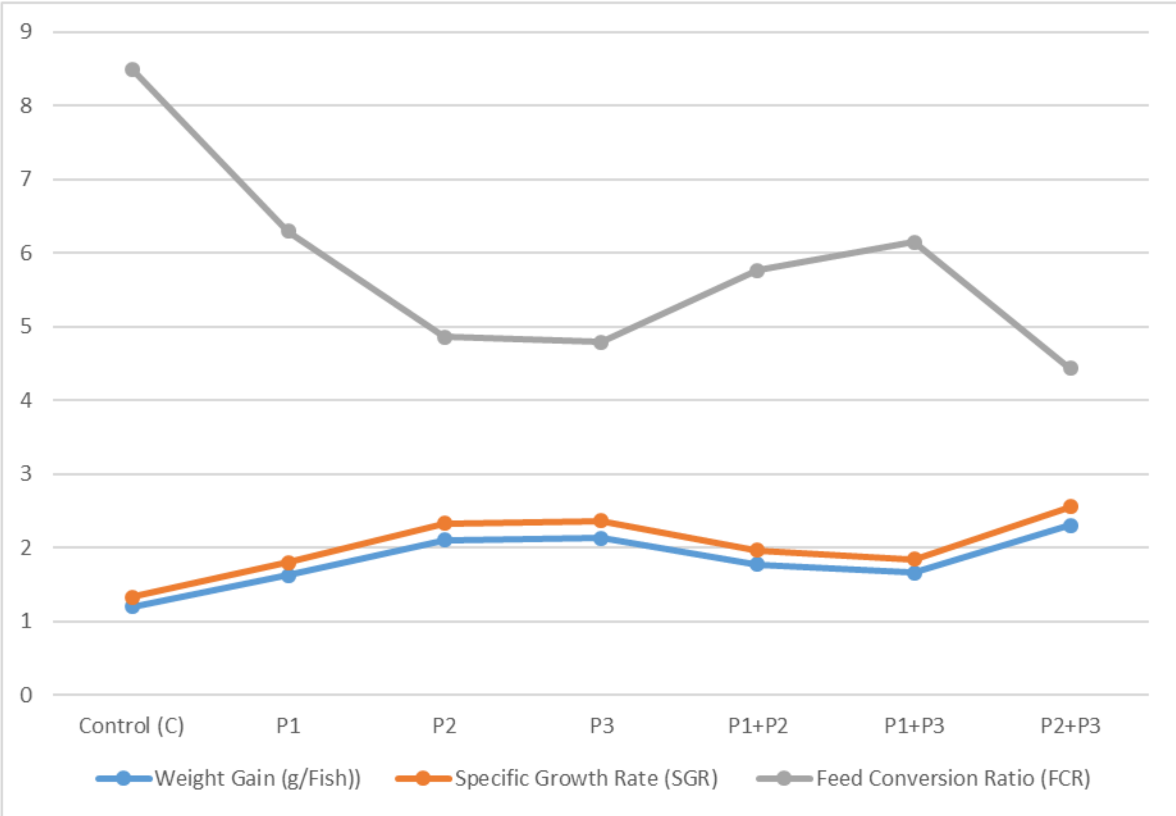

Figure 1: Graphical representation of weight gain, SGR and FCR of *Catla catla* in case of supplementation with 3 different *Lactobacillus* bacteria; *L. delbrueckii* subsp.*lactis* (P_1), *L. rhamnosus* (P_2), *L. Bulgaricus* (P_3).

(Source:Rav*iet al.*, 2013)

In another experiment, *Oreochromis niloticus* fry were supplemented with different treatment of *Lactobacillus*probiotic under laboratory conditions for 70 days without the occurrence of disease (Garg, 2015). The treatments were D_0 (control), D_1 (0.25% probiotic mix), D_2 (0.50% probiotic mix), D_3 (0.75% probiotic mix) and D_4 (1.00% probiotic mix in feed). Highest SGR was found in D_3 treatment while increasing the dose resulted in growth depression and nutrient depletion. Lowest FCR value was found in D_3 treatment rather than others (Table 2).

Table 2. Effect of different levels of probiotics (*Lactobacillus*) supplement on growth performance in *Oreochromis niloticus* fry under laboratory conditions for 70 days treatment

Diets	Parameters					
	Initial Weight (g/Fish)	Initial length (cm/Fish)	Final weight (g/Fish)	Final Length (cm/Fish)	Specific growth rate (%)	Feed conversion ratio
D_0 (Control)	1.22 ± 0.02a	4.00 ± 0.05a	3.02 ± 0.03e	5.77 ± 0.07d	1.29 ± 0.01e	2.07 ± 0.08a
D_1	1.29 ± 0.04a	4.25 ± 0.06a	3.53 ± 0.02d	6.12 ± 0.10 cd	1.44 ± 0.04d	1.99 ± 0.05a
D_2	1.23 ± 0.01a	4.14 ± 0.07a	5.44 ± 0.06b	6.87 ± 0.12b	2.12 ± 0.02a	1.95 ± 0.05a
D_3	1.15 ± 0.03a	4.09 ± 0.06a	6.79 ± 0.24a	7.72 ± 0.12a	2.53 ± 0.06b	1.74 ± 0.08b
D_4	1.20 ± 0.02a	4.20 ± 0.06a	4.13 ± 0.05c	6.19 ± 0.09c	1.76 ± 0.03c	2.04 ± 0.08a

(Source: Garg, 2015)

Based on this experiment, the relationship between different diets and SGR can be graphically illustrated in Figure no 2.

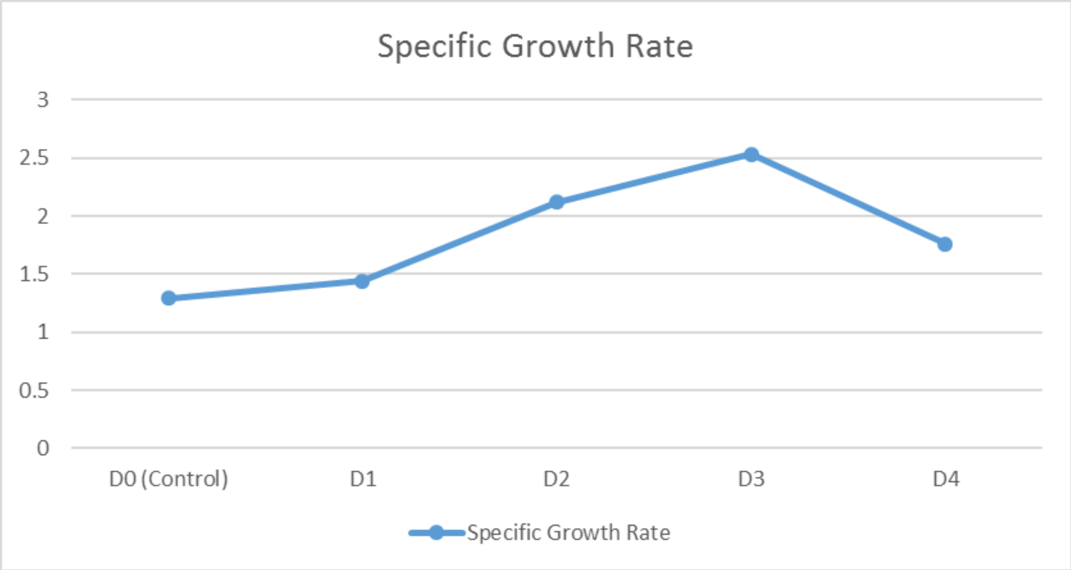

Fig 2: Relationship between dietary probiotics supplement level (D_0, D_1, D_2, D_3 and D_4) and specific growth rate in *Oreochromis niloticus* fry.

(Source: Garg, 2015).

3.6.2 Reproductive Contribution

In zebrafish (*Danio rerio*), administration of *Lactobacillus rhamnosus* induced a significant increase in both estradiol receptor and vtg gene expression which in turn usually results in significant increase in cyp19a expression at the ovarian level (Figure 3) (Gioacchini *et al*., 2011). This gene codes for the enzyme responsible for converting androgen into estrogen. This event resulted in higher GSI (Gonadosomatic Index) found in fish administered with *L. rhamnosus*(Figure 3) and showed the involvement of *L. rhamnosus* in follicle growth.

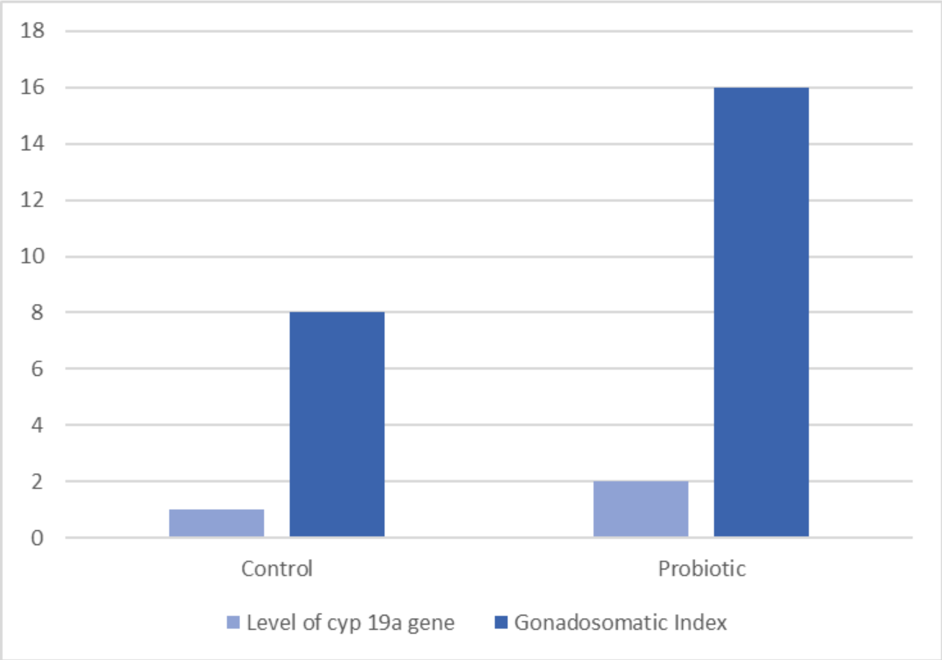

Figure 3:Values of cyp 19a gene and Gonadosomatic Index (GSI) values of zebrafish (*D. rerio*) females fed on *L. rhamnosus* and control diet.

(Source: Gioacchini *et al*., 2011).

The addition of *Lactobacillus* sp. in the swordtail, *Xiphophorous helleri* increased the fry production and reduced the fry mortality during spawning (Abraham *et al*., 2007). As seen in Table 3, significant differences existed in respect of average fecundity per female and average number of fry survived per female. The average fecundity/female and average number of fry survived/female was high in *Lactobacillus* sp inoculated group than in control. The percentage survival of fry was more in *Lactobacillus* sp inoculated group (97.51) than the control (91.16) group thereby reflecting its positive influence on reproductive performance of *X. helleri*.

Table 3. The growth parameters and reproductive performance of *Xiphophoroushelleri*reared in the presence of probiotic treatment and control

Observations	*Lactobacillus* treatment	Control
Average Fecundity (number/female)	53.00 ± 23.57a	39.42 ± 18.72a
Total number of fries	1484	1222
Average Number of Fry survived (number/female)	51.68 ± 22.87b	35.94 ± 18.18 b
Survival Rate of Fry during Spawning (%)	97.51	91.16

(Source: Abraham *et al*., 2007).

3.6.3 Haematological and Immunological Contribution

The haematology parameters monitored of *C. gariepinus* fingerlings fed diets supplemented with and without *L. acidophilus* at the end of the 12-week culture period are presented in Table 4 (Al-Dohail*et al.*, 2009). Significant differences were observed in the haematology parameters between the two treatments. The results indicated that the haematology parameters like haemoglobin, Red Blood Cells (RBC), White Blood Cells (WBC) were higher in the fish supplemented with *L. acidophilus* rather than the diet with null L. acidophilus. So the fish with the probiotic diet were healthier than the other one. Total immunoglobulin was significantly higher in fish fed the probiotics supplemented diet than in the control diet over the 12-week culture period (Table 4).

Table 4. Haematology/immunology parameters of *C. gariepinus* fed diet supplemented with probiotic *Lactobacillus acidophilus* and control diet for 12 weeks

Haematological Parameters	Treatment	
	Control	Probiotic
Haemoglobin (g dL^{-1})	8.46 ± 0.46a	9.62±0.45b
RBC (cells × 10^6 mm^{-3})	2.78 ± 0.05a	3.10 ± 0.08 b
WBC (cells × 10^3 mm^{-3})	23.00 ± 0.82 a	25.00 ± 0.82 b
Immunoglobulin (mg/mL)	7.40 ± 0.33a	9.50 ± 0.14b

(Source: Al-Dohail*et al.*, 2009)

3.6.4 Disease Resistance

An in-vitro experiment regarding probiotic effectiveness against certain fish pathogen was conducted by Jawahar Abraham, Surendra Babu and Tirthankar Banerjee (Abraham *et al*., 2007). *Lactobacillus* sp. was experimented by cross streak technique against 18 opportunistic fish pathogenic strains isolated from ornamental fish. It was stated that *Lactobacillus* sp P21 was quite effective against *Aeromonas hydrophila*, *Bacillus* spp, *Pseudomonas* spp and *Citrobacter freundi* (Table 5). Many of these strains were inhibited at varying levels by *Lactobacillus* sp P21, probably due to the production bacteriocin-like inhibitory substance. *Lactobacillus* bacteria have been known to inhibit many gram-positive and gram-negative bacteria (Tag *et al.* 1976) mainly through production of organic acids, hydrogen peroxide and bacteriocin-like inhibitory substances (BLIS) (Tag *et al.* 1976).

Table 5. *In-vitro* antagonistic activity of *Lactobacillus*sp. against fish bacterial pathogens by cross-streak technique

Bacterial strains (N=18)	Zone of Inhibition (mm)
Aeromonashydrophila(n=10)	0-12.5
Bacillus spp (n=2)	3.0
Citrobacterfreundii(n=1)	1.0
Pseudomonas spp (n=4)	1.0-12.5
P. aeruginosa(n=1)	4.0

(Source: Abraham *et al*., 2007)

L. plantarum subsp. *plantarum*, *L. brevis* and *L. rhamnosus* have disease resistance capacity. The protection activity of each strain against *Aeromonas hydrophila* infection of zebrafish was tested (He *et al*., 2017). Zebrafish were immersed with *Lactobacillus* at 10^7cfu/ml for 14 days. All the three strains decreased the cumulative mortality of zebrafish compared with control group, while LB (*L. brevis*) showed the highest protection (Fig. 4).

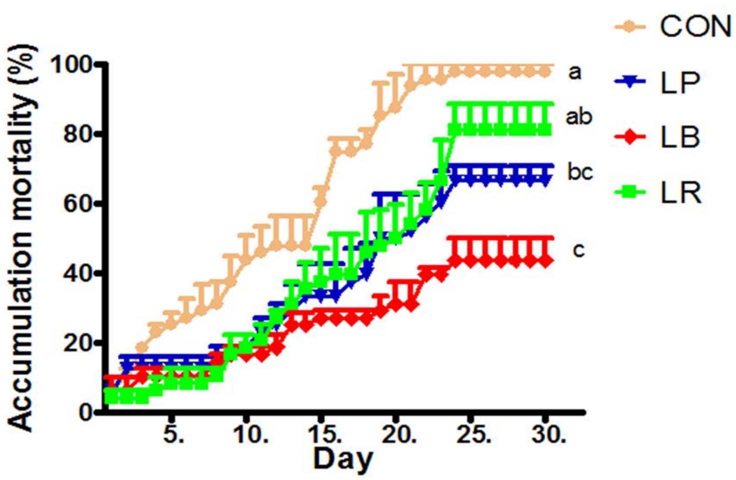

Figure 4.Protection of zebrafish against *A. hydrophila* by the three *Lactobacillus* strains (*L. plantarum*, LP; *L. brevis*, LB; *L. rhamnosus*, LR) and one control (CON) after 14 days im-mersion treatment; cumulative mortality of zebrafish after *A. hydrophila* infection.

(Source: He *et al*., 2017)

3.6.5 Improving Digestive enzyme activity and muscle protein

According to an experiment, *Oreochromis niloticus* fry were supplemented with different treatment of *Lactobacillus*probiotic under laboratory conditions with following dosages: The treatments were D_0 (control), D_1 (0.25% probiotic mix), D_2 (0.50% probiotic mix), D_3 (0.75% probiotic mix) and D_4 (1.00% probiotic mix in feed) (Garg, 2015).

In general, total and specific activity of digestive enzymes (Protease, amylase, lipase and cellulase) remained significantly higher in fish fed diet containing *Lactobacillus* at 0.75% probiotic mix diet in comparison with other treatments and control. Muscle protein was significantly high in fish fed at 0.75% probiotic mix of diet (Table 6).

Table 6: Effect of different levels of probiotic supplement on muscle protein and enzymatic activities (protease, amylase, lipase and cellulase) in *Oreochromis niloticus* fry

Diets	Parameters				
	Muscle Protein (g)	Total protease enzyme activity (mg g^{-1} $h^{-}1$)	Total amylase activity (mg g^{-1} $h^{-}1$)	Total lipase activity (mg g^{-1} $h^{-}1$)	Total cellulase activity (mg g^{-1} $h^{-}1$)
D_0 (Control)	110.85 ± 1.89c	3.51 ± 0.07d	0.22 ± 0.04e	0.09 ± 0.01d	0.93 ± 0.03c
D_1	116.99 ± 1.29b	4.34 ± 0.05c	0.24 ± 0.004d	0.17 ± 0.01 c	1.13 ± 0.02c
D_2	121.03 ± 1.40b	5.44 ± 0.06a	0.30 ± 0.005b	0.37 ± 0.03b	1.36 ± 0.02b
D_3	133.88 ± 2.59a	6.32 ± 0.09a	0.38 ± 0.004a	0.06 ± 0.03a	1.60 ± 0.05b
D_4	118.00 ± 1.75b	5.27 ± 0.06b	0.28 ± 0.006c	0.25 ± 0.03c	1.32 ± 0.11b

(Source: Garg, 2015).

3.6.6 Improved Body Composition

The body composition of the fish can also be affected by the probiotics concentrations in diets. According to an experiment on *Oreochromis niloticus* (Garg, 2015), a specific amount of probiotic can positively result into the improvement of body composition. The accumulation of carcass protein, fat and phosphorus were significantly higher in groups fed diets containing probiotics at 0.5 to 0.75 g per 100g of diet. Carcass ash contents remained significantly low at 0.75 g of probiotics level per 100 g of diet (Table 7).

Table 7. Effect of different levels of probiotics supplement on proximate composition (% wet weight basis) in *Oreochromis niloticus* fry

Carcass Composition	Initial Value	Treatments				
		D_0	D_1	D_2	D_3	D_4
Moisture (%)	71.26 ± 0.17	68.21 ± 0.03a	67.85 ± 0.12b	67.23 ± 0.03d	66.80 ± 0.07e	67.62 ± 0.03c
Crude protein (%)	15.31 ± 0.35	18.26 ± 0.26c	18.56 ± 0.27c	19.79 ± 0.37ab	20.56 ± 0.28a	19.03 ± 0.36bc
Crude fat (%)	2.74 ± 0.04	3.78 ± 0.03d	3.82 ± 0.03cd	3.97 ± 0.03b	4.08 ± 0.03a	3.89 ± 0.03bc
Phosphorus (%)	0.59 ± 0.02	0.60 ± 0.02b	0.59 ± 0.02b	0.64 ± 0.02ab	0.67 ± 0.02a	0.63 ± 0.02ab

(Source: Garg, 2015)

This experiment of proximate composition of *Oreochromis niloticus*was performed under laboratory conditions at 25±1ºC with a duration of 70 days. With following treatments: D_0 (control); D_1 (0.25% probiotic mix); D_2 (0.50% probiotic mix); D_3 (0.75% probiotic mix) and D_4 (1.00% probiotic mix in feed).

Chapter 4

CONCLUSION

The species of *Lactobacillus* can increase the Specific Growth Rate (SGR) of *Catla catla* and *Oreochromis niloticus* fry and decrease the Feed Conversion Ratio (FCR) of the mentioned fishes to the desired level. Such probiotics and their closely related species can contribute to the achievement of optimum SGR and FCR. As a result, fish production will be higher as thus can ensure their overall growth performance.

Lactobacillus can contribute to the reproduction of fish by increasing the fecundity and survival rate of newly born fry of fish. This group of bacteria can also a higher Gonadosomatic Index (GSI) which is beneficial for reproductive purpose.

Supplementation with *Lactobacillus* probiotic bacteria in fish can result in higher hemoglobin, Red Blood Cells (RBC) and White Blood Cells (WBC) in blood which are good indicators of strong physiological condition of fish.

*Lactobacillus*bear significant ability of producing bacteriocin-like inhibitory substances which can contribute a lot to disease resistance. This means that they can overpower the growth of other pathogenic bacteria to inhibit certain diseases.

Proximate composition of a fish body can be brought under optimum condition for fish physiology and morphology if optimum supplementation with *Lactobacillus*can be administered.

Administration of *Lactobacillus* probiotic can enhance the activity of different intestinal enzyme which will contribute to the efficient digestion in fish.

REFERENCES

Abraham, T. J., Babu, C. H. S. & Banerjee, T. Influence of a fish gut bacterium *Lactobacillus* sp. on the production of swordtail *Xiphophorus helleri* (Heckel 1848). *Bangladesh Fish. Res.***11(1)**, 65-74 (2007).

Al-Dohail, M. A., Hashim, R. & Aliyu-Paiko, M. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquac. Res.***40**, 1642-1652 (2009).

Aoki, T., Kanazawa, T. &Kitao, T. Epidemiological surveillance of drug-resistant *Vibrio anguillarum*strains. *Fish Patho*. **20**, 199-208 (1985).

Bailey, M. The mucosal immune system: Recent developments and future directions in the pig. *Dev. Comp. Immunol.***33**, 375-383 (2009).

Balcázar, J. L., Blas, I., Zarzuela-Ruiz, I., Cunningham, D., Vendrell, D. & Músquiz, J. L. The role of probiotics in aquaculture. *Vet. Microbiol.***114(1)**, 173-186 (2006).

Barth, S., Duncker, S., Hempe, J., Breves, G., Baljer, G. & Bauerfeind, R. *Escherichia coli*Nissle 1917 for probiotic use in piglets: evidence for intestinal colonization. *J. Appl. Microbiol.***107**, 1697-1710 (2009).

Bucio, G. A., Hartemink, R., Schrama, J. W., Verreth, J., Bucio G. L. & Zwietering, M. H. Kinetics of *Lactobacillus plantarum 44a* in the faeces of tilapia (*Oreochromis niloticus*) after its intake in feed. *J. App. Microbiol.***107**, 1967-1975 (2009).

Capriles, V. D., Silva, K. E. A. &Fisberg, M. Prebiotics, probiotics and symbiotics: New trend in functional food market. *Rev. Nutr*.**4(6)**, 327-335 (2005).

Denev, S. A. Ecological alternatives of antibiotic growth promoters in animal husbandry and aquaculture. *Dept.Biochem.Microbiol*.**294** (2008).

Dhanaraj, A., Haniffa, M. A., Singh S. V., JesuArockiaraj, A., Ramakhrishanan, C., Seetharaman, S. & Arthimanju, R. Effects of probiotics on growth performance of Koi carp (*Cyprinus carpio*). *J. Appl. Aquac.***22**, 202-209 (2010).

FAO/WHO/OIE. Expert Consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, Republic of South Korea. June 13–16 (2006).

Fujieda, M. & Sakata, T. Glucose but not polypeptone reduces the effect of probiotic preparations to stimulate carbohydrate breakdown and reduce net ammonia production by pig cecal bacteria in-vitro. *J. Med. Food*. **4**, 231-240 (2001).

Fuller, R. Probiotic in man and animals. *J. Appl. Bacteriol*. **66**, 365-378 (1989).

Garg, S. K. Effect of Dietary Probiotic Mix (SPILAC) on Growth Performance and Nutritive Physiology of Nile Tilapia, *Oreochromis niloticus* (Linn.) under Laboratory Conditions. *Int. J. Fish. Aquat. Stud.***3(2)**, 440-446 (2015).

Gioacchini, G., Lombardo, F., Merrifield, D. L., Silvi, S. & Cresci, A. Effects of Probiotics on Zebrafish Reproduction. *J. Aquac. Res Dev.***1:002** (2011).

Giorgio, G., Nina, C. & Yantyati, W. Importance of *Lactobacilli* in food and feed biotechnology. *Res. Microbiol*. **161**, 480-487 (2010).

Haakensen, M., Dobson, C. M., Hill, J. E. & Ziola, B. Reclassification of *Pediococcus dextrinicus* as *Lactobacillus dextrinicus* comb. nov., and emended description of the genus *Lactobacillus*. *Int. J. Syst. Evol. Microbiol*. **59**, 615–621 (2009).

He, S., Ran, C., Qin, C., Li, S., Zhang, H. & Zhou, Z. Anti-Infective Effect of Adhesive Probiotic *Lactobacillus* in Fish is Correlated With Their Spatial Distribution in the Intestinal Tissue. *Nature.J*. **7**, 13195 (2017).

Irianto, A. & Austin, B. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchusmykiss* (Walbaum). *J. Fish Dis*. **25**, 1-10 (2002).

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A. &Pavlova, N. Comparative genomics of the lactic acid bacteria. *Proc. Natl. Acad. Sci. USA*. **103(42)**, 15611–15616 (2006).

Martin, R., Sylvie, M., Jonathan, U., Noura, K., Philippe, L., Bermúdez-Humarán, L. G. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. *Ntl. Cnt. Biotechnol. Info.* **12(71)** (2013).

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. M. T., Bogwald, J., Castex, M. &Ringo, E. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquac*. **302**, 1-18 (2010).

Nayak, S. K., Swain, P. & Mukherjee, S. C. Effect of dietary supplementation and vitamin C on the immune response of Indian major carp, *Labeo rohita*(Ham.). *Fish Shellfish Immunol*. **23**, 892-896 (2007).

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E. G. J., Subaramaniyam, K. & Manikkam, S. Probiotics in aquaculture. *Drug invent*. **5**, 55-59 (2013).

Panigrahi, A., Kiron, V., Puangkaew, J., Kobayashi, T. & Satoh, S. The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchusmykiss*. *Aquac.***243**, 241-254 (2005).

Parker, R. B. Probiotics: the other half of the antibiotics story. *Anim. Nutr. H*. **29**, 4-8 (1974).

Ravi, D., Parthasarathy, R., Muthezhilan, R. & Vijayabharathi, V. The Role of Probiotic Bacteria on Growth and Disease Management of in *Catla catla*(Hamilton, 1822). *Int. J. Agric. Sci. Res.***3(4)**, 121-132 (2013).

Ringo, E. &Birkbeck, H. Intestinal microflora of fish larvae and fry. *Aquat. Res*. **30**, 73-93 (1999).

Ringo, E., Lovmo, L., Kristiansen, M., Bakken, Y., Salinas, I., Myklebust, R., Olsen, R. E. & Mayhew, T. M. Lactic acid bacteria vs. pathogens in the gastro-intestine of fish: a review. *Aquat. Res.***41**, 451-467 (2010).

Salas-Jara, Jose, M., Ilabaca, A., Vega, M. &García, A. Biofilm Forming *Lactobacillus*: New Challenges for the Development of Probiotics. *Ntl. Cnt. Biotechnol. Info*. **4(3)**, 35 (2016).

Silva, E. F., Soares, M. A., Calazansm, N. F., Vogeley, J. L., doValle, B. C. & Soares, R.Effect of probiotic (*Bacillus* spp.) addition during larvae and post larvae culture of white shrimp *Litopenaeus vannemei*. *Aquat. Res*. **44**, 13- 21 (2013).

Suzer, C., Çoban, D., Kamaci, H. O., Saka, S. & Firat, K. *Lactobacillus*spp. bacteria as probiotics in gilthead sea bream (*Sparusaurata* L.) larvae: Effects on growth performance and digestive enzyme activities. *Aquac*. **280**, 140-145 (2008).

Tag, J. R., Dajani, A. S. &Wannekar, L. W. Bacteriocins of gram-positive species. *Bacteriol. Rev.***40**, 722-756 (1976).

Vancanneyt, M., Neysens, P., De Wachter, M., Engelbeen, K., Snauwaert, C., Cleenwerck, I., Van Der Meulen R., Hoste, B., Tsakalidou, E., De Vuyst, L. & Swings, J. *Lactobacillus acidifarinae* sp. nov.& *Lactobacillus zymae* sp. nov., from wheat sourdoughs. *Int. J. Syst. Evol. Microbiol.* **55**, 615-620 (2005).

Verschuere, L., Rombaut, G. & Verstraete, W. Probiotic bacteria as biological agents in aquaculture. *Micro. & Mole. Biol. Rev*. **64(4)**, 655-671 (2000).

Yadav, S. B., Athol, V. K., Peter, J. D. & Wayne, L. B. Probiotics in animal nutrition – Production, impact and regulation. *FAO Anim. Prod. & Health*. **179**, 5 (2016).

Zhou, X., Wang, Y. & Li, W. Effect of probiotic the larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. *Aquac*. **287**, 349-353 (2009).