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# EFFECT OF PROBIOTIC SUPPLEMENTED DIET ON GUT ASSOCIATED BACTERIAL FLORA OF *LABEO ROHITA* (HAMILTON)

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**ABSTRACT**
This study was focused to evaluate the effect of probiotic mixture diet on the growth performance, autochthonous gut bacterial population and extracellular enzymatic activities like amylase, protease and cellulase of *Labeo rohita*. The control diet contained fish meal (FM) and soybean meal (SBM) and the experimental diet contained FM and SBM supplemented with a Probiotic Mixture (PM) (LactoBacil plus: Swiss Garnier Life Sciences) at the rate of (CFU $kg^{-1}$ =$15\times10^8$). The feeding trial was conducted in flow-through 90L circular fibre-glass tanks for 90 days under laboratory condition. After completion of feeding trial the significant difference was found in SGR of fish fed with PM diet than the fish fed with FM and SBM containing control diet. The significant difference also found in PER of fish fed with PM meal which is good for fish health. After feeding trial, it was also found that total log viable counts increased in the hindgut (log viable count = 7.64 $g^{-1}$intestinal tissue) of rohu fed with PM supplemented diet. Highest quantitative amylase activity recorded in the strain LBF6 (52.49±0.77 U), highest cellulase activity showed by the strain LBH6 (23.48±0.31 U) and highest protease activity exhibited by the strain LBH8 (2.29±0.05 U) isolated from PM fed fish gut.

**KEYWORDS**: Growth, Gut bacteria, Gut enzymes, *Labeo rohita*, Probiotics.

## INTRODUCTION

Half of the global population used fish as a principal animal source of protein. Fish also play an important role in human nutrition in India, particularly to people of coastal areas. In India, the major carps are the most preferred farm fishes because of their fast growth and higher acceptability to consumers. The freshwater fish Rohu (*L. rohita*) is one of the most popular species especially in Asia and fetches high price (FAO, 2013). Good and adequate nutrition plays a very important role in the expression of mental, physical and intellectual qualities in humans. The rapid expansion and intensification of carp farming had led to the outbreaks of infectious diseases caused by viruses, bacteria and parasites, inflicting severe loss on fish production. The widespread use of broad-spectrum chemotherapeutants to control these diseases has led to the development of antibiotic-resistant bacterial strains and may cause water pollution in the aquaculture environment (Lunden *et al*., 2002). For this reason, greater emphasis has been placed on improved husbandry and water quality, better nutrition, lower stocking densities and the use of vaccines and immunostimulants in the last decade (Bandyopadhyay and Mohapatra, 2009; Harikrishnan *et al*., 2010). Recently, attention has focused on the use of probiotics with the demand for environmentally friendly aquaculture.

The term probiotic is a word derived from Greek and meaning for life (Zivkovic, 1998). Probiotics are beneficial microorganisms that provide health benefits to the hosts have been used in aquaculture as disease control agents, as supplements to improve growth and in some cases as a means of replacing antimicrobial compounds (Moriarty, 1997). Several previous reports have suggested that probiotic supplementation can reduce disease outbreaks by enhancing the immune system of fish and shrimp and can decrease culture costs by improving the growth and feed efficiency of fish (Mohsen and Xin, 2015; Mohapatra *et al*., 2012; Fernandez *et al*., 2011; Peterson *et al*., 2012). The problems outlined above and recent restrictions on the use of antibiotics have resulted in natural immunostimulants, probiotics and prebiotics being considered as an alternative strategy to disease management and Controlling and preventing (Dimitroglou *et al*., 2011; Guzman-Villanueva *et al*., 2014). And also "Microorganisms promote the growth of other microorganisms". Probiotics as feed supplements benefit the host by improving the feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting

  

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factors and increasing immune response (Verschuere *et al*., 2000; Harikrishnan *et al*., 2010). According to Gildberg and Mikkelsen, (1998) Atlantic salmon and Atlantic cod displayed better colonization capabilities with regard to Atlantic cod fry pyloric caeca than the intestine. Therefore, the aim of this study was to assess the effects of dietary effect of probiotic supplemented diet on gut associated bacterial flora, the growth response of fish as well as evaluation of enzymatic activity of bacteria isolated from the gastrointestinal tract (GI) tract of the fingerling of rohu.

## MATERIALS AND METHODS

***Diet preparation:***

Two diets were prepared using fish meal (FM) and soybean meal (SBM) as the major protein sources (Table 1). The control diet contained FM and SBM and the experimental diet contained FM and SBM supplemented with a Probiotic Mixture (PM) (LactoBacil plus: Swiss Garnier Life Sciences) at the rate of (CFU $kg^{-1}$ =$15\times10^8$). The diets were prepared isonitrogenous (36% crude protein) and isocaloric (11.32 kJ $g^{-1}$). Dry feed ingredients were mixed and the diets were prepared in pelleted form using 0.5% carboxymethylcellulose as a binder. The pellets were prepared by passing the slurry through a pelletizer. The pellets were sun dried for a few days and crumbled prior to feeding and stored at 4 ºC until used.

**Table 1: Composition (g/ kg dry weight) of the diets; [a]Vitamin and mineral mixture (Vitaminetes forte, Roche Products India Private Limited, Mumbai, India).**

| Ingredients | g $kg^{-1}$ diet | |
|---|---|---|
| | FM diet | Experimental diet (PM) |
| **Fishmeal** | 450 | 450 |
| **Soybean meal** | 80 | 80 |
| **Mustard oil cake** | 240 | 240 |
| **Rice barn** | 200 | 200 |
| **Cod liver oil** | 10 | 10 |
| **Vitamin premix[a]** | 10 | 10 |
| **Probiotic Mixtre (LactoBacil plus: Swiss Garnier Life Sciences)** | - | (CFU $kg^{-1}$) $15\times10^8$ |

***Experimental procedure*:**

The feeding trial was conducted in flow-through 90L circular fibre-glass tanks for 90 days under laboratory condition. Each tank was supplied with unchlorinated water from a deep tube well with continuous aeration. *Labeo rohita*, fingerlings were obtained from a local fish farm and acclimatized for 15 days and fed with a mixture of rice bran and mustard oil cake. The fingerlings (Avg. initial weight 8.50±0.12 g) were randomly distributed in the fibre-glass tanks at a stocking density of 15 fish per tank with three replicates for each dietary treatment. The fish were fed once daily at 10.00 h at a feeding rate of 3% of total body weight per day. Weight gain (%), specific growth rate (SGR, %/day), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using standard methods.

***Isolation of Bacterial isolates and culture procedure*:**

Isolation of bacteria was done from the intestine of *L. rohita* fingerlings both prior to and at the termination of the feeding trial. In each case, the test fish were starved for 36 h prior to sacrifice to clean the intestine. The fish were carefully placed aseptically within laminar airflow on ice slabs and their intestines were removed and cleaned with sterile physiological saline solution. The intestine was divided into proximal (PI) and distal intestine (DI) as described by (Ringø and Strøm, 1994). The digestas from the two regions of the gut were squeezed out. Thereafter, both the regions of the gut were cleaned, slit opened by a longitudinal incision, transferred to sterile Petri dishes, and thoroughly flushed with sterilized chilled 0.9% saline in order to remove non-adherent or allochthonous bacteria chilled 0.9% saline in order to remove non-adherent or allochthonous bacteria. The two regions of the alimentary tract were separately homogenized with 10 parts of chilled 0.9% sodium chloride solution (Das and Tripathi, 1991). Bacteria associated with gut were quantified as log total viable count (TVC) per gram intestinal tissue using three different types of agar. The total numbers of resident or autochthonous aerobic bacteria were estimated with plate count agar (Tryptic soy agar, TSA). For isolation and enumeration of protease-, amylase- and cellulase-producing bacteria, the diluted gut

  

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homogenates were spread onto the surface of peptone-gelatin agar, starch-agar and carboxymethylcellulose (CMC)-agar media plates, respectively.

**Enzyme-producing capacity of isolated bacterial strains**

***Screening of isolates by qualitative enzyme production*:**

The isolated strains from the PI and DI of the test fish were screened for the production of extracellular protease, amylase and cellulase on agar plates of the selective media, namely, peptone-gelatin agar, starch-agar, and (CMC) – agar, respectively (Banerjee *et al*., 2013) Qualitative extracellular enzyme activities were assessed based on the measurement of a clear zone (halo) around the colonies as follows: + (low, 5-14 mm halo diameter), ++ (moderate, 15-24 mm halo diameter), and +++ (high, 25-35 mm halo diameter).

***Quantitative enzyme assay*:**

After primary qualitative screening, the selected strains were cultured in selective liquid medium for quantitative enzyme assay. The strains were cultured in 4% tryptone soya broth for 24 h at 37±1 $^0$C and used as the inoculum. Liquid production medium of 20 ml was inoculated with 0.5ml of inoculum obtained from the seed culture and incubated for 48-96 h at the same temperature. The contents of the culture flasks were centrifuged (9,000×*g*, 10 m, 4 $^0$C), and the cell-free supernatant was used for enzyme assay. The protein content of the crude enzyme extract was estimated according to Lowry *et al*., (1951). The quantitative assay of amylase, cellulase and protease was performed using the methods described by Bernfeld (1955), Denison and Koehn (1977), and Walter (1984), respectively. Specific enzyme activity was expressed as a unit (U)/mg protein.

## RESULTS

***Growth performance*:**

The experimental diet with probiotic treated for ninety days. After end of ninety days growth performance and feed utilization of *L. rohita* fingerlings in terms of percentage weight gain feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) fed with fish meal and SBM diet were observed (Table. 2). There was found difference in the FCR of fish, fed with PM diets had better FCR when compared with the fish meal fed diet. The significant difference was found in SGR of fish fed with PM diet than the fish fed with fish meal and SBM containing control diet. The significant difference found in PER of fish fed with PM meal which is good for fish health.

**Table 2:** Growth and feed utilization efficiencies in *Labeo rohita* fingerlings fed experimental diets for 90 days.

| Mean Values | FM diet | Experimental diet (PM) |
|---|---|---|
| Initial weight (g) | 10.35±0.83 | 10.35±0.83 |
| Final weight (g) | 20.84±1.12 | 26.24±1.44 |
| Weight gain (%) | 101.32±0.86 | 148.52±0.91 |
| SGR (%/ day) | 0.87±0.34 | 1.032±0.62 |
| FCR | 3.48±0.04 | 2.32±0.03 |
| PER | 1.58±0.02 | 1.13±0.07 |

***Total Log viable count (TVC) of bacterial strains (g $^{-1}$ intestinal tissue) in the GI tract of Labeo rohita after feeding trial:***

**Table 3:** Aerobic amylase, cellulase and protease producing bacterial log viable counts (g$^{-1}$ intestinal tissue) in the fish gastrointestinal tract before feeding trial.

| Gut region | Log total viable counts /g intestinal tissue | | | |
|---|---|---|---|---|
| | Total bacterial count (TSA) | Amylase producing bacteria | Cellulase producing bacteria | Protease producing bacteria |
| **Proximal Intestine (PI)** | 6.65 | 4.39 | 4.14 | 4.62 |
| **Distal Intestine (DI)** | 6.27 | 4.98 | 3.36 | 4.56 |

  

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Aerobic bacterial count in the GI tract of *L. rohita* on TSA plate don't show more change in the total log viable count (log TVC) in the both PI and DI before the probiotic treatment (Table. 3). And maximum specific enzyme producing bacteria showed that the amylolytic and proteolytic strains were present at the highest population level in the DI and PI respectively. Whereas cellulolytic bacterial count were recorded highest in the PI.

After 90 days of feeding trial, colony forming units (CFU) were counted. The bacterial counts on TSA plate, starch agar, CMC agar and gelatin agar plate are presented in (Table 4). The aerobic bacterial count was highest in the DI (log viable count = 8.64 $g^{-1}$ intestinal tissue) of *L. rohita* fed with probiotic mixture (PM) supplemented diet, while the amylase-producing bacterial count was highest in the PI of *L. rohita* (log = 5.85 $g^{-1}$ intestinal tissue) fed with PM supplemented diet but the cellulase producing bacterial count was highest in the PI of *L. rohita* (log = 4.32 $g^{-1}$ intestinal tissue) fed with PM supplemented diet and also the protease producing bacterial count was highest in the foregut of *L. rohita* (log = 4.78 $g^{-1}$ intestinal tissue) fed with PM supplemented diet after ninety days of feeding trial.

**Table 4:** Aerobic amylase, cellulase and protease producing bacterial log viable counts ($g^{-1}$ intestinal tissue) in the fish gastrointestinal tract after feeding trial.

| Gut region | Feed | Log total viable counts /g intestinal tissue | | | |
|---|---|---|---|---|---|
| | | Total bacterial count (TSA) | Amylase-producing bacteria | Cellulase-producing bacteria | Protease-producing bacteria |
| Proximal Intestine (PI) | FM | 5.48 | 4.35 | 3.84 | 4.66 |
| | PM | 7.14 | 5.85 | 4.32 | 4.78 |
| Distal Intestine (DI) | FM | 6.18 | 4.68 | 3.12 | 4.36 |
| | PM | 8.64 | 5.59 | 3.44 | 4.26 |

***Qualitative enzyme activity in the selected strains:***

After isolation of bacterial isolates by pure culture, qualitative amylase, cellulase and protease assay was carried out with a total of 92 bacterial isolates and isolated strains were screened on the basis of specific amylase cellulase and protease activity (Table. 5 & 6). Many of the bacterial isolates did not produce any visible halo or transparent zone of maltose, glucose or tyrosine hydrolysis in the starch, CMC and gelatin plate respectively after addition of the colour reagent whereas, some isolates produced clear zones of very shorter radius. These bacterial strains were rejected and only the strains which produced good halo were selected.

**Table 5:** Qualitative extracellular enzyme activity in the bacterial strains isolated from the GI tract of *Labeo rohita* after commencement of feeding trial with fish meal.

| | FM Diet | | | |
|---|---|---|---|---|
| | Strains | Amylase activity | Cellulase activity | Protease activity |
| PI | LCF1 | ++ | ++ | ++ |
| | LCF2 | ++ | + | ++ |
| | LCF3 | ++ | + | +++ |
| | LCF4 | + | + | +++ |
| | LCF5 | + | - | ++ |
| | LCF6 | + | +++ | ++ |
| | LCF7 | +++ | ++ | ++ |
| | LCF8 | ++ | +++ | +++ |
| | LCF9 | +++ | +++ | +++ |
| | LCF10 | + | ++ | + |
| DI | LCH1 | + | ++ | ++ |
| | LCH2 | ++ | +++ | +++ |
| | LCH3 | ++ | ++ | + |
| | LCH4 | + | - | +++ |
| | LCH5 | + | +++ | +++ |
| | LCH6 | ++ | +++ | +++ |
| | LCH7 | ++ | +++ | +++ |
| | LCH8 | ++ | ++ | ++ |
| | LCH9 | + | ++ | +++ |
| | LCH10 | ++ | - | +++ |

  

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**Table 6:** Qualitative extracellular enzyme activity in the bacterial strains isolated from the GI tract of *Labeo rohita* after commencement of feeding trial with PM diet.

| PM Diet | | | | |
|---|---|---|---|---|
| | **Strains** | **Amylase activity** | **Cellulase activity** | **Protease activity** |
| **PI** | **LBF1** | +++ | ++ | +++ |
| | **LBF2** | + | + | +++ |
| | **LBF3** | + | +++ | ++ |
| | **LBF4** | +++ | +++ | +++ |
| | **LBF5** | +++ | +++ | ++ |
| | **LBF6** | +++ | ++ | ++ |
| | **LBF7** | ++ | + | +++ |
| | **LBF8** | + | ++ | +++ |
| | **LBF9** | - | + | ++ |
| | **LBF10** | ++ | - | ++ |
| **DI** | **LBH1** | + | ++ | ++ |
| | **LBH2** | ++ | + | +++ |
| | **LBH3** | +++ | +++ | +++ |
| | **LBH4** | +++ | +++ | ++ |
| | **LBH5** | +++ | +++ | + |
| | **LBH6** | +++ | +++ | ++ |
| | **LBH7** | ++ | ++ | +++ |
| | **LBH8** | +++ | ++ | +++ |
| | **LBH9** | + | ++ | ++ |
| | **LBH10** | + | ++ | +++ |

Based on the results of the qualitative amylase, cellulase and protease assay only 40 bacterial isolates; twenty from fish meal and soybean meal fed fish gut (ten from each foregut and hindgut) and twenty from PM (probiotic mixture) fed fish gut (ten from each foregut and hindgut) were selected for further study (Table. 7).

**Table 7:** Selected bacterial strains after the qualitative extracellular enzyme activity.

| Selected strains (total isolates 40) | | |
|---|---|---|
| **Fish** | **Foregut** | **Hindgut** |
| **FM fed fish** | LCF1, LCF2, LCF3, LCF4, LCF5, LCF6, LCF7, LCF8, LCF9, LCF10 | LCH1, LCH2, LCH3, LCH4, LCH5, LCH6, LCH7, LCH8, LCH9, LCH10 |
| **PM fed fish** | LBF1, LBF2, LBF3, LBF4, LBF5, LBF6, LBF7, LBF8, LBF9, LBF10 | LBH1, LBH2, LBH3, LBH4, LBH5, LBH6, LBH7, LBH8, LBH9, LBH10 |

***Extracellular quantitative amylase, cellulase and protease activity of the selected bacterial isolates after feeding trial*:**

The quantitative amylase, cellulase and protease assay was performed with the selected 40 bacterial isolates (Table 8 & 9). The strain LBF6 followed by LBF5 isolated from the foregut of PM fed fish gut exhibited highest amylase activity. Among the strains isolated from the hindgut, highest amylase activity was exhibited by LBH5 followed by LBH4 and LBH6 from the PM fed fish gut, while in case of cellulase, the strain LBF4 followed by LBF5 from the foregut of PM fed group and LCF9 from the foregut of FM fed fish exhibited highest activity and among the strains isolated from the hindgut, highest cellulase activity was exhibited by LBH6 followed by LBH4 from the PM fed fish gut and LCH6 from the foregut of FM fed fish, whereas in case of protease, the strain LBF7 followed by LBF4 from the foregut of PM fed fish and LCF9 followed by LCF3 and LCF8 from the foregut of FM fed fish showed highest activity and among the strains isolated from hindgut, highest protease activity was exhibited by the strain LBH8 followed by LBH7 and LBH2 from PM fed fish gut and LCH7 followed by LCH2 from the fish meal fed fish gut. The isolates detected to have promising amylase activity (U) were LBF6 and LBF5 from the foregut of PM fed fish and LBH5, LBH4 and LBH6 from the hindgut of PM fed fish. The promising cellulase producing bacterial strains were LBF4 and LBF5 from the

  

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foregut of PM fed fish and LCF9 from the foregut of FM fed fish and LBH6 and LBH4 from the hindgut of PM fed fish. The promising protease producing bacterial strains were LBF7 and LBF4 from the foregut of PM fed fish and LCF9, LCF3 and LCF8 from the foregut of FM fed fish and LBH8, LBH7, LBH2 from the hindgut of PM fed fish and LCH7 and LCH2 from the hindgut of FM fed fish.

**Table 8:** Quantitative extracellular enzyme activity in the bacterial strains isolated from the GI tract of *Labeo rohita* after commencement of feeding trial with fish meal.

| Gut region | Strain designation | Specific amylase activity (U/mg protein)* | Specific cellulase activity (U/mg protein)# | Specific amylase activity (U/mg protein)† |
|---|---|---|---|---|
| PI | LCF1 | 10.81±0.36 | 1.38±0.28 | 0.37±0.04 |
| | LCF2 | 5.38±0.27 | 0.23±0.06 | 0.2±0.04 |
| | LCF3 | 9.20±0.73 | 0.53±0.07 | 1.42±0.04 |
| | LCF4 | 4.48±0.27 | 0.19±0.04 | 0.71±0.06 |
| | LCF5 | 2.36±0.20 | 0.04±0.02 | 0.25±0.04 |
| | LCF6 | 1.83±0.11 | 5.39±0.31 | 0.62±0.06 |
| | LCF7 | 20.17±0.67 | 0.77±0.07 | 0.97±0.10 |
| | LCF8 | 4.63±0.38 | 1.63±0.24 | 1.32±0.1 |
| | LCF9 | 11.76±0.33 | 14.25±0.45 | 1.44±0.06 |
| | LCF10 | 1.25±0.21 | 2.57±0.42 | 0.07±0.01 |
| DI | LCH1 | 1.39±0.31 | 0.54±0.18 | 0.29±0.04 |
| | LCH2 | 10.57±0.49 | 1.35±0.29 | 1.36±0.06 |
| | LCH3 | 2.37±0.36 | 0.29±0.04 | 0.11±0.03 |
| | LCH4 | 4.41±0.24 | 0.06±0.02 | 0.84±0.05 |
| | LCH5 | 1.38±0.29 | 5.2±0.45 | 0.08±0.01 |
| | LCH6 | 1.99±0.27 | 12.29±0.56 | 0.98±0.06 |
| | LCH7 | 5.87±0.70 | 2.58±0.13 | 1.48±0.06 |
| | LCH8 | 9.73±0.56 | 0.44±0.07 | 0.37±0.04 |
| | LCH9 | 5.42±0.54 | 0.18±0.06 | 1.02±0.06 |
| | LCH10 | 7.86±0.47 | 0.03±0.01 | 0.49±0.02 |

**Table 9:** Quantitative extracellular enzyme activity in the bacterial strains isolated from the GI tract of *Labeo rohita* after commencement of feeding trial with PM diet.

| Gut region | Strain designation | Specific amylase activity (U/mg protein)* | Specific cellulase activity (U/mg protein)# | Specific amylase activity (U/mg protein)† |
|---|---|---|---|---|
| PI | LBF1 | 25.62±0.89 | 0.83±0.09 | 0.86±0.05 |
| | LBF2 | 4.94±0.56 | 0.24±0.05 | 0.57±0.06 |
| | LBF3 | 8.82±0.56 | 6.79±0.10 | 0.07±0.01 |
| | LBF4 | 28.76±0.56 | 22.20±0.72 | 1.35±0.06 |
| | LBF5 | 48.81±0.84 | 10.42±0.35 | 0.63±0.05 |
| | LBF6 | 52.89±0.77 | 1.19±0.12 | 0.17±0.03 |
| | LBF7 | 15.69±0.30 | 0.51±0.09 | 2.22±0.06 |
| | LBF8 | 9.69±0.74 | 5.12±0.48 | 0.98±0.05 |
| | LBF9 | 2.58±0.26 | 0.36±0.08 | 0.44±0.05 |
| | LBF10 | 11.6±0.41 | 0.05±0.01 | 0.57±0.04 |
| DI | LBH1 | 10.45±0.39 | 0.60±0.11 | 0.27±0.06 |
| | LBH2 | 8.58±0.35 | 0.09±0.02 | 1.19±0.05 |
| | LBH3 | 19.12±0.43 | 1.19±0.15 | 0.79±0.03 |
| | LBH4 | 45.18±0.76 | 8.53±0.24 | 0.11±0.02 |
| | LBH5 | 52.19±0.83 | 2.25±0.12 | 0.09±0.02 |
| | LBH6 | 27.93±0.68 | 23.48±0.31 | 0.43±0.04 |
| | LBH7 | 6.55±0.26 | 1.66±0.11 | 1.63±0.05 |
| | LBH8 | 13.99±0.47 | 0.46±0.13 | 2.29±0.05 |
| | LBH9 | 2.40±0.22 | 1.95±0.21 | 0.22±0.05 |
| | LBH10 | 8.20±0.47 | 0.41±0.10 | 0.69±0.04 |

## DISCUSSION

The definition of a probiotic used in aquaculture differs greatly depending on the source (Merrifield *et al*., 2010b), but generally, probiotics offer potential alternatives by providing benefits to the host primarily via the direct or indirect

  

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modulation of the intestinal microbiota, enhanced immune system and growth, stimulate enzyme activity and improved disease resistance. However, only few studies carried out on fish have focused on contribution of the gut microbiota related to nutrition. Some published reports are available on the application of probiotics in aquaculture and some reviews are also available on the probiotic application like Merrifield *et al*. (2010b), Nayak (2010b), Zhou and Wang (2012), Mohapatra *et al.* (2013), Newaj-Fyzul *et al.* (2014) and Ringø *et al*. (2014c).

In this study dietary inclusion of probiotics mixed diet showed significant effect on growth performance of rohu. At the end of this experiment fish growth was increased and also the other growth parameters PER, FCR, and PER of rohu were better with the PM incorporated diet than with the fish meal and SBM diet. These results support those (Nwanna *et al*., 2017) who used *Lactobacillus plantarum* at 103cfu/g in the food of *Clarias gariepinus* juveniles indicated a significant increase in the body weight gain, fastest growth and also the growth parameters indicated acceptance of probiotic included in the diets. Lara-Flores *et al*. (2003) similarly reported that total feed intake by fish consequently had direct effect on feed conversion ratio, protein efficiency ratio and specific growth rate. Gao *et al*. (2016) also report that fish fed with diet supplemented with *C. butyricum, L. plantarum* and *B. subtili* showed significantly higher FW, WG and SGR compared to fish fed the control diet. The FCR among dietary treatments ranged significant decreasing compared to the control.

Alterations of the fish gut microbiota have been demonstrated with the probiotic application of *Bacillus* spp. (Bagheri *et al.*, 2008; Ghosh *et al.*, 2008), *Vibrio* spp. (Ringø 1999), LAB (Iehata *et al.*, 2009; Lauzon *et al.*, 2010a; Lamari *et al.*, 2013), yeasts (Aubin *et al.*, 2005; Gatesoupe, 2002) and mixed probionts (Gatesoupe, 2002; Lauzon *et al.*, 2010a; Ramos *et al.,* 2013). Probiotic treatments of live feed with *Bacillus* spp. (Avella *et al.*, 2010), LAB (Gatesoupe, 1991, 2002; Villamil *et al.*, 2003) and yeasts (Gatesoupe, 2002) have also contributed to the control of their microbiota. The continual application of probiotic cells via dry feed containing from $10^5$ to $10^9$ colony-forming units $g^{-1}$ has been demonstrated to lead to potentially resident colonization of the intestinal epithelium (Gildberg and Mikkelsen, 1998), intestinal mucus (Merrifield *et al.*, 2010b, 2011b), transient digesta (Bagheri *et al.*, 2008; Ghosh *et al.*, 2008; Ferguson *et al.*, 2010), intestine and stomach (Panigrahi *et al.*, 2004), and pyloric caeca populations (Gildberg and Mikkelsen, 1998). Several investigations have clearly demonstrated that probiotic GI colonization can alter the indigenous GI microbiota composition as well as total population levels in hosts (Strøm and Ringø, 1993; Aubin *et al.*, 2005; Bagheri *et al.*, 2008; Ghosh *et al.*, 2008; Avella *et al.*, 2010; Lauzon *et al.*, 2010a). Aubin *et al.* (2005) enumerated the gut probiotic levels and identified the dominating culturable microbiota by16S rRNA gene sequence analysis. Changes in the relative and absolute abundance of the indigenous bacteria were observed. After a period of 20-days supplemented feeding, the presence of unique genera such as *Buttiauxella* and *Citrobacter* was confirmed in the control diet-fed fish, but *Serratia* was detected only in the *Pediococcus acidilactic* fed fish. In the present study, the bacteria isolated from the GI tract of probiotic supplemented diets were not identified. However, the population of autochthonous bacteria were found to increase in both the foregut and hindgut regions of *L. rohita* after feeding probiotic (LactoBacil plus) supplemented diet. This finding corroborates the findings of Merrifield *et al*. (2010a) with rainbow trout.

Gut microbiota may have an important role in the digestive process of fish (their potential for breaking down of different component like; proteins, lipid, cellulose, phytate and chitin) and identification of these enzyme-producing gut bacteria can help us to choose the best probiotic (beneficial) bacteria that can be a great help for future commercial aquaculture via improvement of fish health, growth and better food management and less feeding cost. Till date, most of the investigations were done on the different enzyme producing bacterial population. In the present investigation, attention also has been focused on the aerobic GI tract autochthonous bacterial enzyme producing ability. Probiotic supplementation resulted to increased amylase activity in some bacterial strains, whereas protease and cellulase activities did not increase appreciably. Gao *et al*. (2016) also reported that fish group fed diet supplemented with *B. subtilis* exhibited the highest protease activity, highest lipase activity was recorded for the intestine of fish fed *C. butyricum* followed by *L. plantarum* and *B. subtilis*, respectively and increase amylase activity fish fed with *C. butyricum, L. plantarum* and *B. subtilis* compared to those fed basal diet of the silver pomfret. Askarian *et al*. (2013) reported that the diversity of most promising enzyme-producing bacteria isolated from the GI tract of Atlantic cod seems to be influenced by the feeding regimes. Fish fed with probiotic supplemented diet manipulate gastrointestinal autochthonous microbe's population, which produce exoenzymes that can increase nutrient digestibility and promote better health conditions (Zhang *et al.*, 2010). Probiotic bacteria secret a variety of digestive enzymes that help fully

  

digest feed, which beneficially affect nutritional status (Sharifuzzaman *et al.*, 2014). In this study, the higher level of total viable bacterial count were increase in the gut of *L. rohita* after feeding probiotic (LactoBacil plus) supplemented diet and growth was also good in the group of fish fed with probiotic mixed diet. Highest enzyme activity was also recorded in fish fed PM diets compared to the FM and SBM mixed diet.

## CONCLUSION

The present study concludes that probiotic mixture diet improved growth performance of *L. rohita*. And intestinal autochthonous total log viable bacterial counts were increased in the probiotic fed fish group. Fish gut extracellular enzyme amylase, cellulase and protease activity was also increased in the fish fed with probiotic mixed food. However, further investigations are necessary to establish the detailed mechanisms that alter the microbial community and probiotic dietary effect on microbes which alter the fish gut extracellular enzymatic activity.

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