A Comparative Study of The Gut Microflora in Juvenile and Adult Stages of An Estuarine Fish, Mugil Cephalus

S Nandakumar[1], M G Sanalkumar[2], R.S.Balamurali[3], Saluja Krishnan[4], Swetha Chandra[5]
[1, 2, 3] Dept of ZOOLOGY
[1, 2] N.S.S. COLLEGE, PANDALAM 689 501,KERALA, INDIA
[3]MAHATMA GANDHI COLLEGE, THIRUVANANTHAPURAM 695 004,KERALA, INDIA

Abstract- *The digestive tract of fish is a microenvironment for various microbes that may be indigenous or transient. Mugil cephalus, commonly known as the flat head grey mullet, found in various habitat have the ability to tolerate wide range of salinities. So its feeding habit also varies according to habitat. Diet has a major role in gut microbial assemblage. An analysis of gut microflora of adult Mugil cephalus showed that the total culturable gut bacterial population was in the decreasing order of amylolytic bacteria, proteolytic bacteria, lipolytic bacteria and cellulolytic bacteria. But in juvenile fishes the population profile were in the decreasing order of lipolytic bacteria, amylolytic bacteria, cellulolytic bacteria and proteolytic bacteria. Juvenile fishes are surface dwellers and have a planktivores diet so their gut microflora contains more lipolytic bacteria but the adult fishes are feeders on detritus, microalgae and benthic organisms which might have contributed for the colonization of amylolytic and proteolytic gut flora. The introduction of beneficial bacteria to commercial aquaculture by incorporating them into fish diets or in the form of bacteria biofilm to achieve colonization in the fish intestinal tract can contribute to healthy growth profile for cultivable fishes.*

Keywords- Gut microflora, lipolytic bacteria, amylolytic bacteria, cellulolytic bacteria, proteolytic bacteria.

I. INTRODUCTION

Gut microflora is the complex community of microorganisms that live in the digestive tracts of animals. The microenvironment of the digestive tract of fish offers a favorable growth for the microorganisms by providing ecological niche for these organisms (Clements and Choat, 1995). The microbiota in the gut of fish may be indigenous or transient and the fish gut hosts a larger bacterial diversity (Ringo, 1999; Martin *et. al*., 2006). *Mugil cephalus*, commonly known as the flat head grey mullet, can be found in tropical, subtropical and temperate rivers, estuaries and coastal waters. They can tolerate a wide range of salinities from freshwater to hyper saline ocean water (Vagner *et. al*., 2008). Juvenile of mullet are primarily carnivores but at the same time the principal food sources of adult fishes are detritus and epiphytic algae. The gut microbiota of fish is highly dependent on the bacterial colonization during early development, environmental conditions, nutrient composition and dietary changes (Olafsen, 2001). Gut microflora of fishes contribute to the nutrition, growth, reproduction, overall population dynamics and vulnerability of the host fish to disease; therefore, this microbial community is highly relevant for aquaculture practice (Round and Mazmanian, 2009). The knowledge about the possible use of microflora as probiotics will increase the quantity and quality of fishes. The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics in aquaculture practice.

II. MATERIALS AND METHODS

Sample Collection: Juvenile and adult fishes of *Mugil cephalus* were sampled alive from four sites at Ayiramthengu, a part of Kayamkulam backwaters, Kerala. The selected sites were Vettathukadavu (901512˚N, 76.4705˚E), Kochiyude jetty (9.1707˚N, 76.4577˚E), Alumpeedika (9.1313˚N, 76.4456˚E) and Muttathumannelkadavu (9.1603˚N, 76.4705˚E). Samples were collected during morning hours and were immediately transported to the laboratory within 2-3 hours of collection in sterile plastic bags containing aerated water taken from the sites. Fishes with standard length in the range 3-5 cm were taken as juveniles and in the range 20-50 cm were taken as adult species (Lee, 1997). Samples were subjected to microbiological analysis. Average weight, total length, gut length and relative gut length of juvenile and adult fishes were measured using standard scale.

Microbiological analysis: Cellulolytic, amylolytic, lipolytic and proteolytic bacteria in the digestive tract of the collected fishes were cultured and identified in carboxymethyl cellulose agar (CMC), Starch agar (SA), Tween 20 agar (TA) and Skim milk agar (SMA) respectively.

Bacteria culture : Bacteria were grown on agar plates for the quantitative assay by adopting pour plate method from the diluted suspension. Fishes for microbiological analysis were ventrally cut aseptically to expose the viscera and gut was cut using sterilized equipments. One gram weight of the intestine samples were then homogenized in a surface sterilized motor and pestle. Then the sample was aseptically transferred to sterilized distilled water to get a serial dilution of 10^{-2}. 1ml each of 10^{-2} dilutions of bacteria sampled from fish gut were poured to the agar surface and spread evenly by rotating the plate and the samples were cultured in quadruplicates. All the procedures during the preparations of culture plate were carried out in laminar flow work station to avoid contamination. The plates were incubated in bacteriological incubator. Bacteria colonies were counted with the help of Digital Colony Counter.

Enumeration and identification: For extra cellular cellulose production, the samples were grown on CMC plates at 37˚C for 24 h and flooded with 0.1% Congo red stain and destained with 1M sodium chloride. Appearance of clear halo due to the presence of hydrolyzed CMC surrounding bacterial colony indicated cellulose production in the medium (Rehman *et al*, 2009). For extracellular amylase production, the samples were inoculated on SA plates and incubated at 37˚C for 48h. The culture plates were then flooded with 1% Lugol's iodine solution. Formation of transparent zone surrounding the colony indicated amylase activity. For determination of lipase activity, the samples were inoculated on TA plates. Formation of calcium laurate white crystals around the colonies confirmed the lipase activity of the colony. For assaying extracellular protease, the samples were inoculated on SMA plates and incubated at 37˚C for 24h. The appearance of clear zone around the colony confirmed the proteolytic activity (Jacobs and Gerstein, 1960).

Statistical Analysis

The data obtained were subjected to descriptive statistics such as mean and standard deviation and for assessing comparative performance, two-way ANOVA was carried out.

III. RESULTS AND DISCUSSION

Analysis of the bacterial flora in the gastrointestinal tract of adult *Mugil cephalus* showed that the total culturable gut bacterial population were in the decreasing order of amylolytic bacteria, proteolytic bacteria, lipolytic bacteria, and cellulolytic bacteria. It could be observed that, the adult fishes showed highest population of amylolytic bacteria with a number of 34.5 ± 1.5× 10^{-2} CFU/g, followed by 28.6 ± 1.2 × 10^{-2} CFU/g of proteolytic bacteria, 26.2 ± 1.0 × 10^{-2} CFU/g of lipolytic bacteria and 24.3 ± 0.5× 10^{-2} CFU/g of cellulolytic bacteria (Table 1).

Table 1: Bacterial count (10^{-2} CFU/ g) of adult Mugil cephalus collected from four different sites (mean ± SD).

Bacteria	SITE 1	SITE 2	SITE 3	SITE 4
Cellulolytic bacteria	19.1 ± 1.0	20.4 ± 1.0	22.6 ± 1.2	24.3 ± 0.5
Amylolytic bacteria	28.0 ± 1.5	30.8 ± 0.5	33.1 ± 0.6	34.5 ±1.5
Lipolytic bacteria	21.3 ± 0.8	23.4 ± 1.3	25.1 ± 0.9	26.2 ± 1.0
Proteolytic bacteria	24.8 ± 1.2	26.1 ± 1.5	22.7 ± 1.0	28.6 ± 1.2

F_{CC}: 8. 521; P<0.05 F_{CR}: 34.795; P<0.05

When the juvenile fishes were examined, the specific enzyme producing bacteria was in the decreasing order of lipolytic bacteria, amylolytic bacteria, cellulolytic bacteria, and proteolytic bacteria. The proliferation of the lipase producing bacteria was found to be maximum in the fishes collected from the site 1 as 40.5 ± 1.5 × 10^{-2} CFU/g and the least number observed was of 35.4 ± 1.2 × 10^{-2} CFU/g. But the amylolytic bacterial flora exhibited highest count in the site 3 which were counted as 23.2 ± 1.3 × 10^{-2} CFU/g and the least count was 20.6 ± 1.0 × 10^{-2} CFU/g. Cellulase producing bacteria showed maximum number in fishes collected from the site 4 with a value of 19.0 ± 1.5 × 10^{-2} CFU/g and the minimum count was 12.6 ± 1.0 × 10^{-2} CFU/g . Proteolytic bacterial flora were also detected and the maximum population density was 10.6 ± 1.3 × 10^{-2} CFU/g and the least number as 7.2 ± 0.6 × 10^{-2} CFU/g.

Table 2: Bacterial count (10^{-2} CFU/ g) of juvenile Mugil cephalus collected from four different sites of Ayiramthengu (mean ± S.E).

Bacteria	SITE 1	SITE 2	SITE 3	SITE 4
Cellulolytic bacteria	12.6 ± 1.0	17.3 ± 1.2	14.3 ± 1.0	19.0 ± 1.2
Amylolytic bacterial	21.8 ± 1.0	19.2 ± 1.0	23.2 ± 1.3	20.6 ± 1.0
Lipolytic bacteria	40.5 ± 1.5	35.4 ± 1.2	37.3 ± 1.4	39.2 ± 1.4
Proteolytic bacterial	7.2 ± 0.6	9.8 ± 0.8	8.4 ± 1.0	10.6 ± 1.3

F_{CC}: 0.634; P>0.05 F_{CR}: 121.610; P<0.05

A comparison between the juvenile and adult fishes reveals that the cellulolytic, proteolytic and amylolytic bacterial population were more in adult fish than the juvenile fish. While observing the lipase producing bacteria, the maximum population density were found in all the juvenile fish than the adult fish. But in the case of the proteolytic bacteria, juvenile fishes exhibit low occurrence than in adults. One of the major factors contributing to the bacterial assemblage in the gut is diet. This indicates that the digestive tract provides favorable ecological niches for these organisms.

Studies of adult *Mugil cephalus* have shown that the main diet are plant materials which consisted range of 60-80%. In estuarine waters, they also feed on detritus, diatoms, algae, and microscopic invertebrates (Blaber, 1976). The adult fishes are normally found in coastal waters (Eschmeyer *et. al.*, 1983). They are usually in schools over sand or mud bottom. They are mainly diurnal, feeding on detritus, microalgae and benthic organisms.

The abundance of amylolytic bacteria associated with gut shall definitely be the taken into account for the enzymes supplemented as a symbiotic relation. Many of the studies have proved that the algae and organic matter forms the major component of the food of adult fish. Algae such as diatoms are an excellent of protein and carbohydrate, with essential amino acids, vitamins, minerals and trace elements. They contain high amounts of simple and complex carbohydrates which provide the fishes with a source of additional fuel. The abundance of algae in the fish gut may contribute to the increased amylolytic bacteria, for epiphytic algae and diatoms contains huge amount of starch in their composition. The gut content analysis of the adult fishes ranging 19-25cm in the brackish water lakes have revealed the presence of zoobenthos, benthic invertebrates and crustaceans.

The high proteolytic bacterial count observed from the gut may be due to the consumption of organic matter, highly proteinaceous benthic invertebrates and crustaceans. Thus the proteolytic bacteria helps in the digestion of complex proteins materials in to simpler amino acids molecules that can be easily absorbed by the fish. The organic matter present in the upper layer of sediment is a mixture of detritus, microphytobenthos and infauna. Detritus usually represents the bulk of sediment organic matter, however the nutritional quality of detritus is highly variable and often low. The adult fishes behave as a sediment filterer and feed mainly on detritus and microphytobenthos (Das, 1997). It is also observed that the adult flat head grey mullets generally feed by grazing on submerged rock and plant surface and the function of inorganic particles in the diet is suggested in the grinding activity to degrade plant cell walls in the pyloric portion. Blaber (1976) hypothesized that the adult *M. cephalus* from South Africa may prefer animal prey to plant materials when available. The least count of cellulolytic bacteria shows the least preference for plant based materials by the fish.

Studies relating to the gut bacteria and the feeding habits of juvenile fish less than 5cms in length is scarce and rare. The peak count of the lipolytic bacteria in the present study has been found in accordance with the planktivorous habit of juvenile fishes. Copepods forms a major food component and this can contribute to the increased presence of lipids in the fish diet. Copepods sequester lipid, oil and fatty acids derived from their main food source, phytoplankton, which is then transferred up the food web (Parrish *et. al.*, 2012). Phytoplankton are the primary producers of lipids and fatty acids, which are then ingested, synthesized and incorporated by copepods and other zooplankton.

Amylolytic bacterial population indicated the presence of algal component in the diet of juvenile fishes. Even if they are surface dwellers, some other studies have shown that the juvenile feed mainly on dinoflagellates, algae, diatoms and crustaceans. The amylolytic bacteria converts the starch components in these feeds in to simple glucose subunits and helps in the digestion process. The intake of plant materials has been proved by the presence of cellulolytic Bacteria. Comparative analysis of the gut microflora in the juvenile and adult fishes reveals that the bottom dwelling habit of adult fishes along with algal consumption reasons for commentable occurrence of amylolytic bacteria and the relative abundance of other enzyme digesting bacteria supports their omnivorous nature too. The good number of lipolytic bacteria in the juvenile fishes show that they are surface dwellers and has a planktivorous diet. The ontogenetic dietary shift from the planktivorous diet to benthic feeding from juvenile to adult stage is clearly established from the present study.

IV. CONCLUSION

Mugil cephalus is regarded as an excellent food fish. Striped mullet are marketed fresh, dried, salted and frozen and has high demand as a food. Global aquaculture production of the mullet was approximately 1, 21365 tons while capture production was 23927 tons in the year 2002. Approximately 20 mullet species have been cultured in a lot of regions of the world. There is good scope for application and utilization of probiotics in aquaculture systems to enhance food assimilation and consequent production by studying the ability of microbial colonies associated with gut of fishes Due to overfishing of wild populations, aquaculture has become an economic activity of great importance around the world.

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