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EFFICIENT AND EFFECT OF DIETARY COMPONENTS ON THE GUT MICROBIOTA OF AQUATIC FISHES – BY HYDRO CHEMISTRY – MODELING – INFORMATION TECHNOLOGY

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ABSTRACT: *Numerous studies have reviewed the structure and function of the fish gut in relation to diet [1] as the gut Microbiota has not been included in these papers, a more discussion is needed. Therefore the main objective of the present review was to summarize the available information regarding the effect of dietary components on the gastrointestinal (GI) Microbiota of fish. It is well known that healthy gut Microbiota is essential to promote host health and well being. The intestinal micro biota of endothermic animals as well as fish are classified as autochthonous or indigenous, when they are able to colonize the host's epithelial surface or are associated with microvillus or as allochthous or transient (associated with digesta or are present in lumen). Furthermore, the gut Microbiota of aquatic animals is more fluidic than that of terrestrial vertebrates and is highly sensitive to dietary changes. In fish, it is demonstrated that [a] Dietary form, [b] Dietary lipid (lipid levels, lipid sources and polyunsaturated fatty acids) [c]protein sources (soybean meal, krill meal and other meal products)[d]functional glycomic ingredients (Chitin and cellulose) [e] nutraceuticals (probiotics, prebiotics, synbioitics and Immunostimulants) [f] antibiotics [g] dietary iron and [h] chromic oxide affect the gut Microbiota in these all, we explain* ***some of them*** *in this paper. Moreover, some information is available on bacterial colonization of the gut enterocytes surface as a result of dietary manipulation which indicates that changes on indigenous microbial populations may have repercussion secondary host microbe interactions. The effect of dietary components on the Microbiota is important to investigate, as the gastrointestinal tract has been suggested as one of the* ***major routes of infection in fish*** *Possible interactions between dietary components and the protective Microbiota colonizing the digestive tract are discussed in this paper*

KEY WORDS: *Gut Microbiota, Microvilli, Poly–β –hydroxibutyrate, Immunostimulants and Antibiotics*

I. INTRODUCTION

Until the 1970s, controversy existed about the role, and even the existence, of an indigenous gut micro biota in fish. However, it is now generally accepted that fish and other aquatic animals have a micro biota in the GI tract [2], which in turn has increased in their diversity and functional relationship. However, the gut micro biota is modulated by dietary manipulation (Table – I) as well as by seasonal variations, stress, individual variations, and different regions of the GI tract, cultured versus wild, triploid versus, diploid, day – to – day variations, male versus female, developmental stages/ life cycle, microbial aspects of live feed, fast versus slow growing, hierarchy formation, starvation, migration from fresh water into sea water and migration from sea water back to fresh water, water quality (Pseudo – green water versus clear water, recirculation versus conventional flow – through, fish farms within a restricted area, environmental and ecological factors, and host ecology and environment (Table – II)

In this context, it is important to evaluate the effect of dietary components on the intestinal micro biota of fish, as gastrointestinal (GI) tract is one of the major parts of entry for some pathogens [3]

In the 1970, 1980 and 1990, numerous investigations were conducted to determine the dietary effects on the intestinal micro biota, and the majority of these studies were based on culture – dependent techniques and the use of physiological and biochemical properties to characterize the gut micro biota. However from 2000 to 2006, there was a shift to use molecular methods to characterize the culturable gut bacteria [4] , but nowadays culture – independent methods have become more common (Table – III). These recent investigation have widened our knowledge about the intestinal micro biota of fish and demonstrated that the microbial diversity of the fish gut is more complex than previously believed

Table – I: Overview of studies investigated the effect of diet on gut micro biota of aquatic animals Only taken for fish		
Dietary Component used	Aquatic Animals Fish only	Reference
Different Dietary lipid sources	Atlantic salmon	Ringo et. al. (2002) [5]
Different Diets	Gold Fish Puffer Fish *Tilapia*	Sugita et. al (1988) [6] Ramachandran 2005 [7] *Bolnick et. al (2014)* [8]
Copper Metal – nanoparticle	*Nile Tilapia*	*Merrifield et. al (2013)* [2]
Sodium butyrate	*Tropical Catfish*	*Owen et. al (2006)* [9]
Organic acid blend (formic, lactic, malice, tartaric and citric acid)	*Red hybrid Tilapia*	*Koh et. al (2014)* [10]
Alginic acid	*Tilapia*	*Merrifield et. al (2013)* [2]
Poly – β- hydroxybutryate	*Tilapia*	*Nhan et. al (2010)* [11]
Betaine	*Hybrid Tilapia*	*He et al. (2012)* [12]
Yeast culture	*Hybrid Tilapia*	*Zhou et. al. (2009)* [13]
Potassium diformate	*Hybrid Tilapia*	*Zhou et. al. (2009)* [13]
Immunostimulants	*Red Tilapia*	*Merrifield et. al (2013)* [2]
Different antibiotics	*Hybrid Tilapia*	*He et al. (2012)* [12]

Even though the traditional culture based technique possesses rather low sensitivity of bacteria colonizing the digestive tract of fish, it is able to indicate differences due to minor dietary alterations. The gut micro biota may function to prevent pathogens from colonization; it is likely that the gut micro biota might be vital importance with regard to fish health. The object of the present paper, to review present information on how dietary supplements affect the population level of gut bacteria and composition, is relevant. This is strengthened by the fact that during the last decade, the aquaculture industry is increasingly demanding sustainable alternative lipid and protein sources to reduce the use of fish meal (FM) and fish oil (FO) [14]

This review firstly presents a short overview of the GI tract of fishes and the techniques most often used for the study of GI micro biota as a background for the succeeding chapters covering impact of the nutrients sources, probiotics, prebiotics, and antibiotics.

Table – II: Overview of Studies investigated the effect on the gut micro biota of fish	
Seasonal Variations	Sugita et al. (1988) [6]
Stress	Ringo et al. (2002) [5]
Individual Variations and different regions of the GI tract	Sugita et al. (1990) [6]
Cultured versus wild	Sugita et al. (1990) [6]
Triploid Versus Diploid	Cantas et al. (2011) [15]
Day – to – Day Variations	Sugita et al. (1990) [6]
Male Versus Female	Iehata et al .(2015) [16]
Different Fish Species fed similar Diet	Li et al. (2014) [17]
Development Stages/Life Cycle	Huang et al. (2014) [18]
Microbial Aspects of Live Feed	Bakke et al. [1]
Fast Versus Slow Growing Fish	Sun et al. (2009) [19]
Hierarchy Formation	Ringo et al. (2002) [5]
Starvation	Xia et al. (2014) [20]
Water Quality	Gatesoupe et al. (2013) [21]
Recirculation Versus Conventional flow – through	Attramadal et al. (2012) [22]
Fish Farms	Diler et al. (2000) [23]
Environmental and Ecological Factors	Sullam et al. (2012) [24]
Host Ecology and Environments	Wong & Rawls (2012) [25]

II. THE GASTROINTESTINAL TRACT – THE DYNAMIC ENVIRONMENT OF THE MICRO BIOTA

2.1 GENERAL CONSIDERATION

The key function of the alimentary tract is its ability to dissolve foodstuffs and process nutrients to make them suitable for absorption by various transport of mechanism in the wall of the GI sections. Besides hydrolytic reactions catalyzed by endogenous enzymes secreted by the pancreas and cells in the gut wall, considered to play the major roles in

digestion, fermentation may also play key roles in digestive processes in fish as in many other monogastrics. The role fermentation in fish is unclear, as research on micro biota in fish intestine is still in its early stages. However, it role is considered to be of minor quantitative importance for nutrient supply in cold water species. The importance for the intestinal micro biota is highly significant for normal functioning of the immune apparatus of the GI tract and the general resistance of the fish towards pathogens and other foreign factors constantly influencing the fish via the intense. The characteristics of the micro biota, products of metabolism etc. depend greatly on the conditions of the intestine, determined by species specific parameters along the GI tract such as anatomy, endogenous inputs of digestive secreta, pH, osmolality, redox potential, compartment size and structure, passage rate and residence time [5]. In this paper will discuss only anatomy, physiological characteristics

2.2 ANATOMY

The GI tract is a tube histologically differentiated in different segment that course through the body. This tube may have a few several hundred sub compartments in which microbes may divide and grow. The GI tract is commonly divided in the following regions: Mouth, Gill arch, Esophagus, Stomach, Pyloric caeca, Mid Intestine (MI), Distal Intestine (DI) and Rectum. For example The GI tract of Atlantic Cod is illustrated in figure – 1. Some fish species lack a typical stomach which in these fish is replaced by a foregut. Pyloric caeca are finger like extensions typical of most teleost fish. They are located in the proximal part of the intestine, MI, and when present, number from a few, as in Atlantic halibut to several hundred as in the Atlantic cod. The structure of the wall of the GI tract varies along the tract, but has in common surface facing the lumen of mucus producing (Goblet) cells between enterocytes. The latter holds digestive and transport apparatus located in microvillus facing the lumen, and being responsible for the uptake of nutrients see figure – 4 (a) and (b). The mucosa lining of the GI tract represents an interface between the external and internal environments and, in conjunction with the associated organs (Example: Pancreas, Liver and Gall Bladder), provides the functions of digestion, osmoregulation, immunity, endocrine regulation of GI tract and systemic functions, as well as the elimination of environmental contaminants and toxic metabolites

Just below the mucosa, we find the sub mucosa which is a layer of connective tissue, blood vessels and nerves. A single or double layer of muscles is located outside the sub mucosa. The serosa forms the outer layer of the GI tracts. In some fish, the compartments may hardly be distinguishable macroscopically, while in other the sections are divided clearly and may be separated by valves or sphincters. The presence of valves and sphincters between the sub compartments of the intestine may greatly influence the residence time of the chime in the compartment and hence for the possibilities of the micro biota to develop.

The Esophagus is, in most fish, short and of small diameter, with the possibilities to expand greatly and with numerous goblet cells aiding in food passage. We will stop here.

There is a great variability of the structure and functional characteristics of the GI tracts among fish species [5] which seems to match to the wide diversity of feeding habits and environmental conditions exploited by fish. The variation is obvious by comparing the GI tract characteristics of carnivorous and herbivorous fish and those from fresh water and sea water.

2.3 PHYSIOLOGICAL CHARACTERISTICS

Fish have ability to adopt the GI tract characteristics rapidly and reversibly to match the changes in functional demands that take place during the life history (Example: Metamorphosis, Anadromous or Catadromous migrations and from day – to – day due to seasonal shifts in diet or environmental conditions Karila et al. (1998) [26]

The mechanisms behind involve a wide diversity of hormones and other signaling molecules secreted by the various cells of GI tract. They modulate the composition of digestive gut wall, exocrine pancreas and liver and allow fish rapidly and reversibly to alter the characteristics of the GI tract and other organ systems to adapt to changes in the contents of the GI tract, such as amounts and types of nutrients, pH, ionic composition, and to environmental conditions. The various components of the digestive secreta to the intestine may serve as substrates for the microbes, but enzymes such as proteases and lipases and antimicrobial components meant to protect the animal, will represent challenges to the micro biota. Information on GI pH of early life stages of marine fish is available [27]. In addition, unpublished observations (A. Krogdahl) made in studies of the GI tract in Atlantic salmon conducted at the Aquaculture Protein Center over some years indicate the pH in a filled

stomach is variable but general as shown in figure 2 & 3. In the pyloric region mid and DI, all observations made showed values about 7 and mostly above 8. The pH of the chime seems to be regulated within fairly narrow ranges. The rather high pH observed in the stomach, compared with that in mammals, may be relevance for microbial survival in the stomach with higher survival during passage of the stomach in fishes. The lack of acidification in the foregut of stomach less fish species makes it even more likely for microbes to survive the passage to the more distal parts of GI tract in these fish compared with fish with stomach.

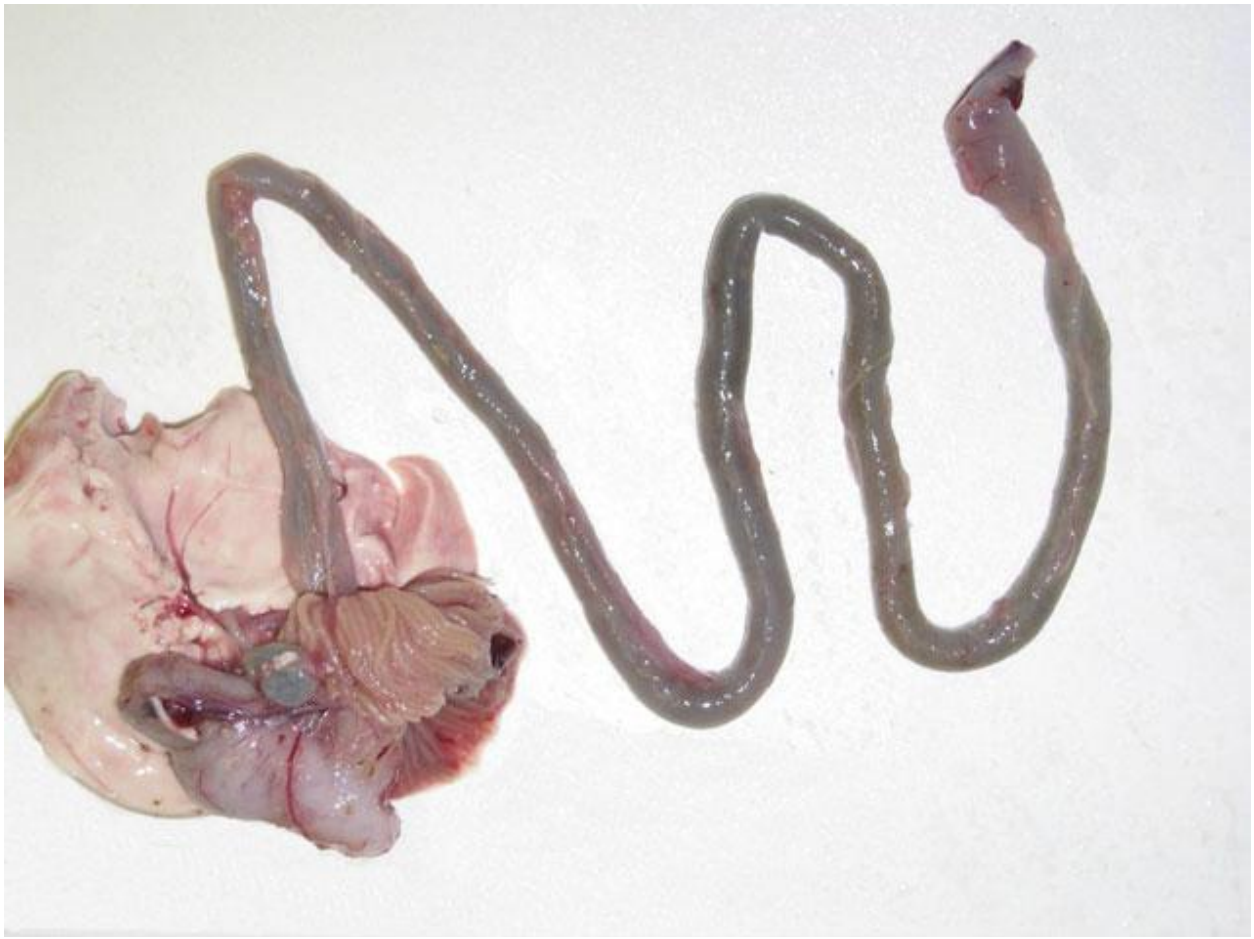

Figure – 1: The gastrointestinal tract of Atlantic cod. Note the many pyloric caeca which may number several hundred in this species. The distal intestine is a pouch closed by sphincters in both ends.

Mucosa associated lymphoid tissue (MALT) in teleost fish is sub divided into gut associated lymphoid tissue (GALT), skin associated lymphoid tissue (SALT) and gill associated lymphoid tissue (GIALT) [27] GALT which represents an essential part of an organism's adaptive defense system is considered to protect the host against pathogens not only by fighting the intruding bacteria but also modulating the composition of the micro biota. Micro biota stability in

animals including fish has been observed. Microbial communities transplanted from mice to gnotobiotic zebra fish altered quantitatively in the direction of the normal biota of the zebra fish species and vice versa. Antibodies, lysozyme and other antimicrobial components in mucus secreted from the wall of the GI tract may play a key role in the apparent stability of the intestinal micro biota. The function GALT depends on diet composition, such as its content of oligosaccharides and the nutritional status regarding essential nutrients, such as selenium [28]. In addition, GALT must develop mechanisms to discriminate between pathogenic and commensally micro organisms

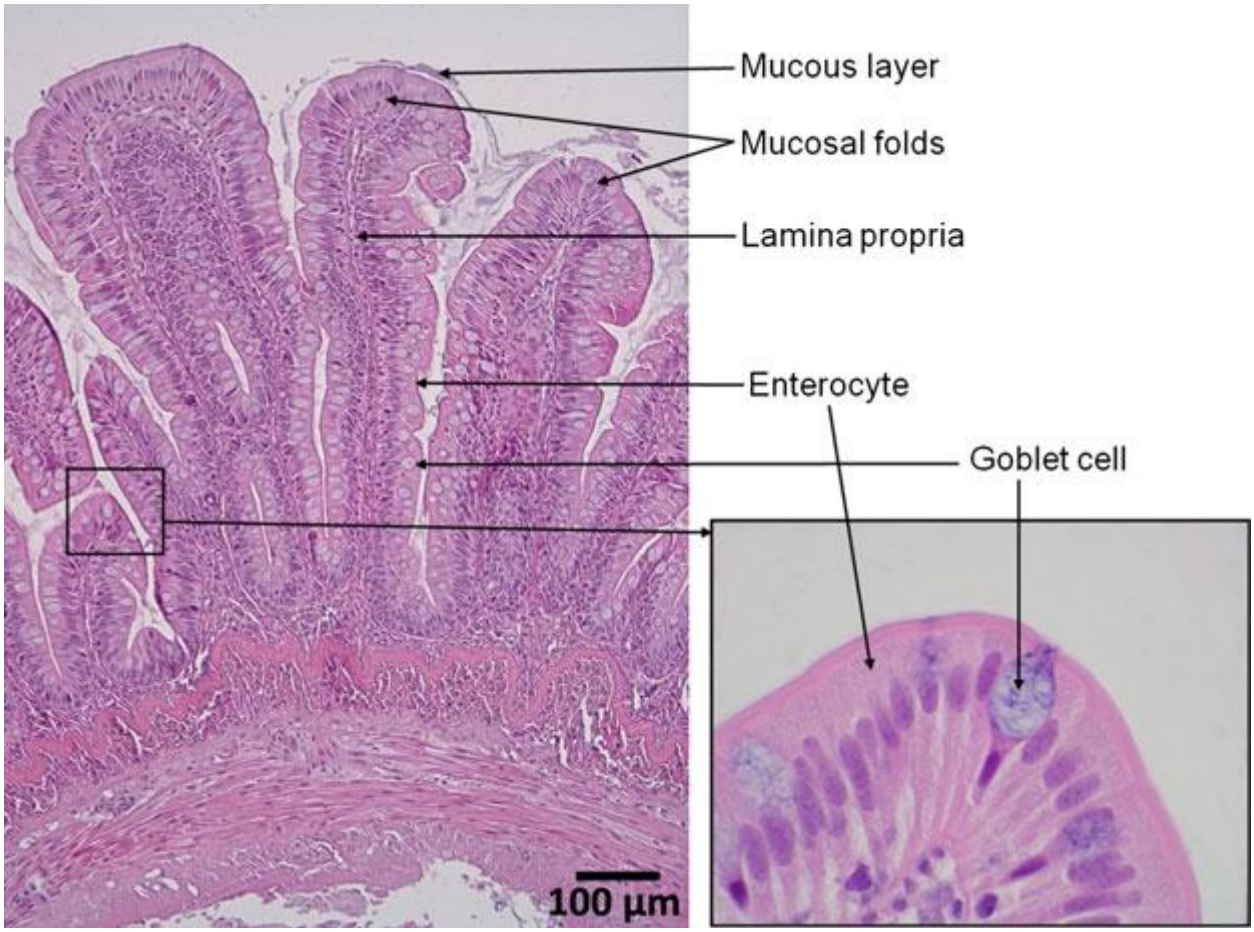

Figure – 2: Structure of the GI tract wall. A histological presentation, stained with haematoxylin and eosin, of the wall of the mid intestine in Atlantic salmon. A layer of mucus secreted by the goblet cells covers the mucosal folds. Cells are dying continuously and released from the top of the folds into the chyme, mixing with unabsorbed food materials as well as components of endogenous secreta**. Photograph: M. Penn**

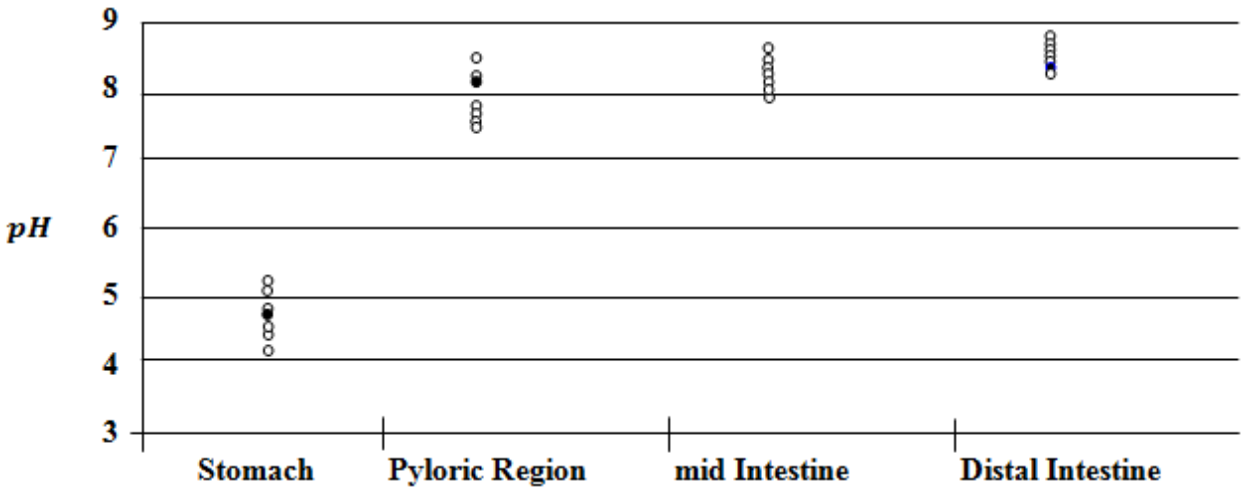

Figure – 3: pH chyme of Atlantic salmon in sea water (H. Holm & A. Krogdahl, unpublished data). The data originate from three feeding experiments, each testing three diets varying in protein content or amino acid supplementation. Each circle represents the mean pH of observations in several fish fed the same diet. Only fish with content in the gut segments were used. No significant effects of diet on pH were observed within experiment.

2.4 MOLECULAR BIOLOGICAL METHODS FOR CHARACTERIZATION OF THE GUT MICRO BIOTA IN FISH

Several different molecular methods are today available for detecting micro organisms is a given sample and monitoring the change in microbial communities, without culture dependent techniques. In the early studies investigating the gut micro biota of fish, conventional culture based methods, were used for review [5] Conventional culture based techniques, even if several different media are used, do not present a “True” picture of the bacterial diversity. Therefore, to present more reliable information about the gut micro biota of fish, molecular methods are necessary.

Culture independent methodologies are useful tools in furthering our understanding of complex ecosystems and have highlighted the limitations associated with culture dependent techniques. Denaturing gradient gel electrophoresis (DGGE), terminal reaction fragment length polymorphism (T – RELP), automated rRNA intergenic spacer analysis (ARISA). Single strand conformation polymorphism (SSCP), 16S rRNA tag pyrosequencing method are examples of such culture independent techniques that have been used to profile bacterial population in a wide variety of ecosystems Lee et al. 1996 [29]. In all of these techniques,

extracted community DNA is amplified using the polymerase chain reaction (PCR) utilizing the primers specific for conserved regions 16S rRNA. Examples of the published papers using culture independent methods in studies evaluating the gut micro biota of aquatic animals and fish are presented in Table – III, those who interest in the field of gut micro biota of fish research.

Table – III: Culture Independent Methods used in studies evaluating the gut micro biota in aquatic animals and Fish (In this table only mentioned Fish)

Method	Species	Microbiota: Allotchthonous (allo) or Autochthonous (auto)	Part of the GI tract investigated	References
DGGE	Puffer fish Hybrid Tilapia Red Tilapia	Allo Auto Allo	Whole intestine Whole intestine Whole Intestine	Yang et al. (2007) [30] Zhou et. al [14] Ferguson et al. (2010) [31]
Biolog Ecoplate ™ and DGGE	Different Fish Species	Allo	Whole intestine	Mouchet et al. (2012) [32]
PCR – TGGE Clone libraries	Coral Reef Fish Japanese Costal Fish	Allo and Auto Allo	Whole intestine Whole intestine	Smriga et al. (2010) [33] Tanaka et al. (2012) [34]
Fish Micro biome	Different species	Allo	S, PC and MI	Sanchez et al. (2012) [35]

Note: S – Stomach, PC – Pyloric Caeca, MI- Mid Intestine

Molecular based methods to describe the microbial communities in a certain sample can be divided into two groups

a. The PCR based techniques which amplify certain fragments of DNA or cDNA using user defined primers and
b. The PCR independent methods which detect bacteria without any gene or cDNA amplification.

Generally the PCR independent methods are less specific and sensitive than PCR based techniques, and they are suitable for profiling bacterial communities. PCR based techniques are qualitative methods when applied to environmental samples due to inherent biasing in PCR amplification. First in this paper we will describe some PCR independent techniques and then the methods which are based on the PCR techniques.

2.5 PCR INDEPENDENT TECHNIQUES

The most common procedure today is to label the probe with a flurophore, called fluorescent in situ hybridizations (FISH). This allows for the simultaneous detection of different micro organisms, using a set of fluorophores with different excitation and emission maxima. The probe can be either RNA or DNA oligonucleotides, and the target can be RNA or DNA. If using DNA at the target, both dead and the viable bacteria will be detected, while RNA as the target will only reveal viable bacteria. Labeling can be performed directly with a fluorescently labeled probe, which is the fastest, cheapest and easiest way. To increasing the labeling sensitivity, which may be relatively low using direct labeling, the probe can be labeled indirectly by enzymatic signal amplification.

2.6 IMMUNOHISTOCHEMISTRY

Instead of using oligonucleotides for the detection of micro organisms, bacteria can be labeled with antibodies which can subsequently be visualized by the use of secondary antibodies. This method has some similarities with in situ hybridization. Immunohistochemistry is highly suitable to follow the infection route of bacterial strains to which a specific antibody has been raised, In addition, bacteria cultured culture in vitro and used for immunization may have a slightly different morphology in vivo, considering that bacteria are affected by the environment in which they grow. This may result in changes in the antigen morphology between in vitro and in vivo growth. At last, Immunohistochemistry does not yield a very high sensitivity and also, which is a time consuming and expensive

process. The advantage of using a monoclonal antibody is that a highly specific antibody can differentiate even between different strains, and it requires less optimization compared with in situ hybridization.

2.7 TRANSCRIPT ANALYSIS WITH AID OF AFFINITY CAPTURE

New methods are continuously being developed to more accuracy determine the composition of microbial communities. One of these is the transcript analysis with aid of affinity capture (TRAC) method, which is a multiplexed and sensitive method for relative quantification of bacteria. The TRAC method is a profiling technique without the need for PCR amplification and may offer a more reliable estimate of the bacterial composition in a given sample than PCR based methods.

2.8 PCR BASED TECHNIQUES

All PCR based methods consists of three basic steps

a. Nucleic acid extraction
b. Amplification of DNA and
c. Analysis (Either quantitatively or qualitatively) of PCR products

2.9 NUCLEIC ACID EXTRACTION

For investigating the presence or absence of bacteria, DNA can be extracted and used as template in either PCR dependent or PCR independent methods. There are, however, different DNA extraction methods and these may influence the relative composition of the DNA pool [13], compared the effects of three different DNA extraction methods (lysozyme digestion, CTAB method and bead mill), it is deal with grass carps for studying the viable portion of the micro biota.

2.10 CLONE LIBRARY CONSTRUCTIONS

Most widely used of 16S rRNA gene and this method to gain sequence information from a given sample, it consists of several steps they may influence the composition of the clones for example, the method used for DNA extraction, the primers used for gene amplification, and the conditions used to amplify the gene. As for all PCR dependent techniques, the user

defined primers determine the amplicon. Usually, primers annealing to highly conserved regions of the 16S rDNA are chosen in order to obtain an amplicon consisting of the highest diversity as possible. The construction of clone libraries is often accompanied by other types of techniques, such as PCR – DGGE, PCR – TGGC or T – RELP, all of which are typical profiling methods. PCR – DGGE and PCR – TGGC combining PCR amplification with separation of the amplicons with either denaturing or temperature gradient gel electrophoresis is widely used technique to determine the bacterial communities in fish and crustacean [14] .

2.11 NEXT GENERATION SEQUENCING TECHNOLOGIES

Pyrosequencing is another method that is used for high throughput sequencing of clone libraries. There are other techniques are also available they are Terminal restriction fragment length polymorphism like as PCR – DGGE, PCR – TGGC, Real time (RT – PCR) a quantitative PCR based method, Single strand conformation polymorphism (SSCP – PCR), rRNA intergenic spacer analysis (RISA), In RISA, the length heterogeneity of intergenic spacer is exploited. The PCR product (A mixture of fragments contributed by community members) is electrophoresed in a polyacrylamide gel, and the DNA is visualized by silver staining. The result is a complex banding pattern that provides a community specific profile with each DNA band corresponding to at least one organism in the original assemblage. This method has only been used in one fish study.

2.12 EFFECT OF DIETARY LIPID

From a microbial point of view, the pyloric caeca is of vital interest as lipid digestion and absorption occur in this organ [5]. However, due to its complex morphology only some studies have investigated the micro biota of pyloric caeca in fish and to our knowledge, no investigation so far has evaluated the effect of dietary lipid on micro biota of pyloric caeca, a topic that merits investigation.

2.13 LEVEL DIETARY LIPID

In their study with rainbow trout (Oncorhynchus mykiss Walbaum), Lesel et al [36] feed the fish two different diets, low $(50\ g\ kg^{-1})$, and high $(160\ g\ kg^{-1})$lipid levels. Differences in faecal bacterial micro biota of fish fed low lipid level consisted on only **Acintobactor spp**.

and Enterobacteria. In contrast *Acintobactor spp., Aeromonas spp. Pseudomonas spp.* and coryneforms were isolated from faeces of fish fed the high lipid level. However, as only 12 isolates from each dietary group were isolated, no clear conclusion can be drawn.

2.14 DIFFERENT DIETARY LIPID SOURCES

Fish oil were for many years the predominating lipid source in diets for carnivorous fish species. However, the increase in aquaculture led to an increased consumption from 16% of available fish oil in 1988 to 81% in Tacon et al.(2002) [37]. This was close to full exploitation. Although studies with substitutes have been done in the past, the prospect of deficiencies spurred extensive work into finding replacements. One obvious choice was vegetable oils. The main reason was that the global production is approximately 100 times higher than that of fish oils (FO) with no prospects of limitations [38]. Secondly they often come at compatible prices compared with FO.

As no information was available about how inclusion of vegetable oils in commercial raw material affects the gut micro biota of fish, [5] investigated the effect of soybean, linseed and marine oils on the hindgut micro biota of Arctic Char. This study showed clear differences in the hindgut micro biota of fish fed different oils (After and prior to challenge with *Aeromonas* salmonicida subsp.) Carnobacteria were only isolated from the hindgut region of fish fed soybean oil (SBO) and linseed oil before challenge, while Carnobacteria spp. and C. Funditum – like strains were isolated from fish fed the same oil after challenge. Furthermore, the ability of carnobacteria to inhibit the growth of A. Salmonicida ssp it was highest in strains isolated after challenge.

2.15 EFFECT OF KRILL, CHITIN, CELLULOSE, RAFFINOSE AND STACHYOSE KRILL AND CHITIN

The second most abundant biomass (10^3, metric ton, Jolles & Muzzarelli 1999 [39]) in the world is chitin consists of a $\beta - 1,4$ linked N −acetylglugosamine residues. Chitosan is obtained from the partial deacytylation of chitin and is therefore a high molecular weight linear composed mainly of 2 amino 2 deoxy D glucose units linked through $\beta(1 \rightarrow 4)$ bonds, and the distinction between chitin and Chitosan is based on the degree of acetylation. Chitin has acetylation values higher that 50%, while Chitosan has lower percentages. As less

information is available on Chitosan as microbial modulators, we recommend that this is merits further investigations.

2.16 CELLULOSE AND EXOGENOUS CELLULASE

Cellulose the most abundant biomass (10^5, metric ton, Wilson & Irwin 1999 [40]) in the world is cellulose and consists of a $\beta - 1,4\ glycosidic\ linkages$. Thus, many cellulose eating animals require the aid of symbiotic micro organisms in their GI tract to digest cellulose and make the energy in this compound available to the host. Information is available on the microbial community in different parts of GI trace of wood eating fish. (Di Maiuta et al. 2013 [41])

For Exogenous cellulase, several studies have shown that the intestinal micro biota of aquatic animals harbors cellulose decomposing micro organisms, including sequences related

to $Anoxybacillus, Bacillus, Carnobacterium, Citrobacter, clostridium, Leuconostoc, Staphylococcus, Acinetobactor, Phaeobacter, Pseudomonas, Rhodobacteraceae, Vibrio$

And$Actinomyces$

2.17 OTHER CARBOHYDRATE SOURCES:

In a recent study Pedrotti et al. (2015) [42] evaluated the dietary effect of different carbohydrate sources, broken rice, dextrin, cassava bagasse, ground corn and wheat bran, on total heterotrophic cultivable autochthonous and amylolytic gut micro biota in DI of Tilapia and jundia (Rhamdiaquelen). The general findings were no difference in levels of total cultivable bacteria among carbohydrate sources within the same fishes. However, jundia fed diets containing broken rice revealed higher total bacterial when cassava bagasse or ground corn were included in the diet. We suggested that culture independent quantitative techniques should be incorporated to evaluate the bacterial changes in future studies

III. EFFECT OF ACIDIFIERS, ACIDIC CALCIUM SULPHATE, SODIUM BUTYRATE, POLY β HYDROXYBUTRYATE AND POTASSIUM DIFORMATE

3.1 ACIDIFIERS

Dietary acidifiers have been reported as a beneficial in aquaculture where they confer benefits such as improved feed utilization, growth and residence to bacterial pathogens.

Historically direct addition, Short chain fatty acid (SCFA), in cultured fish diet were principally in the form of fish silages which can be preserved by the addition of formic acid alone or in combination with propionic acid or sulphuric acid with resent publications focusing on the use of SCFA to increase mineral bio availability in the GI tract [17]

Acidic Calcium Sulphate. It is not available for fish only available on Allotchthonous gut micro biota of Pacific white shrimp

3.2 SODIUM BUTYRATE

Modulation of the gut micro biota by sodium butyrate has been reported in broiler chickens, early weaned pigs as well as for fish, Ringo et al. [5], investigated the effect of the sodium butyrate on the Allotchthonous micro biota of the hindgut of African cat fish. Diets contained either FM as the protein sources of partial replacement with SBM were supplemented with 0.2% and 2% sodium butyrate supplementation. After 15 days of feeding on the experimental diets, the culturable Allotchthonous micro biota was investigated.

3.3 POLY β HYDROXYBUTRYATE (PHB)

The use of PHB as a dietary component for aquatic animals is not inspired by a direct action by which it affects the host or the gut micro biota. The rationale can be found in the release of metabolites in the GI tract with the aim of improving the health status of host or increasing the protection of the host against infections. PHB is a compound that is synthesized by a very of micro organisms mainly under conditions of nutrient limitation and carbon excess. The chemical structure makes it an interesting compound for application in aquaculture settings. It is insoluble in water and consists of an aliphatic C3 polyester backbone with methyl group situated at the position of the molecules. During GI passage, PHB comes in close contact with the micro biota inhabiting the gut. It is likely that PHB induces modifications at the microbial level resulting directly or indirectly in effects the host level. It was and still is thus important to gain insight into the interaction between PHB and the intestinal microbial community as was attempted in several studies.

3.4 POTASSIUM DIFORMATE:

The aim of study of [14] was to investigate the effect of Potassium Diformate (PDF) and two antibiotics on growth performance, feed conversion and gut micro biota of Hybrid Tilapia, PDF is the first substance approved as non antibiotic growth promoter by the Europe Union

and is an alternative substance for growth promoters of Tilapia. Furthermore, dietary antibiotics affected the Tilapia's growth performance possibly through depressing most of the intestinal bacteria.

3.5 EFFECT OF METALS IRON

In our knowledge, only one study has evaluated the effect of iron on the micro biota of fish and also this research only for sea bass larvae and recent study of Sugita et al [6] evaluating the diversity of siderophores producing bacteria isolated from the digestive tract of Japanese fish species. Remaining Copper Chromic Oxide, Phosphorus, Metal nanoparticle all are evaluating the effect of dietary for zebra fish, juvenile jian carp, and Arctic Chars.

3.6 EFFECT OF ANTIBIOTICS

The most commonly used antibiotics in fish farming in the 1970s and 1980 were oxolinic acid, oxytetracyline (OTC), furazolidone, potential sulphomanides (Sulphadiazine and trimethoprin) and amoxicillin. However, the indiscriminate use of those chemicals in disease control in many sections of the aquaculture industry has led to selective pressure of antibiotic resistance in bacteria, a property that may be readily transferred to other bacteria [5]

Use of antibiotic to control pathogenic bacteria can also reduce the numbers of non pathogenic bacteria in the gut, and numerous studies are available on the effect of antibiotics on intestinal micro biota [1], [5], [6], [12], [13]. In their study with rainbow trout, used erythromycin, oxolinic acid (OA), OTC, penicillin G and sulphafurazole to study the effect of antimicrobial compounds in aerobic heterotrophic gut micro biota (Table – IV). A general increase in bacterial population level in the GI tract was observed during 10 days treatment when the fish were administered OA, OTC and sulphafurazole which are commonly used for the treatment of Gram negative pathogens. After the treatment, however, there seemed to be a steady decrease during the following two weeks of period. Conversely, erythromycin and penicillin G, which are used to treat some diseases caused by Gram positive bacteria, caused a rapid reduction in bacterial numbers within the GI tract. Moreover, a dramatic effect was found for penicillin G as the esophagus and stomach appeared to be totally devoid of bacteria through the treatment regime and also the bacterial composition in the GI tract during administration, focusing on some groups of Gram negative bacteria.

Table – IV: Composition (%) of the bacterial population in the digestive tract of rainbow trout during the administration of antimicrobial compounds via medicated food. After Austin & Al Zahrani (1988) [43]

Treatment Regime		Erythromycin			Oxolinic acid			Oxytetracyline			Penicillin G			Sulphafurazole		
Sampling Day	Control	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
Taxon																
Gram negative																
Acintobactor spp.	8	5	0	0	52	40	36	44	40	32	0	10	5	0	0	10
Aeromonas spp.	16	35	20	0	8	0	8	4	0	0	70	30	25	30	20	15
Alcallgenes spp.	0	0	5	5	0	4	0	4	0	0	10	5	0	10	0	0
Eneterobacteriaceae	20	15	25	40	16	8	4	12	8	4	5	0	0	0	5	10
Flavobacterium spp.	4	0	10	10	0	8	12	0	12	18	0	15	0	0	0	10
Methylamines spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Pseudomonas spp.	12	25	5	15	0	4	0	12	0	0	0	10	0	5	10	10
Gram positive																
Bacillus spp.	8	10	15	0	0	0	0	8	8	12	10	5	40	40	40	35
Coryneforms	12	0	10	25	12	8	8	8	12	20	5	10	0	0	5	5
Micrococcus	12	5	5	0	4	20	24	4	12	2	0	0	0	0	15	0
Staphylococcus spp.	8	5	5	20	8	8	8	4	8	12	0	15	30	10	5	5

Currently, molecular approaches and massive sequencing methods have become available; hence these could be important tools to elucidate the diversity of antibiotic resistance genes present in the fish gut. The resistive concept has been used to describe the diversity of antibiotic resistance that exists naturally in particular environment. However the resistive of aquaculture environments has been poorly described and it will require more studies using molecular approaches. These approaches should allow the diversity of antibiotic resistance genes in the gut to be analyzed, even when no antibiotics are used and also permit the effects of antibiotics on bacterial populations to be evaluated

IV. FUTURE PERSPECTIVE

Mucus is secreted from globlet cells, specialized epithelial cells, and in endothermic animals, it is known that through the intestine mucus layer and the loosely adherent mucus layer.

These layers vary in thickness throughout the intestine and evidence indicates the bacteria readily colonize the loosely adherent mucus layer, but not the adherent mucus layer. In aquatic animals, such information is not available and this merits further investigations, as mucus associated bacteria are important in host health.

Adhesion capacity and/or colonization are importance when evaluating the intestinal micro biota of aquatic animals. Even though fish microbiologists have gained some knowledge about adherence of bacteria in the GI tract of fish during the last two decades, it is a long way to compared with the information available from non aquaculture studies.

During the last decade, numerous studies have investigated inclusion of antinutrional factors (ANFs) and genetically modified plants on general biological effects, growth, gut histology and immunology Krogdahl et al. (2010) [40], however per second no information is available about their effect on gut micro biota may modify ANFs, and hence their interactions and biological effects. Furthermore, the intestinal micro biota is undoubtedly an important factor in determining the health status of endothermic animals. Compared to considerable increase in the studies of the effect commensal microbes exert in the mammalian gut from 1996 to 2015 less studies have been carried out on the role played by the GI micro biota in aquatic animals specially for fish health and disease. Therefore the topic of our paper is probably a never end story.

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