

Gut microbial diversity of Teleost

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Teleosts are evolved during the period of Triassic. By the end of the Cretaceous, teleosts present in freshwater and marine habitats. Teleosts (Teleostei) are groups of ray-finned fishes. More than 20,000 species belong to this group. Teleosts are one of the three main groups of rayfinned fish (the other two being the chondrosteans and the holosteans). Teleosts are divided into four subgroups. Which include the Elopomorpha, Osteoglossomorpha, Euteleostei and Clupeomorpha. The Osteoglossomorpha, more commonly referred to as bony tongues, include about 150 living species. The group includes the featherbacks and arowana of South America, the Old World knifefishes, and the elephant trunk fish from Africa. The Elopomorpha, commonly known as the eels, halosaurs and tarpons include about 350 species. The group is diverse in appearance, with the tarpons looking more "fishlike" and the eels and halosaurs looking more "snakelike" in body shape. The Clupeomorpha include about 300 living species of herrings and anchovies. The Clupeomorpha have very fine bones and are extremely supple in their movement. Euteleostei are very diverse and have various subgroups. Among these are the Scomiformes and the Ostariophysi. The Salmoniformes, also known as salmonids, include salmon, trout, charrs, smelts, pike graylings and freshwater whitefishes. Salmonids are among the more primitive groups of teleost fish. Salmonids are large and can grow up to 6 feet in length. They spawn in fresh water and migrate to the sea during nonspawning season. About 10,000 species of fishes including barbs, carp, catfishes, characins, chubs, electric eels, hatchetfishes, knifefishes, loaches, minnows, neon tetras, piranhas and zebrafishes belong to the Ostariophysi which is a very large group of Euteleostei. About half of the living vertebrate species are teleosts. This group is diverse in form and species numbers. The distinguishing features of teleosts are their tails and their jaws. Their caudal fins are homocercal and have two equally-sized lobes that extend from the end of the spine. Teleosts have highly mobile mouth parts. The bones of the upper jaw, the maxilla and premaxilla, are fully movable and this bone structure enables them to protrude their jaw outward from their skull when opening their mouth. The endogenous digestive enzymes are secreted from the lumen of the alimentary canal. These enzymes originate from the oesophageal, gastric, pyloric caeca and intestinal mucosa and from the pancreas of teleost. The presence of endogenous digestive enzymes in fish has been reported in numerous studies (e.g. Fagbenro 1990; Das & Tripathi 1991; Fagbenro et al., 2000). All fish species investigated per se possess the enzyme for hydrolysis and absorption of simple and complex carbohydrates (Krogdahl et al., 2005). It has been found that fish gut microbiota also have positive effects to the digestive processes of fish (De et al., 2015). Several researchers isolated and identified the enzyme-producing microbiota in different fish (De et al., 2014, 2015; Ghosh et al., 2002, 2010; Ray et al. 2010, Ringø et al. 1995). *Bacillus sp.*, *Enterobacteriaceae sp.*, *Acinetobacter sp.*, *Aeromonas sp.*, *Flavobacterium sp.*, *Photobacterium sp.*, *Pseudomonas sp.*, *Vibrio sp.*, *Microbacterium sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, unidentified anaerobes and yeast are possible contributors (Ray et al., 2012). It is difficult to conclude the exact contribution of the gastrointestinal microbiota because of the complexity and variable ecology of the digestive tract of different fish species, the presence of stomach and pyloric caeca and the relative intestinal length. The microbiota present in the gut of fish may be autochthonous or allochthonous.

Normal microbiota: The mixture of microorganisms regularly found at any anatomical site is referred to as the normal microbiota.

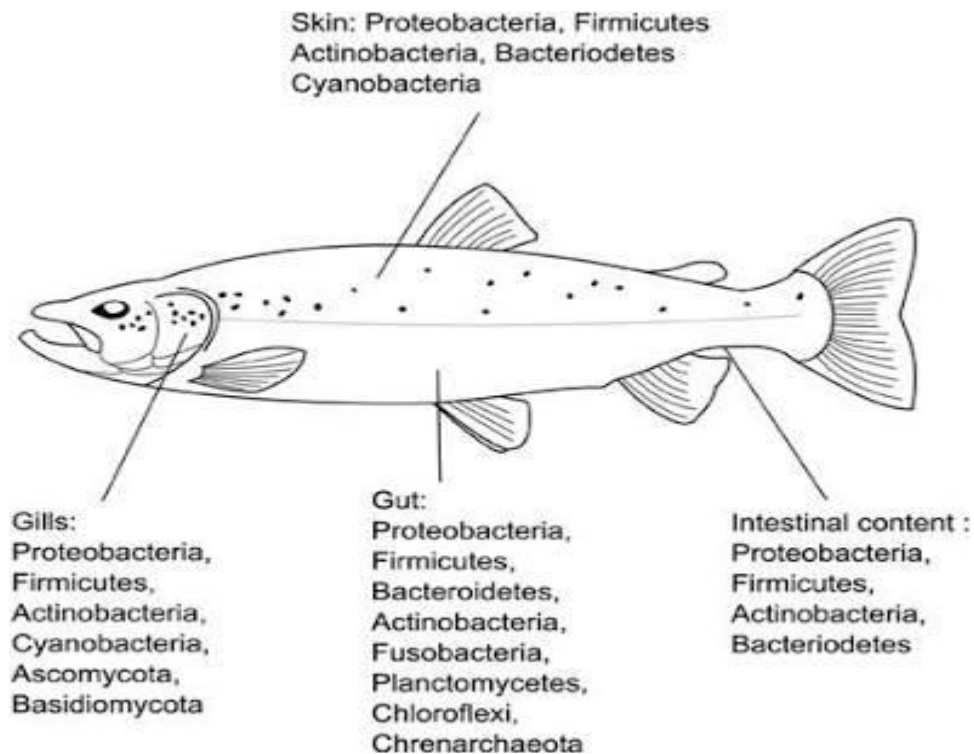


Fig 1. General fish microbiota.

Source: Llewellyn et al. 2014

Gut microbiota: Mixture of microorganisms which is regularly found in the gut is called gut microbiota.

Autochthonous microbes: Microorganisms which are regular normal microflora.

Allochthonous microbes: Microorganisms which entered from the environment but cannot establish as normal microbiota.

The gastrointestinal microbiota of fishes: The gastrointestinal (GI) tract of fish is a complex ecosystem containing different types of microorganisms, consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria (Cahill, 1990; Gómez and Balcázar, 2008). The gut microflora changes with host age, nutritional status, and environmental conditions (Conway et al., 1986; Eddy and Jones, 2002; Verner-Jeffreys et al., 2003). First, a primary transient microbiota is established at the larval stage, which develops into a stable flora at the juvenile stage or after metamorphosis (Olafsen, 2001; Eddy and Jones, 2002). The role of gut microbiota in fish is much less understood compared to humans and other warm blooded animals. In general, the gut microbiota has been suggested to hinder the colonization of pathogenic bacteria (Kennedy et al., 1998; Verschuere et al., 2000; Spanggaard et al., 2001), stimulate immune response (Olafsen, 2001; Gómez and Balcázar, 2008), or produce some beneficial bioactive substances such as essential fatty acids (Ringø et al., 1992), vitamins (Sugita et al., 1991), digestive enzymes (Bairagi et al., 2002; Skrodenyte-Arbaciauskiene, 2007) and antibacterial

substances (Sugita et al., 1998; 2002). Therefore, it is generally accepted that there is a possible symbiotic relationship between fish and gut microbiota (Verschuere et al., 2000).

During rearing, the growth rate of fish varies greatly and can be affected by a variety of factors such as temperature, water quality, territorial defense, unnatural habitats, stocking densities and available nutrition (Sumpter, 1992; Baltz et al., 1998). However, under identical rearing conditions, the growth rate may be affected by “internal factors” such as central nervous system, endocrinological and neuroendocrinological systems (Boeuf et al., 1999). In addition, the gut microbiota could be a new possible internal factor because it plays a very important role in the health and growth of the host (Vine et al., 2006; Comstock, 2007; Mazmanian et al., 2008). Presumably, therefore, fast growing fish might harbor a more favorable gut microbiota.

The gut microbiota has important functions which include metabolism of nutrients, contribution of the colonization resistance, antagonistic activity against pathogens, immunomodulation etc (Denev, 1996; Denev et al.2000; Guarner , Malagelada, 2003 and Rawls et al. 2004). Thus, establishing a healthy microbiota plays an important role in the generation of immuno-physiologic regulation by providing crucial signals for the development and maintenance of the immune system (Salminen et al. 2005). Understanding how the fish immune system generally responds to gut microbiota may be an important basis for targeting manipulation of the microbial composition. This might be of special interest to design adequate strategies for fish disease prevention and treatment (Gomez and Balcázar 2008). The intestinal microbiota possesses antagonistic activity against many fish pathogens and participates in infection-protective reactions (Gutowska et al. 2004; Saha et al. 2006; Skrodenyte-Arbaciauskiene et al. 2006; Sugita and Ito 2006). Intestinal bacteria such as *Aeromonas sp.* and *Vibrio sp.* produce antiviral substances (Yoshimizu and Ezura 1999). The bacterial flora of the GI tract of fishes in general, represents a very important and diversified enzymatic potential. It is capable of producing proteolytic, amylolytic, cellulolytic, lipolytic, and chitinolytic enzymes, which is important for digestion of proteins, carbohydrates, cellulose, lipids and chitin (Bairagi et al. 2002; Gutowska et al. 2004). Total twelve aerobic bacterial isolates from the GI tract of *Lates calcarifer*, ten from *Mugil cephalus*, nine from *Chanos chanos* and five from *Etroplus suratensis* were characterized and identified on the basis of their morphological, physiological and biochemical characteristics (De et al.,2011). Gram positive cocci were prevalent in all the species except *L. calcarifer* where gram positive rods were predominant. Among all the promising isolates, *Geobacillus stearothermophilus* DDKRC4. isolated from the gut of *C. chanos* and *Bacillus sp.* DDKRC1. isolated from the gut of *L. calcarifer* showed maximum specific cellulase activity and *Bacillus subtilis* DDKRC5. isolated from the gut of *C. chanos* showed maximum specific amylase activity (De et al.,2014). pH and bile tolerance ability of *Bacillus subtilis* DDKRC5. was better than *Bacillus sp.* DDKRC1. and both the bacteria have the ability to survive in the gastrointestinal tract in the adverse condition of low pH and high bile concentration and may be used as a potential feed probiotic (De et al.,2011). The enzyme producing microbiota can be used by fish nutritionists as probiotic supplements in the formulated cost-effective fish diet, especially in the larval stages. Further research may establish this point strongly after which their use may be advocated (Bairagi et al. 2002).

Establishment of gut microbiota

The establishment of microflora fully depends upon the environment in which the animal lives in. Microbes entered into the gut from environment through mouth. Microorganism which survives in the gut environment can colonize into the gut wall; others are killed in the gut environment.

Structure of the digestive tract in different animal species differs. Differences are seen mostly in the embryonic stages of development. So, the first factor influencing the formation of gastrointestinal

bacterial communities is the structure of the digestive tract. The formation of regular microflora in the digestive tract of the fish larvae and fry is a complex process and depends on fish spawning, food and microflora present in water.

Stomach microflora

Owing to the very low pH (2-3) of the gastric contents most microorganisms are died. Some microflora mainly *Streptococcus* sp., *Staphylococcus* sp., *Lactobacillus* sp., *Bacillus* sp., yeast etc. may survive if they pass rapidly through the stomach or if the organisms ingested with food are particularly resistant to gastric pH like Mycobacteria.

Small intestine microflora

Because of the combined influence of the stomach's acidic juices and the inhibitory action of the bile and pancreatic secretion, the upper portion of small intestine contains few bacteria, such as *Bacillus* sp., Gram positive cocci e.g. *Staphylococcus*, *Streptococcus* etc., but *Enterococcus* sp. *Lactobacillus* sp, yeast are occasionally found. Anaerobic gram negative bacteria and members of the family Enterobacteriaceae get established in the distal portion of small intestine, where pH becomes more alkaline.

Large intestine microflora

The largest microbial community found in large intestine of fish. The microbes consist primarily of anaerobic, gram negative, non-sporing bacteria such as *Bacteroides* sp. and gram –positive, spore – forming such as *Clostridium* sp. and non-sporing rods like *Lactobacillus* sp..

Table 1. Major microflora found in fish gut

Bacteria	<i>Bacillus</i> sp., <i>Aeromonas</i> sp., <i>Clostridium</i> sp., <i>Staphylococcus</i> sp., <i>Streptococcus</i> sp., <i>Stomatococcus</i> sp. <i>Micrococcus</i> sp., <i>Lactobacillus</i> sp., <i>Pseudomonas</i> sp. <i>Enterococcus</i> sp., <i>Actinomycetes</i> sp., <i>Flavobacterium</i> sp., <i>Enterobacter</i> sp., <i>Proteus</i> sp. <i>Achromobacter</i> sp. <i>Peptostreptococcus</i> sp., <i>Clostridium</i> sp., <i>Pediococcus</i> sp.
Fungi	<i>Aspergillus</i> sp., <i>Yeast</i> , <i>Penicillium</i> sp. , <i>Rhizopus</i> sp. <i>Fusarium</i> sp.

Beneficial effect of gut bacteria

1. The normal microbiota influences their innate immune system. This plays an important role in the disease resistance of fish and is divided into physical barriers, humoral and cellular components.
2. Gastrointestinal microflora take part in the decomposition of nutrients and provide the host with physiologically active materials, such as enzymes, amino acids and vitamins.
3. The microbial population of the gut provides a very important and diversified enzymatic potential, and these enzymes interferes with the processes involved in the metabolism of the host animal.

4. Cellulolytic and amylolytic activity has been found in different microbes (mainly *Bacillus* spp.) isolated from the digestive tract of different fishes which helps in digesting cellulose and other carbohydrates through secretion of cellulase and amylase.
5. Chitinase producing bacteria *Aeromonas* sp., *Pseudomonas* sp. *Enterobacter* sp. and *Vibrio* sp. present in the digestive system of fish helps in digestion of chitin.
6. The enzyme producing microorganisms isolated from fish digestive tracts can be used as probiotics in the diet for fish by isolating intestinal bacteria with favorable properties from mature animals and include these in the feed of immature animals of the same species.

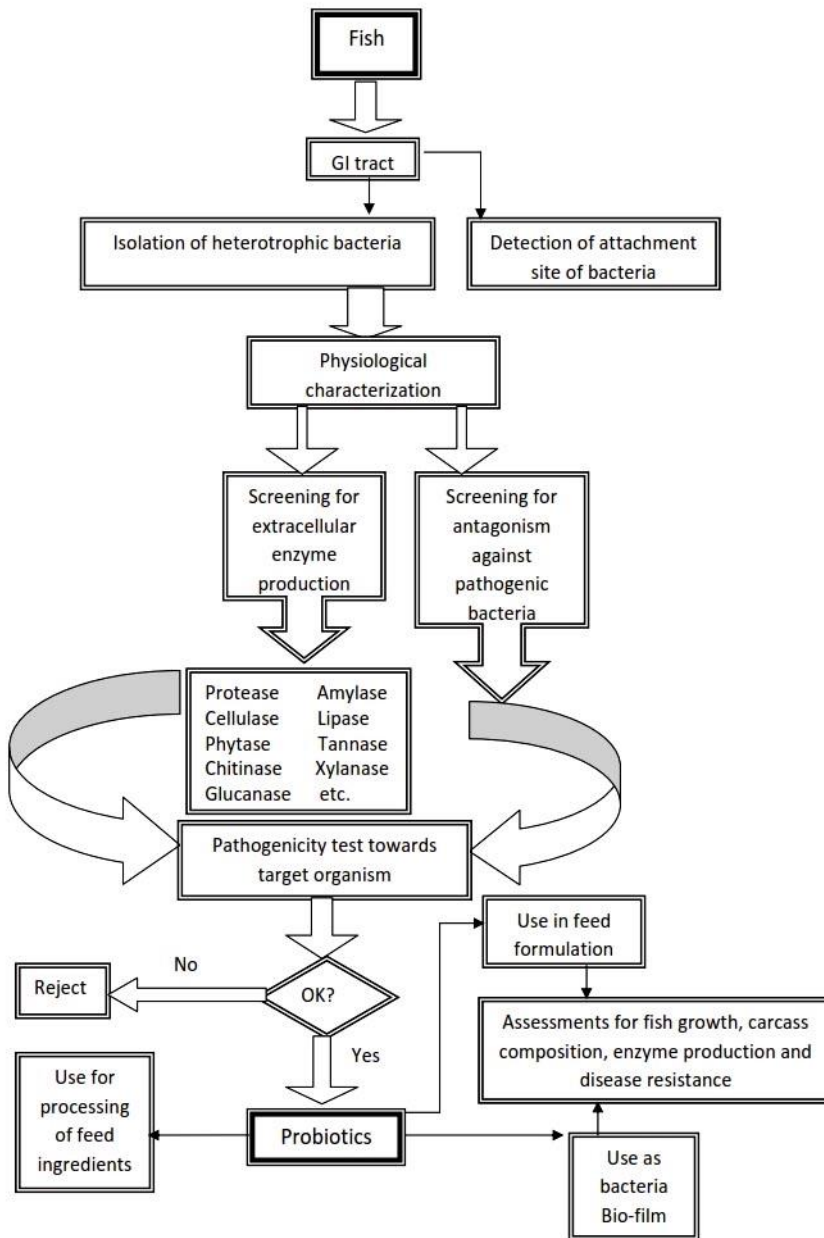


Fig 2. Scheme for using enzyme producing gut microbiota as probiotics
Source: Ray et al. (2012)

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