


# Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish

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## Abstract

Fish gut microbiome confers various effects to the host fish; this includes overall size, metabolism, feeding behaviour and immune response in the fish. The emergence of antimicrobial-resistant (AMR) bacteria and hard to cure fish diseases warrant the possible utilization of gut microbes that exhibits a positive effect on the fish and thus lead to the usage of these microbes as probiotics. The widespread and systematic use of antibiotics has led to severe biological and ecological problems, especially the development of antibiotic resistance that affects the gut microbiota of aquatic organisms. Probiotics are proposed as an effective and environmentally friendly alternative to antibiotics, known as beneficial microbes. At the same time, prebiotics are considered beneficial to the host's health and growth by decreasing the prevalence of intestinal pathogens and/or changing the development of bacterial metabolites related to health. Uprise of sequencing technology and the development of intricate bioinformatics tools has provided a way to study these gut microbes through metagenomic analysis. From various metagenomic studies, ample of information was obtained; such information includes the effect of the gut microbiome on the physiology of fish, gut microbe composition of different fish, factors affecting the gut microbial composition of the fish and the immunological effect of gut microbes in fish; such this information related to the fish gut microbiome, their function and their importance in aquaculture is discussed in this review.

**Key words:** aquaculture, fish metagenome, gut microbes, gut microbiome, immunity, intestinal microbiota.

## Introduction

Gut microbiota is the microorganisms that are positioned in the intestine, encompassing the whole range of biochemical process, and provoke an immune system of the host organism (Gómez & Balcázar 2008; Hanning & Diaz-Sanchez 2015). Better insight on the conditions of this taxonomic and metagenomic research helps to understand the significance of this framework. A microbiota or

microbiome refers to a set of genomes of various microorganisms present in the environment. Microbiota refers to the different microorganisms that are present in the environment, which is frequently used interchangeably that relate to the concentration of habitat-related microorganisms. The chemical and physical parameters of an ecosystem include a habitat that promotes the individual niche space; in short, it determines the microbial interaction along the chemical and physical dimensions of the habitat

**Table 1** Explanation of the terms related to the metagenomics studies of microbiomes

Term	Definition	Examples
Microbes	Microbes are small life forms that are too small for the naked eye to see	<i>Bacillus</i> spp <i>Pseudomonas aeruginosa</i> <i>Citrobacter freundii</i>
Microbiota/microbiome	A microbiota is an environmentally friendly microorganism community within the habitat	Human microbiome Soil microbiome Fish microbiome
Habitat	The locations to discover nutrition, accommodation, safety and sexual partners for the animals	Fish gut Human skin Anaerobic condition Acidic environment
Niche	The chemical and physical aspect of the environment, which determines a particular environment of the species or animals	Anoxic sediment sulphur metabolism, Anaerobic respiration of urinary tract microbes
Ecosystem	The communications between organism and dynamics of biological components of environment	Tropical forest Human body
Metagenomic	A technique for investigating genetic content from microbes	16S rRNA sequencing, Whole-genome sequencing

(Shade & Handelsman 2012; Venkatesh *et al.* 2018). Metagenomics is regarded as a technique for investigating the relationship between those elements in an ecosystem from a molecular genetics outlook. In Table 1, we have provided a number of instances of the definition used in previous research.

Whiteside *et al.* (2015) have provided a description of the terms such as microbiota and microbiome in which the microbiota is linked to the dominant environment; a microbiome is regarded as the linkage between biotic and abiotic factors that lead in relation to the habitat and to the symbiotic environment of the microbes. Metagenomic includes the genetic ability of this microbial taxa in habitat, both genomic and plasmid levels using the 16S rRNA sequencing technique (Whiteside *et al.* 2015). In relation to the microbiome, the essential distinction of the word microbiota is clearly stated in Fig. 1. Considering the terminology, the intestinal microbiome is the composition of the microorganisms of intestinal lumen associated with the microbial community, in relation to obtaining nutritional benefits from the subject that conferring the metabolic and immunological role to the recipient.

The review aims to present a broad spectrum of gut microbial system and its functional effects and influence on aquaculture. The gut microbial diversity is specific to the individual species and various physiological factors such as temperature, pH, nutrients availability and the environmental conditions influenced the gut microbial system and immune system in the broad spectrum of metagenomics, prebiotics and probiotics.

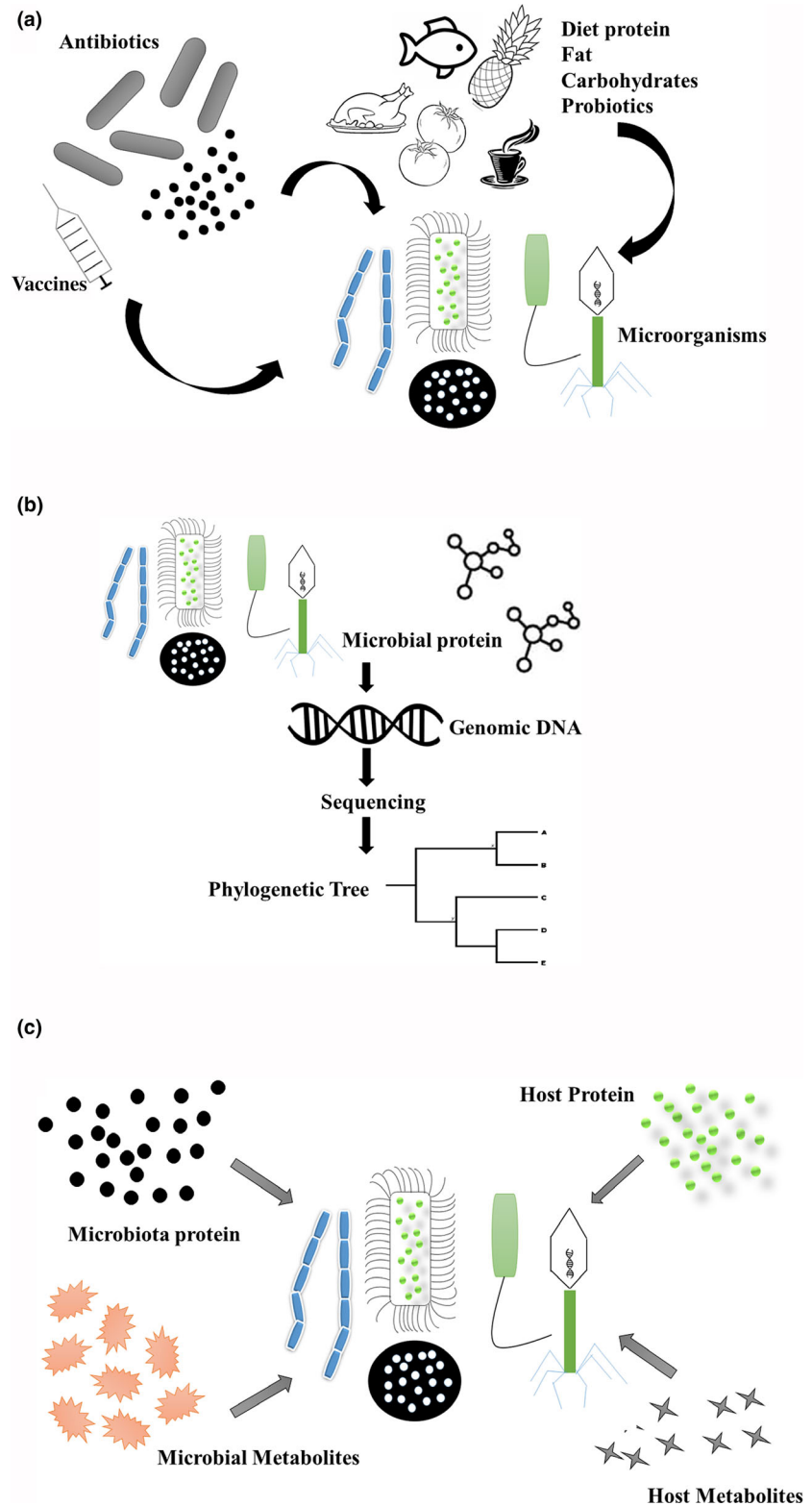
### Intestinal microbiota

The interaction of the microbiome in the gut is dynamic in nature rather than static. Depending on the duration of the

stay of the bacteria residing in the gut, either temporarily or permanently they are referred as transient and persistent microbiota (Shade & Handelsman 2012; Prasanth *et al.* 2018). The transient microbiota comes through the nutritional ingestion mechanism, where the microbes stay in the food and enter into the gastrointestinal system. These microbiota do not last for a long time within the environment as they are surrounded by adhering resident microbes to the gut wall. The resident microbiota lives in the host intestinal membrane have a symbiotic relation to the host (Zhang *et al.* 2016). The detailed description of the microbial composition of gut microbiota in herbivore, carnivore and omnivore fish species is provided in Table 2.

### Source of gut microbiome to newborn and its function

The parameter governing the factors of origin and conservation of gut microbiota is a chaotic model (Koenig *et al.* 2011). The main reason is the broad spectrum of factors involved in the control of microbial gut composition in infant organisms. The origin of gut microbiota in newborn infants is based on the interaction of the microbial community present in the birth canal of human beings. Further, the microbes are introduced into the gastrointestinal system in infants during breastfeeding (Gueimonde *et al.* 2006). Lactose-utilizing microbes are more abundantly present in the gut of the infants that break down the polysaccharides present in the milk. The cellulose-utilizing bacteria are useful for the degradation of plant polysaccharide, which is frequently consumed in human diets. The random colonization of cellulose-utilizing bacteria changes the host behaviour due to the random priming of the immune response of that particular bacteria (Koenig *et al.* 2011). It is initiated in a fish model that environmental microbial



**Figure 1** Illustrating the concept of (a) microbiota, (b) metagenomic and (c) microbiome. (a) Microbiota is described as an ecology-related (nutrient, vaccine, antibiotics, etc.) microbial community; (b) metagenomic is termed as the analysis of microbial composition through molecular study methods such as 16S rRNA genomics or whole genome; (c) microbiome relates to the microbiota genes and genomes combination and interactions as well as protein and host metabolite and microbiotic interactions.

accumulation is significantly correlated with the microbiota composition in the intestine of fish (Jesu *et al.* 2014). In determining the composition of gut microbiota, the

feeding pattern of the fish performs an important measure. Similar challenges as mammals arise while determining the origin of the gut microbiota in fish due to the multiple

**Table 2** Microbial composition of gut microbiota in herbivore, carnivore and omnivore fish

Bacterial species	Fish species	References
<b>Herbivores</b>		
<i>Clostridium</i> sp., <i>Eubacterium desmolans</i> , <i>Papillibacter</i> , <i>Cinnaminovorans</i>	Butterfish <i>Odax pullus</i> Marblefish <i>Aplodactylus arctidens</i>	Clements et al. (2007)
<i>Vibrio</i> sp., <i>Photobacterium</i> , <i>Bacteroidetes</i> , non-vibrio <i>Proteobacteria</i> , <i>Firmicutes</i>	Parrotfish <i>Chlorurus sordidus</i> , Surgeonfish <i>Acanthurus nigricans</i>	Smriga et al. (2010)
<i>Clostridium</i> sp., <i>Epulopiscium</i>	Silver drummer <i>Kyphosus sydneyanus</i> Surgeonfish <i>Acanthurus</i> sp.	Moran et al. (2005) Miyake et al. (2015)
<i>Enterovibrio</i> , <i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Desulfovibrio</i>	Zebraperch <i>Hermosilla azurea</i>	Fidopiastis et al. (2006)
<b>Zooplanktivores</b>		
<i>Vibrionaceae</i> , <i>Pasteurellaceae</i> , <i>Vibrio harveyi</i> , <i>Shewanella</i> sp., <i>Endozoicomonas</i> sp., <i>Pseudomonas</i> , <i>Alteromonas</i> , <i>Psychrobacter</i>	Cardinalfish, Apogonidae Damsel fish, Pomacentridae Herring <i>Clupea harengis</i>	Parris et al. (2016) Hansen et al. (1992) Curson et al. (2010)
<i>Proteobacteria</i> <i>Achromobacter</i> , <i>Vibrio</i> sp., <i>Pseudomonas</i> <i>Psychrobacter</i> , <i>Vibrio</i> sp., <i>Shewanella</i>	Pipefish <i>Syngnathus scovelli</i> Sardines <i>Sardinella longiceps</i> Atlantic mackerel <i>Scomber scombrus</i>	Ransom (2008) Karthiayani and Mahadeva Iyer (1967) Svanevik & Lunestad (2011)
<b>Carnivores</b>		
<i>Clostridium perfringens</i> , <i>Vibrio</i> sp.	Atlantic cod <i>Gadus morhua</i>	Aschfalk and Müller (2002); Star et al. (2013)
<i>Vibrionaceae</i> (larvae & juveniles), <i>Photobacterium phosphoreum</i> (adults)	Atlantic halibut <i>Hippoglossus hippoglossus</i>	Verner-Jeffreys et al. (2003)
<i>Acinetobacter junii</i> , <i>Mycoplasma</i> , <i>Lactobacillus</i> sp., <i>P. phosphoreum</i> , <i>Lactococcus</i> sp., <i>Bacillus</i> sp., <i>Photobacterium</i> , <i>Vibrio</i> sp.	Atlantic salmon <i>Salmo salar</i>	Holben et al. (2002) Hovda et al. (2007) Ward et al. (2009)
<i>Vibrio</i> sp., <i>Pseudomonas</i> , <i>Enterobacteraceae</i> <i>Pseudomonas</i> sp.	Blackfin icefish <i>Chaenocephalus aceratus</i> , Black rockcod <i>Notothenia coriiceps</i>	Newman et al. (1972)
<i>Vibrio</i> , <i>Pseudomonas</i> , <i>Flavobacterium</i> <i>Bacillus</i> , <i>Vibrio</i> , <i>Delftia</i> , <i>Psychroacter</i> , <i>Acinetobacter</i> , <i>Pseudomonas</i>	Bluefish <i>Pomatomus saltatrix</i> Gilthead seabream <i>Sparus aurata</i> Grass puffer <i>Fugu niphobles</i> Grouper <i>Epinephelus coioides</i>	Florin et al. (2013) Sugita et al. (1989) Sun et al. (2009)
<i>Mycoplasmataceae</i> , <i>Photobacterium</i> , <i>Cetobacterium</i> , <i>Clostridiaceae</i> , <i>Vibrio</i>	Red drum <i>Sciaenops ocellatus</i>	Ransom (2008), Givens et al. (2015)
<i>Aeromonas sobria</i> , <i>Pseudomonas</i> <i>Cetobacterium somerae</i>	Sea trout <i>Salmo trutta trutta</i> Siberian sturgeon <i>Acipenser baerii</i>	Skrodenytė-Arbačiauskienė et al. (2008) Geraylou et al. (2012)
<i>Vibrio</i> , <i>Photobacterium</i> <i>Clostridium</i> , <i>Photobacterium</i> , <i>Clostridiaceae</i>	Snapper <i>Lutjanus bohar</i> Southern flounder <i>Paralichthys lethostigma</i>	Smriga et al. (2010) Ramirez and Dixon (2003); Ransom (2008); Givens et al. (2015)
<i>Escherichia coli</i> <i>Aeromonas</i> , <i>Pseudomonas</i> , <i>Vibrio</i>	Speckled trout <i>Cynoscion nebulosus</i> Striped bass <i>Morone saxatilis</i>	Ransom (2008) MacFarlane et al. (1986)
<b>Omnivores</b>		
<i>Clostridium</i> , <i>Mycoplasma</i> , <i>Photobacterium</i> , <i>Propionibacterium</i> , <i>Staphylococcus</i> , <i>Pseudomonas</i> , <i>Corynebacterium</i>	Pinfish <i>Lagodon rhomboides</i>	Ransom (2008); Givens et al. (2015)
<i>Mycoplasma</i>	Long-jawed mudsucker <i>Gillichthys mirabilis</i>	Bano et al. (2007)

confounding variables that occurred during the study (Wu et al. 2012).

With respect to gut microbiota function in an organism, we need to understand the reciprocal adjustment between the intestinal function and the microbes that are vital to the coexistence of these elements. Intestinal activities comprise

two instincts: the intestinal mucosal environment must originally be controlled to adapt nutrient uptake, and a fence within the lumen cavity must be deliberated to prevent the transition of microbes into the intestine cells (Sommer & Bäckhed 2013). The primary function being the innate nature of the intestinal function is easily

achieved, even though the subsequent criteria retain a significant amount of challenge on account of the copiousness of the microbial community, transiently or permanently exist within the intestine (O'Hara & Shanahan 2006). From the view of gut microbiota, as mentioned in this review, the microbiota must produce the host with immunogenic and metabolic function.

### Importance of fish gut microbiome in aquaculture

Aquaculture is termed as a method of breeding, rearing and harvesting aquatic organisms in all types of water environments. The word aquaculture is usually used to describe the activity of fish farming (Aquaculture 2017). As fish in marine and freshwater are increasingly demanded as a food supply chain, in order to ensure the growth of the industry and to satisfy the consumer's requirement, resolving problems in the industry is imperative. It is also necessary to increase the growth of small-sized fish and to progress in the protection of fish against microbial pathogens. The combination of current information in the gut microbiome can resolve both of these targets; hence, the aquaculture industry can be provided with useful elements of gut microbiome function.

### Antipathogenic effects of beneficial bacteria

The widespread use of high density in aquaculture, infectious diseases caused by various pathogens such as bacteria, viruses, fungi, protozoa and parasites are extremely harmful to global aquatic organism farming, resulting in huge economic losses and potential threats to public health (Carrias *et al.* 2012). Vaccines and antibiotics are important therapeutic strategies for bacterial pathogens and have played an integral role in protecting aquatic animals from infection and reducing losses associated with disease (Carrias *et al.* 2012). Additionally, many beneficial bacterial (or probiotic) strains have been developed to treat pathogen-induced bacterial diseases, and this method's efficacy has been proven (Verschuere *et al.* 2000). Among thirty strains of *Vibrio anguillarum*, only one strain developed a significantly higher amount of siderophore (a ferric ion-specific chelating agent promoting iron availability), which was also capable of inhibiting *Vibrio ordalii* (Pybus *et al.* 1994). Smith and Davey (1993) have isolated a *Pseudomonas fluorescens* strain F19/3 that inhibits *Aeromonas salmonicida* in fish by competing for free iron and thus protects stress-induced furunculosis in external locations.

Some beneficial bacteria can produce pathogens that suppress or even destroy inhibitory compounds (Teplitski *et al.* 2009). *Lactococcus lactis* TW34 isolated from marine fish can develop bacteriocin nisin Z, which can inhibit the

growth of the fish pathogen *Lactococcus garvieae* at 5 AU mL<sup>-1</sup>; therefore, it is considered an alternative in the prevention of global aquaculture disease lactococcosis (Sequeiros *et al.* 2015). By screening deep-sea shark-isolated bacteria (*Centroscyllium fabricii*), it possessed gut antagonistic activity (Bindiya *et al.* 2015). Also, it was found that the BTSS-3 strain was most likely related to *Bacillus amyloliquefaciens* and demonstrated antimicrobial activity against pathogenic bacteria like *Salmonella Typhimurium*, *Proteus vulgaris*, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus macerans* and *Bacillus pumilus* (Teplitski *et al.* 2009). Luckily, there have been noticed several other forms of anti-pathogenic compounds produced by beneficial bacteria.

### Bacterial infection: The issues in aquaculture industry

Despite this rise in aquaculture production, clearly, the economic gains in this industry were systematically affected as a result of intrusion through pathogenic bacterial organisms. It consists of multidrug-resistant bacteria with a wide range of host species, which is concerning their natural habitats, thus immediately infect the fish species. The majority of pathogenic bacteria have actually been categorized, but only a few number of bacterial species cause significant financial losses in the aquaculture industry. As an instance, the range of severe infectious diseases transmitted by fish in the aquaculture industry includes photobacteriosis, furunculosis and vibriosis. The enhanced mortality rates of photo bacteria and vibriosis are regarded to account for almost all infectious diseases in aquaculture, which are commonly prominent infectious diseases of fish from brackish water and saltwater. The signs and symptoms of vibriosis have been shown in skin and fins in dark coloration and serious haemorrhage in spleen, body cavity and in the intestine; this occurs mainly because of *Vibrio anguillarum*. Aside from this, severe infectious illnesses in fish are also caused by *Vibrio* comprising *V. vulnificus*, *V. parahemolyticus*, *V. salmonicida*, *V. carchariae*, *V. ordalii*, *V. damsela* and *V. alginolyticus* (Pereira *et al.* 2011).

*Photobacterium damsela* disease is the most serious cataclysmic disease of the aquaculture, which is caused by photobacteriosis. As this bacterium is prevalent in existence, it possesses a broad variety of habitat and enhances the death rate. The presence of white tubercles and lesion on the internal organs of fish is often identified. Necrosis of the spleen, kidney and liver was also noted in fish affected due to *P. damsela* infection (Andreoni & Mag-nani 2014).

In addition, the largest mortality rate for *Aeromonas salmonicida* has been demonstrated for the furunculosis of fish contagious diseases, which are known to cause many



different variations in freshwater and saltwater fish. Furunculosis is externally indicated by haemorrhages that occur in the base of the fish fin due to the lack of hunger, lethargic movement and melanosis, which are collectively lead to infection (Holtén-Andersen *et al.* 2012). *Flavobacterium columnare* has been shown to cause severe damage in aquaculture production, which is another communal infectious disease in the aquaculture industry. The signs shown in fish include a complete necrosis and gill lesions as a consequence of this disease. Concerning the skin, the disease creates mainly a dark discoloration in the centre of the fin and is bound by reddish tinge (Declercq *et al.* 2013).

Haemorrhagic and enteric septicaemia are infectious diseases observed in the aquaculture industry that was triggered by *Edwardsiella* genus; it includes *Edwardsiella ictaluri* and *E. tarda*. These two unique bacterial species were identified in catfish; normally, *E. tarda* was isolated from both freshwater and brackish water fish. However, *E. ictaluri* is normally isolated only in brackish water. Systemic haemorrhagic septicaemia is described as the suppression associated with the immune system of the host developing into necrosis and skin lesions of major internal organs and muscles. Indications of enteric septicaemia are showed by septicaemia haemorrhagic enteritis, well ahead on developing into meningoencephalitis (Sudheesh *et al.* 2012).

Enteric redmouth (ERM) disease is an infection from bacteria that takes place primarily in rainbow trout which are caused by *Yersinia ruckeri* bacterial species. At the early phase of this infection, the death rates are lesser. If the infected fish are untreated from the initial stage, the death percentage rises exponentially and could cause a massive financial loss to the farmer. The study has shown that the death rate in this disease is due to the exposure of stress conditions such as high stocking densities and compromised water quality (Horne & Barnes 1999). Two more major fish bacterial diseases are *Lactococcosis* and *Streptococcosis* that extend throughout the world. This is certainly due to the wide spectrum of host withstanding ability to adapt against *Streptococcus* and *Lactococcus* genus bacteria, which simultaneously confer the diseases. These bacterial diseases have a group of signs such as dark pigmentation, exophthalmos, haemorrhage, congestion and erratic swimming lethargy (Domeénech *et al.* 1996).

The following pathways primarily cover aquatic microbes to avoid pathogen colonization, which are categorized into two groups. The first one to be direct interactions between commensals and pathogens, such as competition for shared nutrients and niches. The same ecological niches need for both pathogens and commensal bacteria to colonize and spread across the gut, and mechanisms have emerging to compete with each other. Commensal bacteria produce bacteriocins and toxins that directly inhibit bacterial species

members of the same or related species. Commensals are often avoiding the pathogenic infection through the modification of the host environment, for example pH (Hammami *et al.* 2012).

The second category comes under the commensal-mediated enhancement of host defence mechanisms. Commensal bacteria are to prevent pathogen colonization and infection indirectly by enhancing host defence mechanisms such as functionally promoting mucosal barrier and enhance innate immune responses. The first line of defence against any pathogen invasion is the epithelial barrier. The promotion of epithelial barrier functions by commensal bacteria, which was supported primarily by indirect evidence that has demonstrated the germ-free mice, and mice deficient in proteins involved in microbial recognition such as Nod2 and the TLR signalling adaptor MyD88 have impaired the production of antimicrobial peptides, particularly by Paneth cells of the small intestine (Kobayashi *et al.* 2005 and Vaishnav *et al.* 2008).

### Usage of antibiotic in aquaculture industry: solution or more issues

Bacterial infection has a predominant position in contributing to economic as well as production loss to the aquaculture industry, as mentioned previously in this review. Chemical therapeutics including antibiotics are essential sensitive solutions for many cases of bacterial infection. Various antibiotics have been used to prevent such outbreaks in aquaculture sectors; specifically, erythromycin, sulfamethoxazole, norfloxacin and trimethoprim are used in aquaculture industry and also in other farmings including cow, pig and poultry industries; nowadays, even in agriculture the usage of antibiotics has become common (Giang *et al.* 2015).

In most cases, a high concentration of antibiotics is employed in the aquaculture industry as a result of high stocking densities and impracticality that the individual treatment plan for infected fish (Resende *et al.* 2012). While the use of antibiotics has addressed the fundamental issues needed for its usage, a distinctive issue occurs in aquaculture farm that is located close to the water source since the excess antibiotic effluent is discharged into the river (Reis & Mollinga 2009). The continuous release of effluent encompassing the surplus antibiotics into the river stream passes into the land soil system over absorption of the effluent by soil assists the development of antibiotic-resistant bacteria within the respective ecosystem (Thiele-Bruhn 2003).

Inadvertently, these bacteria cause specific environmental stress that is encouraging the increase in the surrounding environment of microbe resistance to antibiotics. The unceasing admittance of antibiotics into

the microbial population leads to random chromosomal alteration of microbes (spontaneous mutation), which reduces the sensitivity of the microbe to the particular antibiotic. As a result, the amount of resistant microbes in the ecosystem is increased. This increased exposure to antibiotics continuously minimizes the susceptible microbes (native microbes) and further increases the number of resistant microbes with increased effects of spontaneous mutation and transmission of antibiotic-resistant genes among microbes (Meek *et al.* 2015), which has been exemplified in Fig. 2. The increase or the presence of antibiotic-resistant microbes in the environment, specifically nearby farming lands and water source, allows the acquaintance of persons to these antibiotic-resistant microbes that specifically aggregate the possibilities of diseases by these resistant microbes in the gut and disturbing the health of human populace.

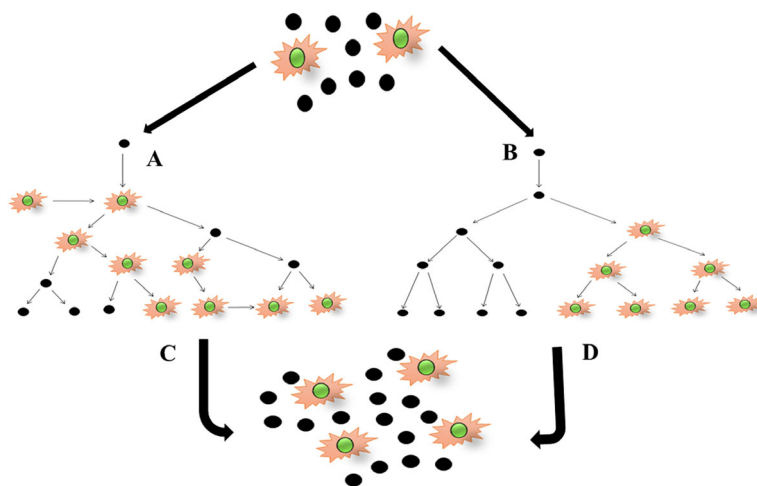
Multiple studies have investigated microbes resistant to antibiotics and the microbial genes responsible for the expression of antibiotic resistance that enhance claims on the adverse effects of antibiotic use on the environment. Meek *et al.* (2015) proposed that in a Brazilian aquaculture farm, four hundred and seven bacterial strains including Gram-positive cocci (GPC), enterobacteria (ENT) and non-fermenting Gram-negative rods (NFR) are isolated and they were tested for antibiotic resistance over minimal inhibitory concentration (MIC) analysis. The analysis showed that a group of bacteria from GPC are unaffected due to penicillin and azithromycin, while NFR and ENT group bacteria are resistant to the antibiotics gentamicin, ampicillin and sulbactam. Apart from this, the presence of multidrug-resistant bacteria is also found more abundant

in the fish pond rather than in the water-fed canal (Meek *et al.* 2015).

Giang *et al.* (2015) conducted a study in Mekong Delta, Vietnam, which projected the presence of aquaculture industry in Mekong managed the use of antibiotics against the bacterial infection in fish; in turn, the aquaculture industry water is released to the utilization of domestic purpose and detected in the river stream occurrence of antibiotic compounds such as trimethoprim (TRIM), enrofloxacin (ENRO), sulfamethoxazole (SMX) and sulfadiazine (SDZ) at the level of  $17 \text{ ng L}^{-1}$ ,  $12 \text{ ng L}^{-1}$ ,  $21 \text{ ng L}^{-1}$  and  $4 \text{ ng L}^{-1}$  correspondingly in the water samples collected from the delta region (Giang *et al.* 2015). Among these, TRIM antibiotics were found to be more in the water sample owing to the TRIM resistance in contradiction to photodegradation. Even though the antibiotic concentration did not maintain any sudden risks in evolving antibiotic-resistant bacteria, conversely constant acquaintance of these antibiotics possibly will cause difficulties in the nearby future. A more suitable technique for handling infectious bacterial diseases in the long term to address these issues, with less detrimental to the ecosystem, is necessary, and this approach is obviously accepted by many scientists as probiotics.

### Effect of antibiotic usage in aquaculture

A microbial organism has been studied by various authors in intestinal tracts of healthy fish for its considered significance in digestion, feeding and the prevention of disease (Navarrete *et al.* 2008). Bates (2006) in his studies revealed that gut microbiota could be involved in important



**Figure 2** (a and b) The number of antibiotic-resistant bacteria in the population is increased by an antibiotic selective pressure. (c) The transmissible gene of antibiotic resistance is carried to certain daughter cells horizontally and vertically. (d) A mutation is carried into each generation in the chromosome gene to resist antibiotics. Figure adapted and modified from (Meek *et al.* 2015).

processes such as epithelial proliferation, promotion of nutrient metabolism and innate immune responses (Bates 2006). Modifications to gastrointestinal microbiota by antibiotics can alter the likely benefit of this host–microbiota interaction or relationship. Therefore, it could help to improve hatchery management to minimize antibiotic use and improve safety for farmed fish if antibacterial compounds alter the gastrointestinal microbiota of farmed fish.

Few types of research have focused, however, on evaluating the impact on the microbial ecology of the fish intestine of antibiotic treatment and have concentrated on antibiotic resistance level during and after antibiotic application (Kerry *et al.* 1997). Antibiotic susceptibility of the pathogens isolated from fish farms are detrimental to antibiotic resistance, which was identified using molecular tools (Kerry *et al.* 1997; Miranda & Zemelman, 2002; Giraud *et al.* 2006; Akinbowale *et al.* 2007; Miranda & Rojas 2007). The gut microbiota and environmental bacteria come in contact with the antibiotics present in fish farm and hatchery wastes when an antibiotic treatment begins (usually via medicated feed). In fact, the treatment of salmonids with different antibiotics (including OTC) has been shown to result in a significant increase in the proportion of the gut microbiota showing resistance to the administered antibiotics (Austin & Al-Zahrani 1988).

Navarrete *et al.* (2010) assessed the effects of oxytetracycline (OTC) treatment on bacterial populations present in the intestines of healthy juvenile salmon. Oxytetracycline was administered via medicated feed to Atlantic salmon held in experimental tanks, and their intestinal microbiota were analysed after culture. Isolates were analysed by restriction fragment length polymorphism (RFLP) and sequencing of 16S rDNA amplicons. Microbiota from the intestines of untreated fish was more diverse, and their main components were *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Psycrobacter* and *Brevundimonas/Caulobacter/Mycoplana*. In contrast, the microbiota of the OTC-treated group was characterized by less diversity and was only composed of *Aeromonas*, clustering with *A. sobria* and *A. salmonicida*. The frequency of resistant bacteria, defined as those capable of colony formation on TSA medium containing 30 µg mL<sup>-1</sup> OTC, indicated that no resistant bacteria were detected (<102 CFU per gram) in the three tanks before OTC treatment. In treated fish, resistant bacteria accounted for 60%, 33% and 25% of isolates from the samples collected on days 11, 21 and 28, respectively. The intestinal microbiota of salmon treated with OTC leads to vanish several bacterial phylotypes, but establishes *Aeromonas* population. Bacteria belong to this genus have been widely isolated from the fish gut (Huber *et al.*, 2004; Romero & Navarrete, 2006) and are considered to be a normal bacterial component. However, some species of *Aeromonas* including *A. salmonicida*, *A. hydrophila*,

*A. caviae* and *A. sobria* are also regarded as common pathogens of fish because they may cause furunculosis and haemorrhagic septicaemia. More recently, Ringø *et al.* (2004) proposed that the digestive tract could represent a port of entry for invading bacteria, especially *Aeromonas*. Compared with the OTC-treated salmon, a more diverse bacterial composition was observed in the untreated salmon. Some authors have suggested that, to maintain a successful culture environment in an aquatic hatchery, it is necessary to maintain a diverse microbial community that includes innocuous and beneficial bacteria (Schulze *et al.* 2006). Therefore, the reduction in the diversity of the intestinal microbiota observed after OTC treatment could facilitate the proliferation or invasion of opportunistic microorganisms, as indicated by the rise of some phylotypes that became prevalent several weeks after treatment. Antibiotic treatment can eradicate susceptible microorganisms and promote opportunists that may occupy ecological niches previously unavailable to them. The occurrence of OTC-resistant bacteria, including *Aeromonas* species, the salmon farming, has been demonstrated previously (Jacobs & Chenia 2007). Mobile resistance determinants have also been detected in this genus (Miranda *et al.* 2003). The presence of bacteria harbouring resistance determinants could be related to the widespread use of antibiotics in aquaculture (Cabello 2006). Some authors have even suggested that common components of the microbiota could disperse resistance genes via horizontal gene transfer because of the high density and proximity of resident bacteria in the gastrointestinal tract microenvironment (Navarrete *et al.* 2008).

### Prebiotic in aquaculture

The promising future of the aquaculture industry will not come without difficulties. Supplements to natural feed improve the efficiency of aquaculture production, reduce treatment requirements, waste disposal, and thus simultaneously improve the quality of the farmed fish. The production of aquaculture is expected to double by 2050, a way to meet global demand while reducing the pressure on wild fishing. By 2030, farming raised fish would account for nearly two-thirds of the world's seafood intake, according to estimates by the United Nations Food & Agriculture Organization (FAO) (Thorpe *et al.* 2018).

Improved rearing methods may lead to the use of antibiotic therapies, which causes a number of possible problems including evolving antibiotic-resistant bacteria and the existence of antibiotic residues in seafood, which further leads to the destruction of the immune system with a serious disease outbreak. Fish are exposed to a number of external stresses and pressures that are unprecedented in contrast to wild what they encounter during intense



cultural operations. The alteration or changes in temperature, photoperiod, salinity, etc., in the farm leads to various stress to the organism as well as immune suppression. In a variety of physiological functions of teleost fish, such as immune responses linked to the neuroendocrine system, environmental conditions that trigger stress that can be the source of some negative effects.

In the aquaculture industry, prebiotics are considered as an environmental-friendly feed additive. The beneficial bacteria present in gastrointestinal tract ferment prebiotics, and the reaction by-products are used to improve the health of the host. Hence, prebiotics that alter gastrointestinal conditions to benefit those existing bacterial species increase growth efficiency and reduce the susceptibility of the pathogen of the host organism which appears an outstanding way to help the growth of the aquaculture industry (Reverter *et al.* 2014).

### Definition of prebiotics

Prebiotics constitute an important dietary supplement to improve both growth and digestive tract microbial activities, which frequently strengthen the immune system and stress tolerance. On the other hand, prebiotics are non-digestible compounds that are metabolized by common bacteria such as *Lactobacillus* and *Bifidobacterium* that promote health. These bacteria are considered beneficial to the health and development of the host by reducing the existence of intestinal pathogens and/or altering the production of bacterial metabolites related to health (Roberfroid 1993; Gibson & Roberfroid 1995; Manning & Gibson 2004). Prebiotics are carbohydrates, which categorized into monosaccharides, oligosaccharides or polysaccharides by their molecular size or polymerization. Until date, common prebiotics established in fish are as follows: inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), arabinoxylo-oligosaccharides (AXOS), chitooligosaccharide (COS), mannooligosaccharides (MOS) and levan.

### Inulin

Inulin is a fructose polymer composed of  $\beta$ -D-fructofuranoses attached by  $\beta$ -2-1-linkage bond and is grouped of sugar-based oligosaccharides. It seems that inulin benefits the intestinal microbiota, particularly in endothermic animal colons (Roberfroid 1993). While inulin is not a natural fibre in fish diets, inulin used in aquaculture stimulates normal bacterial flora in the gut and simultaneously defeat the pathogens and enhances the immune system (Possemiers *et al.* 2009). Oral administration of inulin at the dosage of 10 mg kg<sup>-1</sup> body weight (b.w.) to grass carp (*Ctenopharyngodon idella*)

(24.6  $\pm$  3.5 g b.w.) for 2 weeks resulted in susceptibility against *Aeromonas hydrophila* and *Edwardsiella tarda*; simultaneously, the same dosage of inulin to Tilapia (*Tilapia aureus*) (21.8  $\pm$  3.5 g b.w.) showed the susceptibility against *A. hydrophila* and *E. tarda* (Wang & Wang 1997). Inulin at the dosage of 150 g kg<sup>-1</sup> for 4-week oral administration to Arctic charr (218 g b.w.) (*Salvelinus alpinus* L.) controlled the microbiota *Bacillus* sp., *Carnobacterium maltaromaticum*, *Staphylococcus* sp. and *Streptococcus* sp. (Refstie *et al.* 2006). Atlantic salmon (172 g b.w.) administered with 75 g kg<sup>-1</sup> of inulin for four-week oral administration resulted the decrease in *Marinilactibacillus psychrotolerans*, *C. maltaromaticum* and *Enterococcus faecalis* and also enhanced the growth and relative mass of the gastrointestinal tract of fish (Bakke-McKellep *et al.* 2007). Oral administration (5 and 10 g kg<sup>-1</sup>) of inulin for a week to Gilthead seabream (*Sparus aurata* L.) with initial b.w. 175 g leads to a significant inhibition in phagocytosis and respiratory burst in leucocytes (Cerezuela *et al.* 2008). Gilthead seabream displayed enhanced IgM level, no effect on peroxidase activity and alternative complement activity (ACH50) for 10 g kg<sup>-1</sup> dosage of inulin administered for two to four weeks (Cerezuela *et al.* 2012). Increased lysozyme activity was observed at the dosage administered at the concentration of 5 g kg<sup>-1</sup> for eight weeks in 11 g b.w Nile tilapia (*Oreochromis niloticus*; Ibrahim *et al.* 2010) and 35 g b.w leopard grouper (*Mycteroperca rosacea*; Reyes-Becerril *et al.* 2014).

### Fructooligosaccharides (FOS)

Fructooligosaccharides refer to short and medium  $\beta$ -D-fructans chains in which fructosyl units are bound to a terminal glucose unit by  $\beta$ -(2-1) glycosidic linkage. Dietary FOS inclusion can positively form intestinal microbial communities and modulate the immune role of fish. Multiple studies showed FOS could improve fish's humoral immune response (Mahious *et al.* 2006; Ringø *et al.* 2014). For example, 3 and 6 g kg<sup>-1</sup> of FOS for 56 days to Triangular bream (*Megalobrama terminalis*; 30.5 g b.w.) modulated the immune system by increasing the IgM content and lysozyme (Zhang *et al.* 2013). Administration of FOS (2-4 g kg<sup>-1</sup>) to Ovate pompano, *Trachinotus ovatus* (10.32 g b.w.), increased immunoglobulin and lysozyme on day 56 (Zhang *et al.* 2014), and the same activity was observed in Caspian roach (*Rutilus rutilus*; 0.67 g b.w.) for the dosage administered at 10, 20 and 30 g kg<sup>-1</sup> for the duration of 1-7 weeks (Soleimani *et al.* 2012). Increased lysozyme activity was also observed in Stellate sturgeon (*Acipenser stellatus*; 30.16 g b.w.) for the dosage (10 and 20 g kg<sup>-1</sup>) administered for 11 weeks (Akrami *et al.* 2013).

### Galactooligosaccharides (GOS)

Galactooligosaccharides are composed of 2–20 galactose and glucose molecules, which can be produced by enzyme lactose treatments. Few studies are been carried out using GOS as a prebiotic (Ringø *et al.* 2014). Reports have shown that the administration of GOS at the concentration of 10 g kg<sup>-1</sup> for eight weeks significantly improved the lysozyme activity in red drum (*Sciaenops ocellatus*) at 7 g b.w. (Zhou *et al.* 2010). Atlantic salmon (200.2 g b.w.) fed a diet containing 10 g kg<sup>-1</sup> GOS for four months declined the lysozyme production (Grisdale-Helland *et al.* 2008). However, GOS at 10 g kg<sup>-1</sup> administered to red drum (500 g b.w.) for four weeks boosted the protein ADC and decreased the lipid ADC (Burr *et al.* 2008).

### Arabinoxylan-oligosaccharides (AXOS)

Arabinoxylan-oligosaccharides is a hydrolysis product of arabinoxylans, which can exert different properties depending upon their structure. African catfish (*Clarias gariepinus*) (approximately 20 g b.w.) were administered with 10–20 g kg<sup>-1</sup> of AXOS for ten weeks which resulted in upsurge of acetate, and propionate production; and the same was also observed in Siberian sturgeon (20 g b.w.; Rurangwa *et al.* 2008). Two different forms of arabinoxylans were also identified namely AXOS-32-0.30 and AXOS-3-0.25. Administration of these two different forms of arabinoxylans at the dosage 20 g kg<sup>-1</sup> for twelve weeks in Siberian sturgeon (*Acipenser baerii*; 25.9 g b.w.) enhanced ACH50 and serum peroxidase activity (Geraylou *et al.* 2012). Administration of 20 g kg<sup>-1</sup> AXOS-32-0.30 for four weeks in Siberian sturgeon (48.4 g b.w.) increased ACH50 level (Geraylou *et al.* 2013).

### Chitooligosaccharide (COS)

Chitooligosaccharide is a type of oligosaccharides obtained through chemical and enzymatic chitosan hydrolysis. Because of its lower molecular weight or ready solubility in water, COS has higher activity and more physiological functions than chitosan. COS incorporated diet fed koi (*Cyprinus carpio koi*; 24.9 g b.w.) for eight weeks increased SOD and lysozyme level (Lin *et al.* 2012).

### Mannanooligosaccharides (MOS)

Mannanooligosaccharides derived from the yeast cell wall (*Saccharomyces cerevisiae*), is one of the most frequently evaluated prebiotics in fish (Merrifield *et al.* 2010). MOS improved the lysozyme activity in African catfish (*Clarias gariepinus*; 35 g b.w.) at 10 g kg<sup>-1</sup> within 45 days (Yoshida *et al.* 1995), snakehead (*Channa striata*; 10 g b.w.) at

2 g kg<sup>-1</sup> within 12 weeks (Talpur *et al.* 2014), red drum (*Sciaenops ocellatus*; 7 g b.w.) at 10 g kg<sup>-1</sup> within 8 weeks (Zhou *et al.* 2010), red drum (10.9 g b.w.) at 10 g kg<sup>-1</sup> within 6 weeks (Buentello *et al.* 2010) and Rainbow trout (30 g b.w.) at 2 g kg<sup>-1</sup> within 90 days (Staykov *et al.* 2007).

### Levan

For aquaculture, levan is another fructose polymer which was considered an appropriate prebiotic and immune nutrient. Increased lysozyme activity was observed in Common carp (*Cyprinus carpio*; Rairakhwada *et al.* 2007; Gupta *et al.* 2014), rohu (*Labeo rohita*; Gupta *et al.* 2008) and Orange-spotted grouper (*Epinephelus coioides* H.; Huang *et al.* 2014) due to the levan incorporation in diet (1–50 g kg<sup>-1</sup>) for 75 days, 12 weeks and 45 days, respectively.

### Commercial prebiotic

Grobiotic-A is a mixture of partly autolysed brewer yeast, components of dairy ingredients and products of dried fermentation. The yeast membrane consists of a large number of different polysaccharides in which  $\beta$ -glucans is insoluble in nature. It is widely recognized that the immunological responses in fish can result in yeast  $\beta$ -glucans and  $\beta$ -glucans from other sources too. Studies have proved that the commercial prebiotic at the dosage between 10 and 20 g kg<sup>-1</sup> administered to Hybrid striped bass (19.7 g b.w.) for four weeks has shown no effect on lysozyme activity. However, it increased feed efficiency, growth performance, respiratory bursts, resistance against *Streptococcus iniae* and *Mycobacterium marinum* (Li & Gatlin 2004 and Li & Gatlin 2005). However, red drum (500 g b.w.) treated with 10 g kg<sup>-1</sup> of Grobiotic-A for ten days increased the protein and lipids levels (Burr *et al.* 2008).

### Effect of prebiotic in immune response

Prebiotics directly enhance different innate immune responses. For example, inulin as a prebiotics used in aquaculture promotes healthy intestinal bacteria, suppresses pathogenic organism and enhances immune response. It also stimulates the human immune system through the binding of leucocyte and increasing macrophage proliferation. Though several works conducted in inulin as a prebiotics, the optimum concentration used as 10g kg<sup>-1</sup> due to significant effect on increased IgM level, serum complement, respiratory burst activity and leucocyte phagocytic. After prebiotic feeding, multiple experiments tested the survival of the individual. The results showed that the growth factors of prebiotics normally boost weight gain in daily basis and at a period of interval, specific growth rate, ratio of food conservation, ratio of food efficiency and

protein efficiency; all these impacts may vary depending upon the fish species (Xu *et al.* 2008; Gultepe *et al.* 2012; Hoseinifar *et al.* 2013). After the administration of prebiotics, haematological parameters such as red and white blood cell counting, levels of thrombocyte or lymphocyte, corpuscle volume and haemoglobin content have increased. Also, enzyme activities including lactate dehydrogenase, alanine phosphatase, alanine aminotransferase and aspartate aminotransferase have increased (Hoseinifar *et al.* 2011; Ebrahimi *et al.* 2012; Zhang *et al.* 2013). Diets enriched by prebiotics promoted the levels of enzymes that are associated with weight gain. Nevertheless, the prebiotic administration was not always related to increase digestive enzymes (Anguiano *et al.* 2013).

### Mechanism of action of prebiotic in immune system

Concerning the mechanism of action of prebiotics in the immune system, immunosaccharides are directly activated by interacting with PRRs expressed on innate immune cells such as  $\beta$ -glucan receptor or dectin-1 receptors (expressed in macrophages) are stimulating the innate immune system (Brown *et al.*, 2002). Interaction of ligand–receptor activates the molecules such as NF-kB for signal transduction that stimulate the immune cells (Yadav & Schorey 2002). Furthermore, they can be recognized by MAMPs such as teichoic acid, peptidoglycan, glycosylated protein or the capsular polysaccharide of bacteria, which trigger the immune response (Bron *et al.* 2012; Song *et al.* 2014). Prebiotics therefore tend to activate the innate immune system by stimulating directly the innate immune system or by enhancing the growth of commensal microbiota (Song *et al.* 2014).

### Probiotics

The word ‘probiotic’ is broadly defined as non-pathogenic live microorganism, which is included in food nutrition that has a beneficial impact on the host intestinal health. However, the term was subsequently applied as a useful microorganism that has a positive impact on the general wellness of the host (Ibrahim 2015).

Several investigations have been carried out in the field of probiotics, reinforcing the claim that certain beneficial microorganism has positive impact on animal and human health. The broad variety of research studies has been carried out to study the impact of probiotics on different diseases, including diarrhoea with antibiotic-associated, constipation, abolition of *Helicobacter pylori* infection in human which causes ulcer, moderate inflammatory bowel disease, lessening the occurrence of ventilator-associated pneumonia (VAP). Probiotics is improving the circulatory

system as its effects through human food metabolism (Hungin *et al.* 2013; Bo *et al.* 2014; Ettinger *et al.* 2014).

The implementation of probiotics is not only restricted to metabolic applications in aquaculture but also to overcome the related gastrointestinal imbalance. The study of probiotics has been extended to a number of other physiological and immunological features of aquatic species to improve hatchling survival, hindrance of diseases and innate immunity. A detailed description of aquatic species in which probiotics used is mentioned in Table 3.

### Metagenomic studies of fish gut microbiome

Over recent years, widespread research has been carried out in the fish gut microbiome metagenomic analysis. Detection of bacteria that are unculturable witnessed the real difference between the number of bacterial cells seen under a microscope and the actual wide range of Petri plate’s colonies. This was the origin of the identification as ‘The Great Plate Count Anomaly’ (Stewart 2012). As a result of this variance, the reliance upon the bacterial culture method has decreased considerably, because cultured bacteria comprise a bacterial population of only 0.01 to 1 percentage. In addition to this, advances in scientific methods such as 16S rRNA taxonomical marker and polymerase chain reaction (PCR) method are massive leap in genome sequencing technology (next-generation sequencing) that has greatly contributed to the overtaking of metagenomic vibrant replacement from cultural-dependent methodology to culturally independent strategy (Garza & Dutilh 2015).

Indeed, in the last few years, the culture-independent technique created by scientists was the most appropriate and preferred method for metagenomic research. In general, the major objective of metagenomic research is to develop probiotic applicants for economically and nutritionally important fish. This is accomplished by altering the microbial accumulation of the metabolic function, which in addition to improve the host organism’s metabolic and immunological performance. More studies are being carried out in metagenomic studies on the gut of the fish such as factors influencing the composition of the gut microbiome and the physiological effect of this microbiome on the host fish.

### Goals of fish gut microbiome metagenomic research

Metagenomic studies of fish gut microbiomes varied extensively. Two main themes of the fish gut microbiome are a factor that affects microbiota composition and the influences of gut microbiota on host species. It is agreed, as in most cases, that the composition of the microbiota is a dynamic system and that the origin of the gut microbiome

**Table 3** Probiotic species used in aquatic industry

Probiotic organism and aquatic species used	Results	References
<i>Aeromonas hydrophila</i> – <i>Oncorhynchus mykiss</i> (Rainbow trout)	↓ <i>Aeromonas salmonicida</i> infection	Irianto and Austin (2002a)
<i>Agarivorans albus</i> F1-UMA – <i>Haliotis rufescens</i> (Abalone)	↑ Survivability	Silva-Aciades <i>et al.</i> (2011)
<i>Aeromonas media</i> A199 – <i>Crassostrea gigas</i>	↓ <i>Vibrio tubiashii</i> infection	Gibson (1999)
<i>Alteromonas</i> CA2 – <i>Pacific oyster</i>	↑ Survivability	Douillet and Langdon (1994)
<i>Arthrobacter</i> XE-7 – <i>L. vannamei</i> (Pacific white shrimp)	* Intestinal microbes	Li <i>et al.</i> (2008)
<i>Aeromonas sobria</i> GC2 – Rainbow trout	⊕ <i>Lactococcus garvieae</i> and <i>Streptococcus iniae</i> . <i>Aeromonas bestiarum</i> and <i>Ichthyophthirius multifiliis</i>	Pieters <i>et al.</i> (2008); Brunt and Austin (2005)
<i>Burkholderia cepacia</i> Y021 – <i>Crassostrea corteziensis</i> , (Lions-pay scallop)	↑ Growth and survival	Granados-Amores <i>et al.</i> (2012)
<i>Bacillus pumilus</i> – <i>P. japonicus</i>	↑ Larval survival	El-Sersy <i>et al.</i> (2006)
<i>B. subtilis</i> UTM 126 – <i>Litopenaeus vannamei</i>	⊕ Vibriosis	Das <i>et al.</i> (2005)
<i>Dunaliella tertiolecta</i> – <i>Artemia</i>	⊕ <i>Vibrio campbellii</i> and <i>V. proteolyticus</i>	Marques <i>et al.</i> (2006)
<i>Enterobacter amnigenus</i> – Rainbow trout	↑ Resistance towards <i>Flavobacterium psychrophilum</i>	Burbank <i>et al.</i> (2011)
<i>Lactobacillus fructivorans</i> and <i>L. plantarum</i> – <i>S. aurata</i>	↑ Production of HSP70	Carnevali <i>et al.</i> (2004)
<i>Lactococcus lactis</i> AR21 – Rotifers	↑ Growth and protects against <i>V. anguillarum</i> infection	Harzevili <i>et al.</i> (1998)
<i>L. rhamnosus</i> – <i>O. niloticus</i>	⊕ <i>E. tarda</i> infection	Pirarat <i>et al.</i> (2006)
<i>Rhodococcus</i> SM2 – Rainbow trout	↑ Immunity and protection against <i>V. anguillarum</i>	Sharifuzzaman and Austin (2010)
<i>Streptococcus phocae</i> P180 – <i>P. monodon</i>	↑ Growth ⊕ <i>V. harveyi</i> infection	Swain <i>et al.</i> (2009)
<i>Shewanella putrefaciens</i> – <i>Sparus aurata</i> L	↑ Growth of juveniles	De la Banda <i>et al.</i> (2012)
<i>Yarrowia lipolytica</i> – <i>Pinctada mazatlanica</i>	↑ Growth	Aguilar-Macias <i>et al.</i> (2010)

↑- Increase/Promote; ↓- Decrease; ⊕- Protection; \*- Alter.

is not often studied. As in most cases, the source of intestinal or gut microbiomes is not often studied since the microbiota composition is usually acknowledged to be a dynamic system rather than a constant system. Therefore, the factors affecting the composition of fish gut microbiota have to be examined from now on.

Nutritional patterns and ecological influence such as pollutants, reef settlement, trophic levels and behaviour of host fish species were explored according to factors that influence the composition of fish gut microbiota that hypothesized to regulate the host fish (Baldo *et al.* 2015; Estruch *et al.* 2015; Brown-Peterson *et al.* 2015; Miyake *et al.* 2015; Eichmiller *et al.* 2016; Liu *et al.* 2016). Furthermore, research has also been performed in the determination of the physiological change to gut microbiota and the genetic factor of the host fish on the impact of target fish gut microbiota and vice versa (Li *et al.* 2013; Smith *et al.* 2015). In addition, investigative trials were conducted on gut microbiota to identify the primary microbiota of fish

species (Roeselers *et al.* 2011; Star *et al.* 2013; Parris *et al.* 2016).

### Importance of metagenomics study in aquaculture industry

Nitrogen and phosphorous metabolites and organic matter abound in aquaculture, making aquaculture an ideal platform for microorganisms to develop (Martínez-Córdova *et al.*, 2009). It is believed that, for this reason, the prevalence of microbial DNA could be even greater in aquaculture facilities. The above scenario shows that the microbiological knowledge of aquaculture currently represents perhaps only a small part of a whole universe. It is difficult to demonstrate without genomic techniques the full diversity and hypothetical function of uncultured microorganisms; metagenomics and functional genomics in combination with chemical ecology may answer these questions (Riesenfeld *et al.* 2004).



Aquaculture microbial studies concentrate on understanding the symbiotic and antagonistic interrelations between the microbes and eukaryotes, such as fish, crustaceans and molluscs. Metagenomics can provide a deeper insight into those ties by associating with host or environment specific host species the information revealed by the extracted DNA (Suttle 2007; Gianoulis *et al.* 2009). New sequencing technologies and bio-informatics technologies enable the diversity of intracellular bacteria to be examined as well as specific genomic information from such communities to be elucidated. In the field of metagenomics, researchers have investigated the diversity and quantity of different microbes or genes in the spatially temporal pattern and have identified stronger associations between certain microbial communities and the host genotype (Monchy *et al.* 2011; Gilbert *et al.* 2012; Quince & Lundin 2013).

- Metagenomics could provide more evidence for the understanding of the microbial diversity in aquaculture facilities. By studying hypervariable regions of 16S rDNA for prokaryotes and 18S for eukaryotes, we can now understand the wide diversity of these microorganisms (Not *et al.* 2009; Hugerth *et al.* 2014).
- Metagenomics can be used to evaluate antibiotic resistance in bacterial communities according to the objectives of the analysis in two ways: functional metagenomics or sequence-based metagenomics, that is shotgun type (Schmieder & Edwards 2012).
- Functional metagenomics include the cloning and transmission of biologically derived DNA into a bacterial host to identify the roles of genes that may not be elucidated by analysing their sequences (Schmieder & Edwards 2012)
- Sequence-based metagenomics include the random sampling of whole environmental DNA samples. To identify resistance genes or mutations in specific genes involved in antibiotic resistance, the sequences obtained are compared to databases. In addition, mutations of multiresistance were found in aquatic microbes affected by agro-industrial activities such as aquaculture; the same microbial community may have enhanced tolerance to antibiotics, metals, ammonia compounds and other antibacterial chemicals due to mutation and further horizontal gene transfer (Zhang, *et al.* 2009).
- Viral species have special mechanisms of replication, transmission and persistence. Identifying viral processes can help to identify pathogenic species and mutations quickly, which are useful information for diagnosis, prevention and treatment development. However, the identification of pathogens as well as the current diagnostic techniques is restricted by an incomplete picture of the immense complexity of viruses and the limitations of traditional methods of detection.
- Shotgun metagenomics of clinical or random environmental samples are a promising alternative that circumvents conventional methods limitations. While this method has usually been used to study genomic diversity, it may also be useful in clinical detection of viral pathogens (Rosario *et al.* 2009; Bibby *et al.* 2011)
- Virus studies using metagenomic methods were recently promoted due to the quality and quantity of genomic information obtained with next generation sequencing; while the Sanger sequence provides significantly lower results, it was also used as an ID tool (Finkbeiner *et al.* 2008; Svraka *et al.* 2010). Compared to other methods including PCR or microarrays, metagenomics demonstrated better efficiency and accuracy of detection of multiple genomes (Yozwiak *et al.* 2012).
- Metagenomics libraries for particular biodegradation genes can be constructed, amplified and screened. This technique tested by cloning genes into bacteria and incubated in highly toxic compounds medium (George *et al.* 2010).
- New molecules can be provided with different functions through metagenomics, such as new enzymes or bioactive molecules, which can provide therapeutic applications or economic strategies (Lorenz & Eck 2005).

### Host fish species

The host species themselves are further considered and postulated in the determination of the fish gut microbiota; this parameter involved in a variety of confounding variables. Fish behavior, habitat salinity and intrinsic feeding tendency were among the various factors that could contribute to this parameter. For such a range of fish species, their gut microbiota is being examined. The different fish species under examination in the relevant studies are shown in Table 4. In the last ten years, the gut microbiome of carp fish species has been extensively researched. The carp fish are recognized comprehensively as invasive species, because of the enormous quantity of food they need of and it is recognized that their feeding model disturbs the food chain and disrupts the environment (Eichmiller *et al.* 2016). Besides, carp species are the trophic extent of this genus grass carp from herbivorous group and filter feeders such as bighead carps and omnivorous such as common carp and crucian carp (Liu *et al.*, 2016). Subsequently, zebra and cichlid fish species have been ascetically studied. Cichlid fish are identified for their capability to acclimatize at extremely precise niches in the ecology, consenting them to be distinctive feeders, such as nourishing on eyes or scales (Baldo *et al.* 2015). In biomedical studies in the last few years, zebrafish is a model organism that comes from ray-finned fish and is pre-eminent in vertebrate Actinopterygii



of Cypriniformes order, and is omnivorous species (Roeselers *et al.* 2011). Fish gut microbiota metagenomic studies are also conducted on cods and seabreams among them gilthead seabream and Blunt snout bream fish were studied. Primary, these fish were herbivorous and then impending carnivorous (Estruch *et al.* 2015; Liu *et al.* 2016). Major histocompatibility complex II (MHC II) was found to be lacking in Atlantic cods, which was formerly speculated in Gnathostomata as a conserved domain, constructing it is an exciting applicant for research of gut microbiome as the absence of MHC II, an immuno-regulating complex which possibly subsidize to the alteration in gut microbiota of the species (Star *et al.* 2013). Apart from these, other types of fish species including Gizzard shad, freshwater drum, damsel fish, mandarin fish, cardinal fish, threespine stickleback, top mouth culter and southern flounders are too researched.

### Factors influence gut microbes

The environment is among the most important key determinant faecal microbiota of silver carp, bighead carp and common carp, as demonstrated by the variation in fish gut microbiota content obtained from various sources from rivers, lakes and laboratory-reared. This correlation between the environment and the components of gut microbiota may be caused by multiple factors; for example, the availability of wild plant material in lakes and rivers may increase the feeding quantity of such content which aggregate arabinoxylan oligosaccharide in the fish which in turn act as a prebiotic for the ergo, *Clostridaceae* bacteria. *Clostridaceae* bacteria are likely to propagate thriving in the gut environment (Eichmiller *et al.* 2016). However, the composition of the gut microbiota during the larval stage of the fish must play more prominent role in determining the environmental influence (Stephens *et al.* 2016). From a different standpoint, profile analysis of T-RFLP exhibited the composition of zebra fish gut microbiota, which was different among fish captured from the wild in diverse locations and laboratory-reared fish of different locations. Unexpectedly, in determining the gut microbiota composition, the location of the laboratory-reared fish performed a major part than the wild fish collected (Roeselers *et al.* 2011). Environmentally ascribed factors, such as pH, NO<sub>3</sub>-N, PO<sub>3</sub>-P and conductivity, are also significantly correlated with fish gut microbiota composition (Giatsis *et al.* 2015). In addition, the existence of pollutants such as crude oils in the environment also disturbs the gut microbiota composition. In southern flounder, as evident from the proliferation of *Deltaproteobacteria*, *Gammaproteobacteria*, *Epsilonproteobacteria* and *Sphingobacteria* amid the decline of *Alphaproteobacteria* and *Clostridia* in its gut with crude oil.

It is suggested that the trophic level of the host fish species has a major impact on the gut microbiota composition of fish. Evident from Liu *et al.* (2016) studies that fish from distinct trophic levels, namely herbivores, carnivores and omnivores (including filter feeders), were specified to have distinct contents of gut microbiota. Herbivorous fish appeared to harbour *Leptotrichia*, *Clostridium* and *Citrobacter* in abundance, while the carnivorous fish gut existed rich in *Halomonas* and *Cetobacterium*, and the omnivorous fish were harbouring *Halomonas*, *Clostridium* and *Cetobacterium*.

In different stages of fish development, the composition of gut microbiota varies (planktonic and mature). The nature of the fish diet as they grow is conflicting with these phases, with mature fish getting more complex are all through the planktonic stage (Parris *et al.* 2016). During the larval and juvenile stages, zebrafish gut microbiota was considerably distinct, with the genotype of the fish as a cause of the gut microbiota composition is suggested with the reared environment and the same feeding material (Stephens *et al.* 2016). The environment performs a significant part in influencing the composition of gut microbiota at the larval stage of codfish rather than feed (Bakke *et al.*, 2013).

In addition, the position of the foregut, midgut and hindgut is assumed to be one of the variables influencing the gut microbiota composition of the fish. Changes in gut microbiota composition may be attributed to the environment on the basis of the consumption, and the composition varies as they transfer over the gut and some are engaged in the mucosal layer and, approximately, some are eliminated as waste. The modifications, however, are not well known, and further studies are needed to strengthen this proof (Ye *et al.* 2014; Estruch *et al.* 2015).

In determining the composition of gut microbiota in fish, host genotype performs an important role. One such instance is the host's immunological genotype that affects the composition of the gut microbiota. Fish with advanced genetic heterozygosity exhibited poorer complexity in the microbiota variation, specifying the role of MHCII in the control of the microbial population in gut fish (Smith *et al.* 2015).

Biotic (e.g. genotype, physiological status, pathobiology, lifestyle) and abiotic (e.g. environmental) factors may affect the fish gut microbiota and influence its composition and diversity, as well as its function and metabolic activity, thus affecting feeding, growth, energy storage and health of the fish (Ghanbari *et al.*, 2015). Previous studies have shown that microbes in water can affect the microbiota GI fish (Tanasomwang & Muroga 1988; Wang *et al.* 1993). The intestinal microbiota composition of the grass carp (*Ct. idellus*) is related to two main factors influencing GI microbiota in the production of water and sediment. Hagi *et al.*

**Table 4** Gut microbiota/microbiome studies in host fish species

Host Fish species	Research outcomes	References
Freshwater drum ( <i>Aplodinotus grunniens</i> ) Common carp ( <i>Cyprinus carpio</i> ) Silver carp ( <i>Hypophthalmichthys molitrix</i> ) Bighead carp ( <i>Hypophthalmichthys nobilis</i> )	Factors affecting carp microbiome characterization	Eichmiller <i>et al.</i> (2016)
Bighead carp ( <i>Hypophthalmichthys nobilis</i> ) Silver carp ( <i>Hypophthalmichthys molitrix</i> ) Crucian carp ( <i>Carassius auratus</i> ) Common carp ( <i>Cyprinus carpio</i> ) Top mouth culter ( <i>Culter alburnus</i> ) Mandarin fish ( <i>Siniperca chuatsi</i> ) Grass carp ( <i>Ctenopharyngodon idellus</i> ) Blunt snout bream ( <i>Megalobrama amblycephala</i> ) Damsel fish ( <i>Pomacentridae</i> ) Cardinalfish ( <i>Apogonidae</i> )	Fish gut microbiota and metabolic activity influenced by the host trophic level	Liu <i>et al.</i> (2016)
Haptri ( <i>Haplochromis trifasciatus</i> ) Hapmic ( <i>Haplochromis microlepis</i> ) Plestr ( <i>Plecodus straeleni</i> ) Permic ( <i>Perissodus microlepis</i> ) Perecc ( <i>Perissodus eccentricus</i> )	Fish health and ecology significantly influence by fish-associated microbial communities Investigation on the adaptation of gut microbiota in the process of the host adaptive radiation	Parris <i>et al.</i> (2016) Baldo <i>et al.</i> (2015)
Gizzard Shad ( <i>Dorosoma cepedianum</i> ) (Silver Carp ( <i>Hypophthalmichthys molitrix</i> ) Gilthead seabream ( <i>Sparus aurata</i> )	Fish gut microbiota differentiate in Asian and American carp based on the food source Replacement in fishmeal had a significant impact on gastrointestinal microbiota	Ye <i>et al.</i> (2014) Estruch <i>et al.</i> (2015)
Zebrafish ( <i>Danio rerio</i> )	Gut microbial communities interactions with zebrafish that have been experimentally analysed	Roeselers <i>et al.</i> (2011)
Grass carp ( <i>Ctenopharyngodon idella</i> )	Factors affecting the gut microbiome of grass carp and its effect on metabolism	Ni <i>et al.</i> (2014)
Baikal omul ( <i>Coregonus migratorius Georgi</i> ) Baikal whitefish ( <i>Coregonus baicalensis Dyb.</i> ) Tilapia ( <i>Oreochromis niloticus</i> )	Comparison between sympatric species and their first-generation hybrid crosses The effect of rearing environment on water bacterial communities which is present in the gut of Nile tilapia larvae	Belkova <i>et al.</i> (2017) Giatsis <i>et al.</i> (2015)
Zebrafish ( <i>Danio rerio</i> )	Intestinal microbiota in zebrafish has distinct communities throughout development and increasingly different from the surrounding environment	Stephens <i>et al.</i> (2016)
Atlantic cod ( <i>Gadus morhua</i> )	The host-specific selection is determined in the composition of the core microbiota	Star <i>et al.</i> (2013)
Southern flounder ( <i>Paralichthys lethostigma</i> )	A sediment-oil mixture has a negative impact on the health of fish species	Brown-Peterson <i>et al.</i> (2015)
Acanthuridae	The gut microbiota of surgeonfish is strongly influenced by diet	Miyake <i>et al.</i> (2015)

(2004) reported that the composition of the intestinal lactic acid bacteria (LAB) varied with seasons in four species of fish, namely silver carp (*Hypophthalmichthys molitrix*), common carp (*Cy. carpio*), catfish channel (*Ictalurus punctatus*) and deep-bodied crucian carp (*Carassius cuvieri*). It seems that the intestinal microbiota of fish from estuarine environments is more similar to that of freshwater fish, while the intestinal microbiota of fish from mixed salinity ecosystems is more similar to the intestinal microbiota of saltwater fish. Nevertheless, it is difficult to evaluate the exact role of salinity in forming the intestinal microbiota,

as they used different fish from freshwater or marine water. Zhang *et al.* (2016) recently revealed that the gut microbiota composition of Nile tilapia born under varying salinity was further investigated. The results showed that in high-salinity conditions, the abundance of *Devosia*, *Pseudomonas* and *Cetobacterium* increased. Feeding habit is also an important factor influencing GI microbial diversity, and an increasing trend in diversity was observed following the order of carnivores, omnivores and herbivores (Ward *et al.* 2009; Larsen *et al.* 2014; Li *et al.* 2014; Miyake *et al.* 2015).

## Physiology and immune role of fish gut microbiome

Few beneficial effects of gut microbiota are conferred on host fish, and such impacts can be classified systematically as physiological and immunological impacts. The following functions of bacteria in the gut microbiota of fish are exposed on the basis of latest studies carried out on this sector.

The gut microbiota physiological impact on the host fish is perceived through a number of interactions of metabolic ability and host metabolism of the gut microbiota. This relationship is defined by the failure of host fish to break down certain compositions in food, such as xenobiotic compounds or cellulose, used as a medium of energy by the host fish's gut microbiota, and the metabolites generated from this use as digestible energy source by the fish. Another component that contributes to the physiological effect on the fish gut microbiota is the synthesis or part of the synthesis of an essential supplement like a vitamin.

*Verrucomicrobiae* acquires the capability to break down  $\beta$ -glycans to  $\beta$ -glucose over the activity of cellulase. The presence of these bacteria now suggests that it is important for the digestion of plant cellulose in the gut of fish, and this feature has been demonstrated in carps and further strengthened by reduced cellulase activity in antibiotic-treated fish, and cellulase action in fish gut is effectively determined by gut microbiota (van Kessel *et al.* 2011). *Pseudomonas fluorescens* and *P. putida* were established as xenobiotic degrading bacteria (Austin *et al.* 1995). *Clostridia* was demonstrated to be one of the major representatives in fish gut microbiota. It is renowned for the synthesis of propionate, short fatty acid chains and butyrate; these compounds are synthesized in the host GI system and supplied to the host for better growth (Eichmiller *et al.* 2016). In addition, the fish diet is also considered to affect the composition of fish gut microbiota, in particular, the bacteria *Clostridia*, as shown in most of these bacteria in the gut microbiota of cichlid fish consuming the primary food component as the scales connected with other identical fish niches. These bacteria are connected with collagen-degrading enzymes because collagen is the primary component of the scales that are consumed by the particular niches of cichlid fish (Baldo *et al.* 2015). In many fish, gut microbiota, *Fusobacteria*, is also found to dominate with the *Cetobacterium* genus in the fish gut, being the most dominant genus of this category (Roeselers *et al.* 2011; Ye *et al.* 2014; Liu *et al.* 2016). This bacterium is notorious for the biosynthesis of vitamin B12 in fish, which is vital for fish development (Eichmiller *et al.* 2016).

The fish immune system is much similar to that of the higher vertebrate's immune system that comprises the innate and adaptive immune system. The adaptive

immune system can acquire memory through the production of antibodies, whereby the presence of antigens in monocytes and macrophages facilitates the innate immune system through cytokine and chemokine receptor profiles; the antigen-containing bodies are subject to elimination. This offers immune resistance against a multitude of pathogenic bacteria that adversely affect fish health (Gómez & Balcázar 2008). However, some pathogenic bacteria are not permitted from the exchange of any property of causing the disease to the fish, by means of the innate host resistance mechanism before any immunological response is activated. The innate resistance of the host involves a number of biological molecules, for example antimicrobial peptides, bile, mucosal layer, proteases and stomach acids (Huisinget *al.* 2003). Some beneficial bacteria that comprise the gut microbiota of fish also lend the host fish for certain immunological benefits by modulating the fish innate immune system, by interacting with host NK cells, neutrophils and monocytes. In addition, the method of action in which beneficial bacteria help to modulate the host fish immune response to pathogenic bacteria involves competition for nutrients, stimulating the nonspecific immune system of host fish, antagonism of pathogenic bacteria in excess of secretion of antimicrobial molecules and rivalry for adhesion site. The conclusion was that lactic acid bacteria (LAB) such as *Lactobacillus sakei*, *L. lactis*, *L. rhamnosus* and *Clostridium butyricum* increased host fish immunoglobulin levels, and thus, the siderophore-producing biological control agent such as *P. fluorescens* was also effectively implemented in the gut. This might rule out the pathogenic bacteria *Aeromonas salmonicida* strain from stress-inducible furunculosis infection in Atlantic salmon presmolts. Also, it is to limit the death of rainbow trout infection due to *Vibrio anguillarum*. Temporary, the immersion of the fish in the bacterial suspension of probiotics and long-standing exposure of the fish in rearing water or a mixture of these two procedures resulted in a significant reduction in mortality following the experimental test (Zorriehzahra *et al.* 2016). In Ecuador, at a commercial shrimp hatchery, *V. alginolyticus* strain has been used as a probiotic and the same was applied in a bath treatment to Atlantic Salmon, which was maintained in freshwater. Up to 21 days after initial probiotic implementation, *V. alginolyticus* was encountered in the intestine. The experiment exposed that the probiotic application to Atlantic salmon controlled the decline of mortality once exposure to *A. salmonicida* and to a reduced the level after acquaintance to *V. ordalii* and *V. anguillarum* (Austin & Newaj-Fyzul 2017). *V. alginolyticus* is a strain that has been shown to be a probiotic agent in the treatment of fish infection with *V. ordalii*, *V. anguillarum* and *A. salmonicida* (Austin *et al.* 1995). *Pediococcus acidilactici* originating from LAB is considered

to secrete organic acids and a range of biomolecule bacteriocin that has an adverse effect on a variety of pathogenic bacteria (Ferguson *et al.* 2010). Another research has focused on endogenous intestinal microbes, *Bacillus*

*circulans*, in Siberian sturgeon, which displayed substantial enhancement of complete stimulation of immune response and growth performance of the fish (Geraylou *et al.* 2013).

**Table 5** Studies on the gut microbial composition of microbiota/microbiome in corresponding fish gut

Bacterial composition in the Fish gut	Studies outcome	References
Proteobacteria Firmicutes Fusobacterium	Factors affecting the characterization faecal microbiomes of common, silver and bighead carps	Eichmiller <i>et al.</i> (2016)
Proteobacteria Firmicutes Fusobacteria Bacteroidetes	The structure, gut content enzyme activity and composition of gut microbiota, metabolic capacity influenced by host trophic level	Liu <i>et al.</i> (2016)
Gammaproteobacteria Pseudoalteromonadaceae Endozoicimonaceae Vibrionaceae shewanellaceae	Identifying transition in microbiome structure across host life stage	Estruch <i>et al.</i> (2015)
Firmicutes Proteobacteria Actinobacteria	The microbial composition of the gut was analysed after the total fishmeal replacement by vegetable-based feed in the sea bream	Baldo <i>et al.</i> (2015)
Firmicutes Fusobacteria Proteobacteria Bacteroidetes Actinobacteria Planctomycetes Verrucomicrobia	Effect of host adaptive radiation in the gut microbiota of cichlids	Ye <i>et al.</i> (2014)
Proteobacteria Fusobacteria Firmicutes Cynobacteria Bacteroidetes Actinobactericae	Gut microbiota analysis differentiates in food sources including bacteria ingested by Asian and American carp	Roeselers <i>et al.</i> (2011)
Proteobacteria Fusobacteria Firmicutes Actinobacteria Bacteroidetes	The zebrafish intestinal habitat selected for specific bacterial taxa differences in host provenance	Ni <i>et al.</i> (2014)
Proteobacteria Fusobacteria Firmicutes Proteobacteria Firmicutes Actinobacteria Cyanobacteria	Gut microbiome metabolism in grass carp is affected by feeding	Belkova <i>et al.</i> (2017)
Proteobacteria Firmicutes Actinobacteria Cyanobacteria	Through water microbial management the steering of gut microbial communities could be possible	Giatsis <i>et al.</i> (2015)
Proteobacteria Firmicutes Bacteroidales erysipelotrichales Clostridiales Alteromonadales Deferribacterales	Zebrafish intestinal microbiota was increasingly different from the surrounding environment and from one another	Stephens <i>et al.</i> (2016)
Gammaproteobacteria Deltaproteobacteria Epsilonproteobacteria Alphaproteobacteria Clostridia	Composition of core microbiota is determined by the host-specific selection	Star <i>et al.</i> (2013)
Firmicutes Proteobacteria	Chronic exposure of oiled sediments produces adverse effects in bacterial population structures	Brown-Peterson <i>et al.</i> (2015)
Firmicutes Proteobacteria	Surgeonfish change in feeding behaviours, which in turn has a strong impact on the reef ecology.	Miyake <i>et al.</i> (2015)

## Microbial load in fish gut

As mentioned earlier, the microbial composition of host fish varied due to several variables; and the microbial loads of different fish found in the related study are shown in Table 5. Most of the gut microbiota of fish comprises of *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Firmicutes* and *Proteobacteria* cumulatively in phyla term with more than 80% concentration.

A study directed at defining the effects on the gut microbiota composition of invasive carp fish in wild as well as in captive environments concluded that *Fusobacteria*, *Proteobacteria* and *Firmicutes* phyla in the gut microbiota were available in the carp fish with a combined concentration of 76.7%. Five mutual OTUs were found to consist of 40% of the core faecal microbiota between laboratory and wild carp fish (Eichmiller *et al.* 2016). Another study aimed at identifying the correlation between the composition of gut microbiota and wild freshwater trophic level fish indicated the fact the most prevalent bacterium genus observed in all fish was *Proteobacteria*, with a comparative abundance of 45.52% in herbivorous fish and in carnivorous fish 32.82%, in omnivorous fish about 37.32% and 38.13% in filter-feeding fish. This is preceded by *Firmicutes*, the most common bacterial species, with a comparative abundance of 21.16%, 21.83%, 22.38% and 27.13%, in filter-feeding fish, carnivorous, herbivorous and omnivorous, respectively. Other bacterial species, such as *Actinobacteria*, *Acidobacteria*, *Crenarchaeota*, *Bacteroidetes*, *Verrucomicrobia*, *Cyanobacteria* and *Planctomycetes*, were relatively abundant in a range between 0.89% and 8.26%. More noteworthy is the fact that, at the level of the genus, the comparative abundance of the *Leptotrichia*, *Citrobacter* and *Clostridium* bacteria was found with the capacity to degrade cellulose, which was significantly higher in *Halomonas* bacterial species, *Cetobacterium* and herbivorous fish. Liu *et al.* (2016) reported that the protease producing bacteria were rich in carnivorous fish species. The gut microbiome of pre-settlement-reef and postsettlement reef of cardinalfish and damselfish was completed by discovering indicator taxa for the two species specified life phases. Throughout the fish pre-settlement stage, the guts were initiated to be harbouring *Kordia* (Flavobacteriia), *Oceonospirillum* (Gammaproteobacteria), *Arcobacter* (Epsilonproteobacteria), *Halomonas* (Gammaproteobacteria) and *Idiomarina* (Gammaproteobacteria) in downward order relating to the strength of the indicator of the bacterial taxa. Meanwhile, in postsettlement, fish harboured bacteria from *Planctomycetes* (Pirellulaceae), *Firmicutes* (Epulopiscium), *Gammaproteobacteria* (Vibrionaceae, Portiera), *Verrucomicrobia* (Coraliomargarita) and *Alphaproteobacteria* (Kiloniellales, Hyphomicrobiaceae; Parris *et al.* 2016).

The lab-reared zebrafish gut microbiota composition was subjective to the different stages of the fish development, where the change from the larval stage to the juvenile phase was noted in the microbiota structure, and an alteration in the microbial composition was detected throughout the changeover from the juvenile to the adult stage. In this, considerably extensive bacteria such as *Fusobacteria* and Class CK-1C4-19 bacteria in the gut of adolescent zebrafish were found to be in tiny proportion during the larval and juvenile stages, indicating the development of zebrafish physiology plays a major role in determining the microbial composition of the intestine (Stephens *et al.* 2016).

## Conclusion

Metagenomic research of fish gut microbiome provided numerous data on the physiological and immunological features of the gut microbiota of various fish. Gut microbiota influences the fish metabolism and modulates the fish immunogenicity with respect to pathogenic microbes. Fish gut microbial composition differed depending on habitat, species and feeding behaviour. The gut microbes that have been shown to have a positive effect on the health of the fish that can be used as a probiotic candidate awaiting further study.

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