Recent studies on probiotics as beneficial mediator in aquaculture: a review

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Abstract

Background: The diseases in fish and other economic aquatic species is a great concern, and every year it causes a huge loss in aquaculture sectors. The use of probiotics might be a good option to reduce the disease risk and to enhance the productivity.

Methods: We have gathered information from various important research and review articles related to fish diseases, probiotics, and gut microbial community. We have tried our level best to represent the up-to-date information in a concise manner.

Results: In this present review, we have demonstrated the various beneficial aspects of probiotics in aquaculture sectors. Probiotics are considered as novel functional agents that have potential implications in influencing the gut microbiota of any aquatic organism. Researchers have already documented that probiotics play a wide spectrum functions (such as decrease diseases and stress, enhance immunity, modulate gut microbiota, helps in nutrition, improve water quality, etc.) in host body. Furthermore, the beneficial effects of probiotics contribute to increase feed value and growth of the animal, and improve spawning and hatching rate in aquaculture system. Here, we have discussed each and every functions of probiotics and tried to correlate with the previous knowledge.

Conclusion: The reports regarding the efficacy of probiotics and its detailed mechanism of action are scarce. Till date, several probiotics have been reported; however, their commercial use has not been implicated. Most of the studies are based on laboratory environment and thus the potentiality may vary when these probiotics will be used in natural environments (pond and lakes).

Keywords: Probiotics, Aquaculture, Antibiotics, Stress, Reproduction, Gut microbiota, Mucosal immunity

Background

Aquaculture is the fastest growing food industry in several countries like China, India, Norway, etc. According to Food and Agriculture Organization (FAO), the aquaculture production reached 106 million tonnes with an estimated cost of USD 163 $ in the year 2017 with a growth rate of 6.6. The production/captured of finfish was recorded to be highest in Asian countries, followed by Americans countries, Europe, and Africa. Aquatic animals maintain a close relationship with their external environment, which enhance the risk of diseases susceptibility (Banerjee & Ray, 2017). Furthermore, high stocking density, water pollution, insecticides containing agricultural drain-age water, and unscientific feeding enhance the risk of bacterial, fungal, and viral diseases in cultured animals (Banerjee & Ray, 2017). In intensive culture system, disease outbreak is a major difficulty that decreases the profit in food industries, as well as hampers the socio-economic condition of the country (Bondad-Reantaso et al., 2005).

The use of antibiotics in aquaculture as a preventive measure associated with the evolution and spread of several resistant human pathogens like *Aeromonas* sp., *Escherichia tarda*, *Escherichia coli*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, and many more (Allameh et al., 2016; Brogden et al., 2014). In a review, Lakshmi, Viswanath, and Sai Gopal (2013) have provided the information regarding the resistance
development in aquatic pathogens under long-term antibiotic pressure (Lakshmi et al., 2013). Thus, the uses of certain antibiotics in aquaculture industries have been restricted in several countries like the USA and Canada. So, the use of probiotics along with dietary supplementation is a very fruitful strategy to combat pathogenic agents through a variety of mechanisms as an alternative driving force of antibiotic treatment (Bandyopadhyay et al., 2015; Wu, Jiang, Ling, & Wang, 2015). The term ‘probiotic’ came from Greek words ‘pro’ (= favor) and ‘bias’ (= life) which are live organisms (usually bacteria or yeast or combination of both) and taken with food to confer beneficial effects to host in various ways (Fuller, 1989). The concept of probiotics, in the field of aquaculture, is fundamentally different from those which are used in terrestrial organisms depending upon certain critical influencing factors. It is now well established that probiotics play a vital role in maintaining the gut health by modulation of microbial community structure (Nayak, 2010). The microbes also proliferate independently of the host animal in response to diseases (Bondad-Reantaso et al., 2005; Irianto & Austin, 2002). The first experimental attempt of the probiotic application in aquaculture was made by Kozasa (1986), considering the beneficial effects of probiotics on humans and poultry (Kozasa, 1986). The rapid evolution of probiotics in aquaculture is well established due to the adverse effects of antibiotic resistance (Lakshmi et al., 2013). Thus, the uses of antibiotics and broad spectrum chemicals restricted in several countries like the USA and Canada. The goal of this review is to summarize and evaluate the current information on the efficacy and mechanism of action of probiotics for the enumeration in a complex microbial community in aquaculture.

**Main text**

**Application methods of probiotics**

Based on the mode of action, probiotics can be divided into two broad categories: (a) gut probiotics: which are administrated orally along with food to improve the gut associated beneficial microbial flora (Table 1) and, (b) water probiotics: these types of agents proliferate in water medium and exclude the pathogenic bacteria from the specific medium by consuming all available nutrients, resulting in elimination of the pathogenic bacteria through starvation (Table 2).

**Candidates as probiotics**

Recently, the application of probiotics is a very popular practice in aquaculture sectors and it is mainly isolated from fish gut. Among several bacterial candidates, lactic acid bacteria (LAB), *Bifidobacterium*, and *Streptococcus* (Giri et al., 2013) gain more popularity. Despite the fact that implication of probiotics is relatively a very new approach but it has gained attention due to their potential activity in controlling different physiological activities of aquatic organisms. Thereafter, many probiotics such as *Aeromonas media*, *Bacillus subtilis*, *Lactobacillus helveticus*, *Enterococcus faecium*, *Carnobacterium inhibens*,

**Table 1** Gut probiotics and their beneficiary effects on aquatic organisms

| Name of the probiotics | Beneficial effects | Reference(s) |
|------------------------|--------------------|--------------|
| Lactobacillus rhamnosus | Enhance immunity and reduce disease susceptibility | Nikoskelainen, Ouwehand, Bylund, Salminen, and Lilius (2003) |
| Lactobacillus plantarum | Enhance stress tolerance | Taoka, Yuge, Maeda, and Koshio (2008) |
| Lactobacillus rhamnosus | Improve blood quality | Panigrahi et al. (2010) |
| Streptococcus sp. | Improve feeding efficiency and growth rate | Lara-Flores and Olvera-Novoa (2013) |
| Bacillus subtilis | Enhance cellular immunity | Sánchez-Ortiz et al. (2015) |
| Bacillus subtilis + Lactococcus lactis + Saccharomyces cerevisiae | Enhance survival rate, foster metabolism, enhance weight | Abareethan and Amsath (2015) |
| Bacillus amyloliquefaciens | Enhance antibody concentration, reduce stress | Nandi et al. (2018) |
| Bacillus subtilis + Lactobacillus rhamnosus | Enhance the food digestibility | Munirasu, Ramasubramanian, and Arunkumar (2017) |
| Lactobacillus sp. | Reduce pathogen load, provide protection against *Aeromonas hydrophilia* | He et al. (2017) |
| Bacillus cereu | Protect from *Aeromonas hydrophila* infection | Dey, Ghosh, and Hazra (2018) |
| Different species of Bacillus, *Anthrobacter*, *Paracoccus*, *Acidovorax* etc | Reduce pathogen load and provide nutrients | Nandi et al. (2018) |
| Alcaligenes sp. AFG22 | Enhance volatile short chain fatty acids | Asaduzzaman et al. (2018) |
etc. are considered to be significantly effective at present. However, Gram-negative facultative symbiotic anaerobes such as *Vibrio*, *Pseudomonas*, *Plesiomonas*, and *Aeromonas* were also reported to be potential probiotic candidates present in the gastro-intestinal tract (GIT) of fish and shellfish (Lakshmi et al., 2013; Verschueren, Rombaut, Sorrgeloos, & Verstraete, 2000). Apart from these discussed laboratory-based probiotics, various experimentally approved commercial probiotics are also available in the market which is also effective in aquaculture (Table 3).

**Screening of probiotics**

Although, probiotics have been used in aquaculture due to their broad spectrum biological activities but the

| Table 2 Water probiotics and their role in maintaining water quality |
|-----------------------------|-----------------------------|-----------------------------|
| Name of the probiotics | Beneficial effects | Reference(s) |
| Bacillus spp. | Reduces the load of ammonia and nitrite | Porubcan (1991) |
| Enterococcus faecium Z14 | Improves water quality and enhances immunity | Wang and Wang (2008) |
| Lactobacillus acidophilus | Improves water quality | Dohail, Abdullah, Roshada, and Aliyu-Paiko (2009) |
| Bacillus NL110, Vibrio NE1 | Reduces ammonia and nitrite concentration | Mujeeb Rahiman, Yousuf, Thomas, and Hatha (2010) |
| Nitrosomonas sp., Nitrobacters sp. | Reduces the concentration of ammonia, phosphates and nitrite in culture pond | Padmavathi, Sunita, and Veeraiah (2012) |
| Rhodopseudomonas palustris, Lactobacillus plantarum, Lactobacillus casei, Saccharomyces cerevisiae | Reduces nitrate load, maintain water pH and enhances dissolve oxygen concentration | Melgar Valdes, Barba Macías, Alvarez-González, Tovilla Hernández, and Sánchez (2013) |
| Paenibacillus polymyxa | Enhances immunity and reduces pathogenic stress | Giri, Sukumaran, and Oviya (2013) |
| Lactobacillus rhamnosus | Reduces pathogen load in culture tank | Talpur et al. (2013) |
| Pseudomonas sp. | Enhances transcription rate of anti-microbial peptide | Ruangsi, Lokesh, Fernandes, and Kiron (2014) |
| Bacillus spp. | Promotes the growth of beneficial algae and reduces the growth of harmful algae | Lukwambe et al. (2015) |
| Nitrosomonas sp., Nitrobacters sp. | Reduces pathogen load in culture pond and increases dissolved oxygen content | Sunitha and Krishna (2016) |

| Table 3 Commercial probiotics for aquaculture available in the market |
|-----------------------------|-----------------------------|-----------------------------|
| Product name | Company name | Composition |
| Prosol | Prosol Chemicals | *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus plantarum* |
| Progut | Lincoln Pharmaceuticals | Yeast cell wall, Mannoproteins, Betaglucans, nucleotides and peptides |
| Aqualact | Biostadt India | Information is not available |
| Lact-Act | Geomarine Biotechnologies | *Lactobacillus sporogens* |
| Enget | Microtech | *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis* |
| Grobact | Tropical Bionarine System | *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Saccharomyces boulardii*, *Bacillus coagulans*, *Streptococcus thermophilus*, *Bifidobacterium longum*, *Bifidobacterium bifidum* |
| Prolacto | Drug International | *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus bulgaricus* and fructooligosaccharides |
| ProbioDiet | Prowin Bio-Tech | Saccharomyces sp., *Lactobacillus* sp. and *Bacillus* sp. |
| Hydroxyeast Aquaculture | Agranco Corp | *Streptococcus faecium*, *Lactobacillus acidophilus*, Yeast, *Bifidobacterium* sp. and probiotics |
| Biotix Plus | Matrix Biosciences | *Lactobacillus* sp. |
| AquaStar | Biomin | *Pediococcus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Bacillus* sp. |
| Biocom Plus | VXL Drugs and pharmaceuticals | Information is not available |
| NatuRose | Artemia International | *Haematococcus pluvialis* |
| Enterotrophic | National Centre for Aquatic Animal Health, India | *Bacillus cereus*, *Arthrobacter nicotianae* |
| Nitro-PS+ Micro-Pro | Asian Bio Tech | Information is not available |
| Pond Plus | Novozymes | Different kind of heterotrophic bacteria |
| Eco-Pro | symbiozymes | *Rhodopseudomonas palustris* |
selection methods of inappropriate microorganisms lead to failure of many related researches. Screening of probiotics is the first and foremost crucial step that has to be achieved through a step by step fundamental scientific research. Till date, several probiotic candidates have been reported by different research groups; however, their use is restricted in laboratory scale. A full-scale trial of these probiotics is important to commercialize these products in the market. In order to select the potential probiotics, knowledge about the mechanisms of its action is essential (Pandiyan et al., 2013). It is widely accepted that a probiotic must contain some definite features in order to aid the correct establishment of effective agents (Priyodip, Prakash, & Balaji, 2017; Thakur, Rokana, & Panwar, 2016). The selection criteria of probiotic include the following: (a) it should be harmless to the host; (b) it must be non-invasive, and non-carcinogenic; (c) it should reach effectively at the host’s target site; (d) it should contain plasmid without antibiotic and virulence resistance genes; (e) it should be colonized for a stable time period and replicate within the host; and (f) it should actually work in host model system as opposed to in vitro findings.

However, the probiotic screening to date is concentrated on the search for active agents against a pathogen which induce the interruption in the aquatic environment. In in vitro screening for potential probiotics, most of the researchers employ identification of inhibitory or antagonistic activity (Kesarcodi-Watson, Kaspar, Lategan, & Gibson, 2008; Sahu, Swarnakumar, Sivakumar, Thangaradjou, & Kannan, 2008). To screen for inhibitory substances in vitro, four methods are commonly applied; the double layer method, the well diffusion method, the cross-streak method, and the disc diffusion method. The basic principle of all these methods is based on the fact that a bacterium (producer) produces an extracellular substance which is inhibitory to itself, or another bacterial strain (indicator) (Kesarcodi-Watson et al., 2008; Priyodip et al., 2017). The methods used in aquaculture include some major steps: (a) a background knowledge about the application of probiotics; (b) attainment of alleged probiotics; (c) both in vivo and in vitro assessment of their pathogenicity; and (d) a long-term practical evaluation of the treated probiotics. Recently, a number of fast and sensitive molecular tools are also used for selection and evaluation of probiotics includes ERIC-PCR and PCR-DGGE/TGGE techniques, FISH, and 16S rRNA gene sequencing (Qi, Zhang, Boon, & Bossier, 2009; Wu et al., 2015) (Fig. 1).

**Beneficial effects and mode of action of probiotic in aquaculture**

The risk of disease enhancement in aquaculture industries fosters the probiotic research for developing sustainable aquaculture. With the increased public concern on the use of antibiotics, it is not surprising to increase a rapid growth of the probiotic for aquaculture. Food and Agricultural Organization (FAO) has now recommended the application of probiotics for the improvement of aquatic environmental quality by reducing the mortality (Subasinghe, 2005), or by increasing the resistance against putative pathogens of host (Irianto & Austin, 2002). The beneficial effects are temporal on occasion, depending on the time of application (Verschueren et al., 2000). The effectiveness and mode of actions of many probiotics used recently in aquaculture are summarized in Table 4.

**Maintenance of water quality**

Probiotics help to improve water quality due to their ability to participate in the turnover of organic nutrients in aquaculture (Wang & Wang, 2008; Wang, Zheng, Liao, Huang, & Sun, 2007). Organic enrichment and nitrogenous wastes, including ammonium and ammonia (NH$_3$), are a serious concern in aquaculture, for example in pond rearing of catfish (Sahu et al., 2008). To date, the information regarding the maintenance of the balance of NH$_3$/NO$_2$/NO$_3$ in pond by probiotic candidates is limited (Wang et al., 2007) (Fig. 2). There is a strong tendency to combine different photosynthetic bacteria, *Bacillus*, nitrifiers, and denitrifiers together; therefore, probiotics are often labeled as multifunctional and can be applied to various species under diverse culture conditions (Wang & Wang, 2008). Apart from these, probiotics are more efficient in transforming the organic matter to CO$_2$ (Fig. 2); therefore, it is suggested to maintain their high levels in production ponds to reduce the organic carbon load and to enhance the water quality and fish health.

**Augmentation of growth and survival rate**

Probiotic is also used to promote the growth of different cultivated species in aquaculture. In Javanese carp (*Puntius gonionotus*), *Enterococcus faecalis* causes significant weight gain when supplemented at $10^7$ and $10^9$ cfu g$^{-1}$ diet compared to the control group of carp (Allameh et al., 2016). The microorganisms are able to colonize within the GIT due to their higher multiplication rate than the rate of expulsion after the administration over a long period of time. Probiotics are added constantly to fish cultures to maintain the health by enhancing the expression of several immunological factors, and to reduce the pathogen load to the gut mucus layer by occupying the physical space (Banerjee & Ray, 2017). Furthermore, probiotic candidate also play a vital role in nutrient enhancement in host. Hamdan et al. (2016) have reported the enhancement of crude lipid, total protein, and body weight in Nile tilapia (*Oreochromis niloticus*) fed with probiotic strain of *Lactobacillus* sp. (Hamdan et al., 2016).
This also depends on factors such as water quality, hydrobionts species, enzyme levels, and genetic resistance. Tan, Chan, Lee, and Goh (2016) have also reported that growth and survival rate increase in *Xiphophorus helleri*, *Xiphophorus maculates*, and *Poecilia reticulate* fed with probiotic supplemented food containing *Bacillus subtilis* and *Streptomyces* sp. (Tan et al., 2016).

**Improvement in nutrient utilization**

Probiotic microorganisms have beneficial effects in GIT of aquatic animals in the digestion of dietary nutrients as well as in production of energy. The most commonly used probiotic preparations in this purpose are the lactic acid bacteria (Ringø et al., 2018). It is found in large numbers in the gut of healthy animals and, in the words of Food and Drug Administration (FDA), is generally regarded as safe (GRAS status) (Giri et al., 2013). However, this increased nutrient digestibility are due to the elevated level of digestive enzymes (protease, amylase, cellulose, phytase, etc.) produced by the probiotic altered gut-associated microbial community in the host (Banerjee, Nandi, & Ray, 2017; Burr & Gatlin, 2005; Ghosh, Banerjee, Moon, Khan, & Dutta, 2017). For example, few bacteria (viz. *Rhodobacter sphaeroides* and *Bacillus* sp.) participate effectively in the digestion processes by activating protease, lipase, amylase, and cellulase enzymes significantly in white shrimp (*Litopenaeus vannamei*) (Wang & Wang, 2008) and in bivalves (Sahu et al., 2008). Additionally, a few recent studies have shown that probiotics may also stimulate the nutrient absorption by increasing the surface area of the host GIT, based on quantitative changes in histological measurements of the area of intestinal fold, enterochromaffin cells, and microvillus (Zhou, Buentello, & Gatlin, 2010) (Fig. 2). It is also suggested that *Lactobacillus brevis* and *Bacillus subtilis* are capable of producing higher amount of enzyme phytase (up to 1,354,906.6 U/mL) which helps to utilize the plant product phytate, chemically known as *myo*-inositol hexaphosphate (Priyodip et al., 2017). Till date, several bacterial candidates (*Pseudomonas* sp., *Brevibacterium* sp., *Microbacterium* sp., *Agrobacterium* sp., and *Staphylococcus* sp.) have been reported.
| Tested aquatic animals | Potential probiotics | Dose and method of administration | Observations | Mode of action | Reference(s) |
|-----------------------|----------------------|-----------------------------------|--------------|---------------|---------------|
| Abalone (Haliotis discus hannai Ino) | *Shewanella* colwelliana WA64, *Shewanella* olleyana WA65 | 10 (9) cells/g probiotics; addition to diet | Enhanced cellular and humoral immune responses | Immunostimulation | Jiang, Liu, Chang, Liu, and Wang (2013) |
| Marron (Cherax tenuimanus) | *Bacillus* sp., *Shewanella* sp. | 10 (8) CFU/g diet | Improved tail muscle indices, total haemocytes counts | Improved nutritional value | Ambas, Suriawan, and Fotedar (2013) |
| Wild shrimp (Penaeus monodon) | *Bacillus* cereus | 0.1–0.4%/100 g diet | Increased growth and survival rates, stimulated respiratory burst and lysozyme activities | Improved nutritional value | NavinChandran et al. (2014) |
| Indian major carp Catla (Catla catla) | *Bacillus* amyloliquefaciens FPTB16 | 1 × 10^7, 1 × 10^8, and 1 × 10^9 CFU/g diet | Stimulated cellular immune response; myeloperoxidase content, lysozyme activity | Immunostimulation | Das, Nakhro, Chowdhury, and Kamilya (2013) |
| Indian major carp rohu (Labeo rohita) | *Bacillus* subtilis, *Terribacillus saccharophilus* | 1 × 10^7 CFU/g diet | Increased humoral immune response, activities of serum phagocytes, respiratory burst | Immunostimulation | Giri et al. (2013); Kalarani, Sumathi, Roshan, Sowjanya, and Reddy (2016) |
| Nile tilapia (Oreochromis niloticus) | *Lactobacillus* plantarum AH 78 | 0.5, 10, or 20% (w/w) with diet | Significantly up-regulated the expression of cytokine genes IL-4, IL-12 and IFN-γ | Immunostimulation | Hamdan, El-Sayed, and Mahmoud (2016) |
| Grass carp (Ctenopharyngodon idella) | *Shewanella* xiamenensis A-1, S. xiamenensis A-2 and *Aeromonas veronii* A-7 | 1 × 10^8 cells/g of bacteria, addition to diet | Enhanced phagocytic and lysozyme activities, complement C3, expression of immune-related genes (IL-8, IL-1β, lysozyme-C, and TNF-α) | Immunostimulation | Wu et al. (2015) |
to contribute in nutritional and metabolism physiology in Arctic charr (*Salvelinus alpinus*) (Ringø, Dimitroglou, Hoseinifar, & Davies, 2014). Different bacterial strains in the form of probiotics also contribute significantly by modulating gut microbial population of the host organisms especially by synthesizing the fatty acids, minerals, vitamins, and essential amino acids (Nayak, 2010; Newaj-Fyzul, Al-Harbi, & Austin, 2014). Different bacterial strains in the form of probiotics also contribute significantly by modulating gut microbial population of the host organisms especially by synthesizing the fatty acids, minerals, vitamins, and essential amino acids (Nayak, 2010; Newaj-Fyzul, Al-Harbi, & Austin, 2014).

**Effects on phytoplankton**

Probiotic bacteria play vital role in controlling algal growth, particularly of red tide plankton (Qi et al., 2009). Bacteria antagonistic toward algae will be undesirable in green water larval rearing technique in hatchery where unicellular algae are cultured, but will be advantageous when undesired algae species are developed in the culture pond.

**Bacteriostatic effects of probiotics**

Probiotic bacterial populations may release a variety of chemical substances that have a bactericidal or bacteriostatic effect on both Gram-negative and Gram-positive bacteria. These inhibitory substances belong to different origin such as proteinaceous substance (lysozyme and different kind of proteases), chemical (hydrogen peroxides), and iron-chelating compound like siderophores (Giri et al., 2013). LAB produces a compound—bacteriocins that can alter inter-population relationships by influencing the outcome of competition for chemicals, or energy (Kesarcodi-Watson et al., 2008; Ringø et al., 2018). These inhibitory substances play an important role in controlling algal growth, particularly of red tide plankton (Qi et al., 2009). Bacteria antagonistic toward algae will be undesirable in green water larval rearing technique in hatchery where unicellular algae are cultured, but will be advantageous when undesired algae species are developed in the culture pond.

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role in pathogen inhibition and proliferation, and thereby reduce the pathogen load. The information about the inhibitory substances produced by probiotic bacteria are given in Table 5.

**Stimulation of decolonization of pathogenic bacteria**

One possible mechanism for preventing colonization by pathogens is physical competition for attachment sites on the gut mucosal layer in host. It is known that the ability to adhere to mucus and wall surfaces is necessary for bacteria to become established in fish intestines (Cruz, Ibáñez, Hermosillo, & Saad, 2012; Roeselers et al., 2011). Since bacterial adhesion to tissue surface is important during the initial stages of pathogenic infection, competition for adhesion receptors with pathogens might be the first probiotic effect (Chabrillón, Arijo, Díaz-Rosales, Balebonz, & Morínigo, 2006). In general, probiotic microorganisms possess mucus binding proteins which help in the acceleration of the binding process. In an investigation, Mackenzie et al. (2010) have reported the differential expression pattern of a key receptor mub in different strains of *Lactobacillus*, and have compared their binding efficacy in the gut mucosa (Mackenzie et al., 2010).

**Augmentation in the immune system**

Probiotics play the beneficial role as immunostimulatory to assist in the protection of aquatic cultured species by reducing the impact of diseases and entrance of pathogens (Dawood & Koshio, 2016; Hai, 2015). Thus, its use as an immunostimulant is very practical approach to improve the success of the aquaculture. Many authors have compared their binding efficacy in the gut mucosa and reported the differential expression pattern of a key receptor *mub* in different strains of *Lactobacillus*, and have compared their binding efficacy in the gut mucosa (Mackenzie et al., 2010).

### Table 5 Production of inhibitory substances by probiotic candidates

| Probiotic candidates       | Inhibitory substances | Inhibitory pathogens                  | Reference(s)                        |
|----------------------------|-----------------------|----------------------------------------|-------------------------------------|
| *Vibrio anguillarum* / VLA4335 | Siderophore           | *Vibrio ordalii*                       | Pybus, Loutit, Lamont, and Tagg (1994) |
| *Vibrio* sp.               | Siderophore           | *Vibrio splendidus*                    | Gatesoupe, (1997)                   |
| *Pseudomonas fluorescens*  | Siderophore           | *Vibrio anguillarum*                   | Gram, Melchiorsen, Spanggaard, Huber, and Nielsen (1999) |
| *Photobacterium leognathi*, *Vibrio scophthalmi* and *Enteroborii norvegicus* | Siderophore | N/D                                    | Sugita, Mizuki, and Itoi (2012)      |
| *Lactobacillus murinus* AU06 | Bacteriocin           | *Vibrio* sp., *Micrococcus*            | Elayaraja, Annamalai, Mayavu, and Balasubramanian (2014) |
| *Pediococcus acidilactici* L-14 | Pediocin PA-1        | N/D                                    | Araújo et al. (2016)                |
| *Bacillus subtilis* LR1     | Bacteriocin           | *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Bacillus mycoides* and *Pseudomonas fluorescens* | Banerjee et al. (2017)               |
| Strains H4 (not identified) | Bacteriocin           | *Pseudomonas stutzeri*                 | Feliatra et al. (2018)               |

*N/D* not detected
**Effects on reproduction**

The use of probiotics on disease resistance ability is well documented, but research on the effects and action mechanism of probiotics on the reproductive performance of aquatic animals are lacking (Fig. 2). Very few studies have attempted to demonstrate the role of probiotic supplementation on reproductive performance in aquaculture (Abasali & Mohammad, 2011; Ghosh, Sinha, & Sahu, 2007), using various strains like *B. subtilis*, *Lactobacillus acidophilus*, *Lactobacillus casei*. It is well documented that probiotics influence reproduction in different factors like fertilization, gonadosomatic index, fecundity, and production of fry from the females (Abasali & Mohammad, 2011). Recent study also reported that probiotics increase the daily numbers of ovulated eggs compared to control levels with higher hatching rate and faster embryonic development in zebrafish (Gioacchini et al., 2013). However, rigorous experiments still need to be established for the utilization of probiotics to increase the production rate of aquatic animals.

**Other functions**

Very few recent investigations also highlight the effects of probiotics on some major physiological processes in aquatic organisms. In European seabass, it helps to increase the body weight by stimulating the mRNA transcription of insulin-like growth factor (IGF)-I (Carnevali, Sun, Merrifield, Zhou, & Picchietti, 2014). Additionally, it is now profoundly accepted that probiotics reduce the concentration of the stress hormone cortisol and activate the expression of antioxidative enzymes (superoxide dismutase, catalase, and glutathione peroxidase) to increase the stress tolerance (Zolotukhin, Prazdnova, & Chistyakov, 2014). Moreover, the use of probiotics in fish farming to reduce the production cost, mortality, and thus ultimately affects the income. Several researchers have proved that probiotic feeding in fish from their first stage of life (larvae) is profitable due to diseases load is low in later stage (Table 6), but the delivery of probiotics during early stage is quite difficult. The protection of hatchling or larvae is the most challenging issue in aquaculture. So, the manipulation of probiotics and different types of food in aquaculture

**Table 6** Interaction between probiotics and different types of food in fish farming

| Fish species larvae | Probiotic feed                                                                 | Beneficiary effects                                                                 | References                                                                 |
|--------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Scophthalmus maximus | Lactic acid bacteria enriched Brachionus plicatilis                           | Resistant against wide range of Vibrio sp.                                           | Gatesoupe (1994)                                                          |
| Scophthalmus maximus | Lactic acid bacteria enriched Brachionus plicatilis                           | Promoted colonization on the gut and enhanced survival rate                         | Makridis, Fjellheim, Skjermo, and Vadstein (2000)                         |
| Sparus aurata       | Lactobacillus fructivorans and Lactobacillus plantarum enriched dry feed or live feed (Brachionus plicatilis and Artemia salina) | Enhanced colonization on the gut epithelial surface and significantly reduced the mortality rate during larval rearing and fry culture | Carnevali et al. (2004)                                                   |
| Gadus morhua        | Life feed enriched probiotic bacteria Phaseobacter galleaeensis                | Reduced the pathogenic load during larviculture                                      | D’Alvise et al. (2012)                                                   |
| Seriola lalandi     | Live feed (B. rotundiformis and B. plicatilis) and Artemia sp.) enriched with Pseudoalteromonas sp. | Enhanced survival rate of the larvae.                                               | Sayes, Leyton, and Riquelme (2018)                                       |
| Scophthalmus maximus | Bacillus amyloliquefaciens enriched Branchionus plicatilis and Artemia sinica | It improves the microbial community in live feed and ultimately conveys the beneficial effects to larvae | Jiang et al. (2018)                                                      |
| Centropomus undecimalis | Bacillus licheniformis and Bacillus amyloliquefaciens enriched feed            | Improved water quality, fish health and rearing tank environment                   | Tamecki, Wafapoor, Phillips, and Rhody (2019)                             |
Microbiota by inoculating probiotic strain and their uses is a promising alternative. However, in later stage, probiotic-enriched formulated artificial balanced diet is good for fish health and the application of it is very easy. Moreover, farmers have to be careful of three main constraints (Vadstein et al., 2018; Vine, Leukes, & Kaiser, 2006) viz., (a) leaching of feed which reduces the availability of probiotic to the host. Thus, dose standardization and regular monitoring is required. (b) Probiotic candidate confers beneficial effects to the host only when it is active or live under different appropriate environmental conditions, so farmers have to be concerned about these facts. (c) Nature of various ponds differ depending on the physicochemical parameters and natural feeds (zooplankton and phytoplankton). So, application, types, and dose of probiotics will be varied accordingly.

**Probiotics and fish gut microbial community**

Gut environment provides a favorable niche for indigenous microorganism by providing space, attachment sites, and nutrition. Balanced microbial communities are very important for maintaining gut health (Banerjee & Ray, 2017; Giatsis et al., 2016). During disease condition, the natural microbial communities in the gut are disrupted, which creates several health-related problems. Fish lives in such a condition which is surrounded by a huge population of pathogenic bacteria, fungi, and deadly virus (Egerton, Culloty, Whooley, Stanton, & Ross, 2018). Restoration of gut microbial communities through dietary probiotic supplementation is an effective method to improve fish health (Han et al., 2015). However, selection of probiotics varies greatly from one fish species to another to properly maintain the good to bad ratio of bacteria in the gut mucosal surface. Till date several bacterial candidates have been tested for probiotic potential; however, few candidates of *Bacillus* sp., *Micrococcus* sp., *Enterococcus* sp., *Phaeobacter* sp., *Shewanella* sp., lactic acid bacteria, and *Pseudomonas* sp. have gained popularity in manipulating gut flora in fish (Lobo et al., 2014; Merrifield et al., 2010a, b). In an investigation, Asaduzzaman and co-workers have reported the beneficiary effects of three probiotics (*Shewanella* sp. AFG21, *Bacillus* sp. AHG22, and *Alcaligenes* sp. AFG22) in *Tor tambroides* which are able to shift the microbial composition toward good bacterial populations (Asaduzzaman et al., 2018). Several researchers reported that probiotic significantly induced many fold gut microbiota to produce several metabolites including volatile short-chain fatty acids (VSCFs), which play a vital role in maintaining gut health in fish (Fig. 3) (Allameh, Ringø, Yusoff, Daud, & Ideris, 2017; Asaduzzaman et al., 2018; Burr & Gatlin, 2005). Researchers also reported that probiotic modulation of gut microbiota is not restricted to fish age and maturation, as probiotics confer beneficial effects to all age group ranging from larvae to adult (Merrifield & Carnevali, 2014). A previous study reported that probiotic supplemented diet in rainbow

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**Fig. 3** Effects of probiotics on metabolites production by gut microbial flora
trot was very effective to enhance the population of beneficial bacterium *Bacillus subtilis* (Newaj-Fyzul et al., 2007). They also reported that colonization of *B. subtilis* on the gut epithelial surface conferred protection (boost immunity, reduced oxidative stress, increased serum lysozyme concentration, and enhance phagocytic activity of specialized cell) against pathogenic strain of *Aeromonas* sp. The finding of Newaj-Fyzul and co-workers (Newaj-Fyzul et al., 2007) was further supported by the study conducted by Bagheri, Hedayati, Yavari, Alizade, and Farzanfar (2008), who used commercial probiotic product (Bioplus) containing a mixture of *B. subtilis* and *Bacillus licheniformis*. In the same direction, an investigation conducted in four fish species (*Poecilia sphenops*, *Xiphophorus maculates*, *Poecilia reticulate*, and *Xiphophorus helleri*) fed with *B. subtilis* containing diet and reported the population enhancement of *B. subtilis* on the intestinal mucosal surface (Ghosh, Sinha, & Sahu, 2008).

Recently, the effects of two probiotic strains *Bacillus subtilis* and *Rhodococcus* sp. have evaluated on gut microbiota of *Oreochromis niloticus* (Martinez Kathia et al., 2018). The results of their study clearly indicated a significant shifting of gut microbial community (increasing percentage of proteobacteria and bacteroidetes) in probiotic fed fish compared to control. Furthermore, study also reported that bacteria belongs to phylum proteobacteria are important members as they are involved in mineralization of organic compounds and nutrient recycling process in fish (Cardona et al., 2016). However, the gut microbiota restoration capability of two probiotics also tested in diseased black molly (*Poecilia sphenops*) treated with antibiotics (Schmidt, Gomez-Chiarri, Roy, Smith, & Amaral-Zettler, 2017). Results of their study indicated that both the probiotic candidates (*Phaeobacter inhibens* S4Sm and *Bacillus pumilus* RI06-95Sm) were able to restore the microbial community back to the normal. Among several probiotic strains, lactobacillus groups as probiotics in aquaculture have been studied extensively. It is well established that lactobacilli has high colonization property and thus retain for a longer time on the gut epithelial surface, and confer greater beneficial effects to host and gut microbiota (Merrifield & Carnevali, 2014). Researches on germ-free fish model indicated that probiotic along with environmental factors have high impact on gut microbiota modulation in term of antibody production, stress release, and resistance colonization (Kelly & Salinas, 2017). The microbial manipulating property of probiotic on gut mucosal surface depends on several external/environmental (water quality, temperature and pH) and internal (fish age, binding strength of the probiotic, duration of probiotic supplement diet, delivery system, etc.) factors. Alteration in any of these factors may hamper the probiotic efficiency. The cross talk between host and microbe on the gut epithelial surface is a complex phenomenon and is responsible to maintain a healthy environment. Restoration of gut microbiota in patient using fecal microbial therapy (microbiota collected from healthy individual) to solve several diseases is common practice in human (Aas, Gissert, & Bakken, 2003). The probiotic research in mammal including human is at peak level; however, such depth of research is still lacking in the case of aquaculture.

**Probiotics and mucosal immunity**

Apart from systemic immunity, fish possess a well-defined mucosal immunity which is very important for protection and survival. Till date, the mucosal immunity in fish has been studied mostly in teleost fish (Lazado & Caipang, 2014). Mucosa-associated lymphoid tissues (MALT) in teleosts can be divided into three broad categories: skin-associated lymphoid tissue (SALT), gut-associated lymphoid tissue (GALT), and gill-associated lymphoid tissue (GIALT). However, lymphoid tissue mucosal immunity (nasopharynx-associated lymphoid tissue (NALT)) has recently been discovered by Salinas (2015). Immunomodulation by probiotic bacteria is a vital process which confers strength to fish for combating with surrounding pathogen in the water, as well as inside the body. The mucosal secretion in fish contain a wide spectrum of anti-microbial peptides (AMPs) such as AJN-10 (Liang, Guan, Huang, & Xu, 2011), Gadscudin-1 and -2 (Browne, Feng, Booth, & Rise, 2011), Piscidin 3 (Dezfuli, Giari, Lui, Lorenzoni, & Noga, 2011), and YFGAP (Seo, Lee, Go, Park, & Park, 2012), which have direct role in pathogen inhibition (Fuochi et al., 2017; Gallo & Nakatsuji, 2011; Gomez, Sunyer, & Salinas, 2013). Skin mucus layer act as a first defence barrier in fish, as it is in direct contact with water. Among the lymphoid tissues, GALT is the most important one and interestingly in fish it lacks Peyer's patches like mammal. However, GALT contains the other important components (plasma cells, macrophages, lymphocytes, etc.), which are necessary for defense (Lazado & Caipang, 2014). It was reported that probiotic modulate the mucosal immunity in fish by increasing the population (10–30%) of granulocytes and lymphocytes cells which is related to cell mediated mucosal defense (Lazado & Caipang, 2014). Furthermore, an investigation on GALT of seabream (*Sparus aurata*) also reported that oral administration of a mixture of probiotic strains (*Lactobacillus plantarum* and *Lactobacillus fructivorans*) enhanced the production of antibody and G7' granulocytes cells (Picchietti et al., 2007). In general, plasma cells of fish produce three types of antibodies: IgM, IgD, and IgZ. The action of IgT/IgZ is thought to be associated with the gut mucosal immunity in fish (Salinas, Zhang, & Oriol Sunyer, 2011). Whereas, IgM is a general immunoglobulin responsible for combating invaded pathogen and the level of this antibody is elevated in the gut mucus in fish fed with
Conclusion and future perspectives

The current researches improvise and optimize the utilization of probiotics in aquaculture industry. Notably, the future application also looks bright due to the ever-increasing demand of probiotics for aquacultured animals. Further investigations will demonstrate the techniques to screen host specific probiotic strains from aquaculture rearing system to manage significantly its quality and functional properties. Furthermore, research should also focus on studying the effects and mechanism of action of probiotics on the reproductive performance and gonadal development of aquatic organisms in an industrial scale hatchery system. Probiotic bacteria confer a broad spectrum of beneficial effects to host, but still there are certain limitations. For example, antimicrobial compounds or bacteriocins produced by probiotic candidates against pathogenic bacteria are not species specific. Thus, strain improvement is necessary to enhance the efficiency of probiotic bacteria. There are several molecular biology techniques such as recombinant technology, mutagenesis, etc. that are available to improve the genetic makeup of the probiotic strain. However, application of these techniques is limited to probiotic candidates used for aquaculture. Future investigation must be done to solve these serious issues and to prepare effective probiotics.

Table 7 Effects of probiotics on fish mucosal immunity

| Probiotic candidates/products | Fish species | Effects on mucosal immunity/morphology | References |
|------------------------------|--------------|----------------------------------------|------------|
| Lactococcus lactis, Lactobacillus sakei, and Leuconostocmesenteroides | Oncorhynchus mykiss | Enhance phagocytic activity of gut mucosal leucocytes | Balcázar et al. (2006) |
| Pediococcusacidilactici | Oncorhynchus mykiss | Increase surface area for absorption by increasing villi length | Merrifield et al. (2010a, b) |
| GP21 and GP12 | Gadus morhua | Lower down caspase-3 and lactate dehydrogenase activity of the pathogen infected gut epithelial cells | Lazado, Caipang, Brinchmann, and Kiron (2011) |
| Bacillus subtilis FPTB13 and chinin | Catla catla | Foster the production of skin mucosal lysozyme, alkaline phosphatase, myeloperoxidase content and total protein content | Sangma & Kamiya, (2015) |
| Shewanellaputrefaciens | Sparus aurata | Enhance the activity of skin mucosal lysozyme and complement C3. Enhance the expression of nonspecific cytotoxic cell receptor protein 1 and natural killer cell enhancing factor. | Cordero, Morcillo, Cuesta, Brinchmann, and Esteban (2016) |
| Bacillus coagulans and Lactobacillusplantarum | Danio rerio | Enhance intraepithelial lymphocytes cell population. Up-regulation of TNF-α and IL-10 | Wang, Ren, Fu, and Su (2016) |
| galactooligosaccharide (prebiotic) and Pediococcusacidilactici | Cyprinus carpio | Enhance immunoglobulin concentration in skin mucus | Modanloo, Soltanian, Akhlaghi, and Hoseinifar (2017) |
| Vitace® and Primalac® | Rutuluskutum | Modulate mucosal immunity and enhance digestive enzyme activity | Mirghaed et al. (2018) |
| Lactobacillus plantarum and Cordyceps militaris spent mushroom | Oreochromis niloticus | Enhance the activity of skin mucus lysozyme and peroxidase | Van Doan, Hoseinifar, Dawood, Chitmanat, and Tayyamath (2017) |
| Bacillus amyloliquefaciens (GB-9) and Yarrowialipolytica lipase2 (YLL2) | Hybrid sturgeon (Acipenserschrenkii x Acipenser baerii) | Enhance mucus lysozyme activity and leukocytes phagocytic activity | Fei et al. (2018) |
| Lactobacillus casei and Aganicus bisporus | Danio rerio | Uregulated the expression of mucosal immune genes (TNF-α, LYZ, and IL1B) and anti-oxidant genes like SOD, CAT | Safari, Hoseinifar, Dadar, and Khalili (2018) |
Abbreviations
AMPs: Anti-microbial peptides; CFU: Colony forming unit; FAO: Food and Agriculture Organization; FDA: Food and Drug Administration; GALT: Gut-associated lymphoid tissue; GALT: Gut-associated lymphoid tissue; GIT: Gastro-intestinal tract; IFN: Interferon; IGF: Insulin-like growth factor; IHNV: Infectious hematopoietic necrosis virus; IL: Interleukin; LAB: Lactic acid bacteria; LCDV: Lymphocystis disease virus; MALT: Mucosa-associated lymphoid tissues; MAMPs: Microbial-associated molecular patterns; NALT: Nasopharynx-associated lymphoid tissue; PCR: Polymerase chain reaction; PRR: Pathogen pattern recognition receptors; SALT: Skin-associated lymphoid tissue; TNFα: Tumour necrosis factor alpha; VSCFs: Volatile short-chain fatty acids

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The authors declare that they have no competing interest.

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