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REVIEW

Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives

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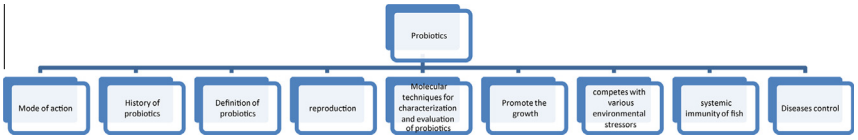
GRAPHICAL ABSTRACT



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ABSTRACT

The increase in the human population in addition to the massive demand for protein of animal origin forced the authorities to seek for additional sources of feed supplies. Aquaculture is the world worth coming expansion to compensate the shortage in animal protein. Feed in aquaculture plays an important role in the production cycle and exert threshold on both practical and economic aspects. Feed additive sectors are expanding day after day to achieve better growth and health for fish and shrimp and to meet the potential requirements of the culturists. Probiotic proved its successes in human and animal feeding practices and recently gained attention in aquaculture; it has beneficial effects in diseases control and competes with various environmental stressors as well as to promote the growth of the cultured organisms. Probiotics have the privilege to manipulate the non-specific innate immunity among fishes, hence help them into resist many pathogenic agents and are actively used worldwide. The present review is an informative compilation of the probiotics, their mode of action and their useful effects on fishes. The review also highlights the status of probiotics in aquaculture of Egypt, probiotic recent prospective for the possible role of probiotics in fish external and internal environment.

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Mai D Ibrahem works as a Professor in the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Egypt. Her researches focused on health enhancement through disease prevention rather than disease treatment, this was carried out through *Aeromonas hydrophila* vaccine production and application, ecology of viral infection, probiotics application, production and manipulating the different stressors and environmental toxicants that adversely affects fish health and immune system.

Introduction

The global production of farmed fish and shellfish has tremendously increased in the last decenniums and the growth is projected to increase [1]. The world needs for fish and fishery products are vision to expand to more than 2 million tones by 2020 [2]. At the same time, natural fisheries stocks are maximally deteriorated and stocks of many fish species are in decline attributed to illegal and over-fishing. Some wild fish species became more and more attractive as potential aquaculture species, such as tilapia (*Oreochromis niloticus*), African catfish (*Clarias garipienis*), cod (*Gadus morhua*), turbot (*Psetta maxima*), and tuna (*Thunnus* spp.) [3], hence, farming of such species can fulfill consumer demand that no longer can be met by wild capture fisheries alone. It is therefore expected that the anticipated expansion of the consumer demand for fish and fishery products will predominantly be met by aquaculture, which was projected to account for 41% of global fish production in 2015 [2]. Fishes in culture systems are humbled by various obstacles which include both infectious and non-infectious factors [4]. There is no line of demarcation between fish and their surrounding environment as fish interact involuntary with it. The fact of functional feed represents an emerging new era in aquaculture industry, where diets are designed to extend beyond satisfying the basic nutritional requirements of the cultured organisms [5]. As preventing or reducing the risk of disease is preferable to treating disease. Search for health-enhancing additives as probiotics is of premium importance. Probiotics were originally proposed as supplements for the human diet [6]. The tradition of using probiotic microorganisms to promote human and animal health is now backed by strong scientific evidence for some clearly defined and well characterized strains [7]. In aquaculture, probiotics have been proposed as a major nutritional factor influencing gastrointestinal physiology and function [8]. This development introduces many challenges, but also creates new opportunities for food and nutrition scientists to improve food quality and develop new products with specific health benefits for different hosts. The administration of probiotics appears to be a very promising research area for nutrition, biological control and disease prevention in aquaculture [9].

History and definition of probiotics

The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) defined probiotics as living microorganisms, which, once administered in appropriate amounts, confer a health profit on the host. Stimulation or improvement of the defense system may be a mode of action by that probiotic exerts a helpful impact to the host [10]. Probiotics definition was initially commissioned to Lilly and Stilwell [11] who expressed probiotics as substances secreted by one organism that stimulate another organism. The nomenclature was then employed in 1971 by Sperti [12] who delineated tissue extracts that stimulate microbes' growth. The word was later described by Parker [13] in 1974 that advanced the definition by adding the word organisms, thereby describing probiotics as ''Organisms and substances that exert beneficial effects on the host by balancing its intestinal microbes.'' The definition was re-improved by Fuller [14] in 1989 whose explanation was as ''a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance.'' The term, probiotic was also defined by Gismondo et al. [15] as ''for life,'' originating from the Greek words ''pro'' and ''bios.'' Recently, scientific data proved that the application of probiotic to the host get beyond its effects on the intestinal region to other desired effects [16]. Gram et al. [17] broadened the definition by removing the restriction to the improvement to the intestine: ''a live microbial supplement which beneficially affects the host animal by improving its microbial balance.'' Moreover, Salminen et al. [16] addressed probiotics as any live and dead microbes or their cellular fractions exerted beneficial effects on the host. Biswas et al. [18] recorded an *in vitro* modulation of immune response in the head kidney cells, organ responsible for immunity, of the Japanese puffer fish (*Takifugu rubripes*) after supplementation of heat-killed probiotics isolated from the Mongolian dairy products.

Definition of probiotics in aquaculture

The nature of the aquatic species and their intimate interaction with environment forced to a more complicated and precise definition for probiotics, in aquatic hosts, there is no line of demarcation between microbial community inside and outside the host, this is because of the constant interaction with the ecosystem and the host functions. Cahill [19] proved that the bacteria present in the aquatic environment influence the composition of the gut microbiota and vice versa. In aquatic environments, the probiotics must be defined to cope with the nature of this sector. Verschuere et al. [20] suggested the probiotics to be outlined as live microorganism adjunct that have useful effects on the host by modifying the host-associated or close microorganism community, by guaranteeing improved use of the feed or enhancing its nutrition worth, by enhancing the host response toward malady, or by rising the quality of its close setting. Apart from the demand of the probiotic to be a live culture, this definition may be a protracted approach of describing a probiotic, so a probiotic is an entire or elements of a micro-organism that is helpful to the host health. Lately, probiotic was outlined as a live, dead or element of a microbic cell that once administered via the feed or to the rearing water advantages the host by rising disease resistance, health standards, growth performance, feed optimization, stress and tolerance response, that is possibly achieved via rising the microbic balance of the hosts or the close surroundings [15,16,21,22]. Taoka et al. [23] investigated the impact of live and dead probiotic cells, introduced either through food or in rearing water of closed re-circulating system, on the

non-specific immune system of *Oreochromis niloticus*. The probiotics treatment increased the non-specific immune parameters like lysozyme activity, migration of neutrophils and plasma bacteriocidal activity, leading to improvement of resistance to *Edwardsiella tarda* infection. Specifically, per os administration of live cells perceived to be more practical compared with alternative probiotic treatments like in food administration of dead probiotic cells or provide of live probiotic cells to the rearing water. The viability of probiotic microorganism may be a key issue to induce additional potential effects of probiotics used for fish production. The intensive interaction between the culture surroundings and the host in cultivation implies that vast of probiotics are obtained from the surroundings culture and in some way from feed, as suggested by the definition of Fuller [14]. Therefore, a changed definition was projected by Verschuere et al. [20] that allowed a broader application of the term "probiotic" and addresses to the objections created earlier. A probiotic is outlined as a live microbic adjunct that incorporates a helpful impact on the host by modifying the host-associated or close microbic community, by making certain improved use of the feed or enhancing its nutritionary worth, by enhancing the host response toward illness, or by up the standard of its close surroundings. Probiotics might embrace microbic genera that serve repressive actions as forestall harmful pathogens from proliferating into the intestinal tract, forestall infective agent attachment on the superficial structures, and within the culture surroundings of the esthetic species, probiotic supplementation in feed aids in digestion [24], stimulate the immune system of the host [25]. Probiotic genera improve water quality [26]. It is important to indicate that microorganism that is delivering essential nutrients to the esthetic species while not exerting a lively perform within the host or in its surroundings should not be thought of as probiotic [27]. Once the host or its surroundings encompasses a well stable microorganism community, the appliance of the chosen probiotic microorganism typically must be applied on a daily scheduled mode so as to attain the specified positive effects desired from it. Probiotics contribute considerably to the health and zoo-technical performance in a nutrition manner, and it is generally not possible to separate feeding of aquatic organisms from environmental management.

Modes of action

There have been several hypotheses for probiotics mode of actions in the host, most of the following actions have been observed during *in vitro* experiments; however there are needs to emphasize that the efficiency of a selected probiotic *in vitro* may significantly change when administered to the host in its natural environment, probiotic organisms are influenced by more complex factors among which selective ingestion [9], the manipulation in the intestinal tract [24] and the more complex microbial interactions and/or nutritional environment are of premium importance. We can rely on the aforementioned factors in the success or failure of the probiotic in maintaining its *in vivo* physiology. In general, there is still an incomplete correlation bond between *in vitro* and *in vivo* experiments to explore the claimed mechanisms of probiotic actions. The following are reviews for the different action modes and applications of probiotics in aquatic hosts.

Competitive exclusion

Bacterial behaviors vary according to their interactions. Antagonism is a natural phenomenon; as it comforts the balance between competing beneficial and potentially pathogenic microorganisms. The gastrointestinal tract microbiota of aquatic animals can be radically modified by the presence of other microorganisms. Therefore, antagonism constitutes a viable tool to reduce or eradicate the presence of opportunist pathogens.

Competition for adhesion sites and colonization

Prevention of disease occurrence can be awaited through inhibition of etiological agents from gut colonization and reaching their target organs, thus interfere with disease cycle completion. Possible mode of action of bacterial probiotic is competition for adhesion sites in the gut or other tissues in the digestive tract which antagonist the colonization mechanism of the pathogenic bacteria and prevents the adhesion [15].

Successful probiotic bacteria are usually able to colonize and adhere to the intestinal mucosa as it prevents the place establishment of pathogens, in addition it stimulates their removal from the infected intestinal tract [24]. Vine et al. [24] demonstrated a competitive exclusion effect with five probiotics versus two pathogens on fish intestinal mucus. They found that the presence of one of the probiotics on the mucus inhibited the attachment of one of the pathogens tested. Balcazar et al. [28] recorded that the method of probiotic establishment can be summarized in three steps, attraction, association into the surface secreting gel and ended by attachment to animal tissue cells. Adhesion and organization to the tissue layer surfaces are attainable protective mechanisms against pathogens through competition for binding sites and nutrients, or immune modulation. They believe the influencing factors for the colonization of microorganisms into Host-related factors: body temperature, redox potential levels, enzymes, and genetic resistance, and microbe-related factors: effects of antagonistic microorganisms, proteases, bacteriocins, lysozymes, hydrogen peroxide, and the formation of ammonia, diacetyl, and alteration of pH values by the production of organic acids. Gatesoupe [29] recorded that a microorganism is able to colonize the alimentary canal when it can persist there for a long time, for example, addition of *Bacilli* spp. into the water for 20 days, result in its domination for up to 500th of the total normal micro-flora. Lara-Flores and Guzman [30] tested the attachment ability of some bacteria, *in vitro* and *in vivo* and suggested that a potential probiotic can dislocate the pathogenic bacteria through its ability to attach to the mucus; this character is highly associated with the competition for essential nutrients and space. Lactic acid producing bacteria, Gram-positive and Gram-negative bacteria superposed as probiotic for their ability of adhesion. Divya et al. [31] proved the colonization ability of probiotic bacteria namely *B. coagulans*, *B. mesentericus*, and *Bifidobacterium infantis* in the gut of *Puntius conchonius*, a freshwater ornamental fish. The results also cleared the significant competitive inhibitory effects of the probions to the pathogenic gut microbes.

Competition for nutrient and energy sources

The hypothesis of competition on energy sources and adhesion sites helps in the selection phenomena can be proposed as one

mode of action for probiotics. Theoretically, competition for nutrients can play an important role in the composition of the microbiota of the intestinal tract or the surrounding environment of cultured aquatic species [16]. Increasing some strains of bacteria such as *Lactobacillus* and *Bacillus* by way of a probiotic may thereby decrease the substrate available for other bacterial populations [32]. The impact was not solely caused by extra cellular product, however conjointly needed the live microbial cell, though further testing is needed, they hypothesized that the protecting impact most likely resulted from competition for energy sources and for adhesion sites.

Competition for iron

Siderophores are bacterial products that have affinity for the uptake and transport of ferric ion [33], iron is an essential element for most organisms, serving as a cofactor for various enzymes. Siderophores also play important roles in bacterial chemical communication [34]. In the marine environment, some bacteria acquire siderophore produced by the other strains for their own growth [35] in a process known as siderophore piracy [36]. It was assumed that during the ultimate competition for iron, bacteria can aggravate the siderophore biosynthesis and utilization machineries to overcome siderophore piracy or to enable use of siderophores for specific inter–strain chemical communication [37,38]. Siderophores are low molecular weight (1500), ferric ion-specific chelating agents which can dissolve precipitated iron and make it available for microbial growth. The biological value of siderophores resides in their capacity to capture the essential nutrient from the environment and deprive competitors of it [39,40]. Successful bacterial pathogens are able to compete successfully for iron in the highly iron-stressed environment from the tissues and body fluids of the host Verschuere et al. [20]. Pybus et al. [41] investigated an *in vitro* study for thirty strains of *V. anguillarum* as effective probiotics against *V. ordalii*, a common pathogen of salmon, by the deferred-antagonism test. Only one strain (*V. anguillarum* VL4335) inhibited strains of *V. ordalii in vitro*, and this effect was diminished as iron salts were added to the culture medium, indicating that the growth inhibition was conditioned with iron deficiency. Gatesoupe et al. [42] recorded that the addition of the bacterial siderophore, deferoxamine to *rotifers* increased the resistance of turbot larvae to infection with the pathogenic *Vibrio* spp. The addition of a siderophore producing *Vibrio* strain added an additional protection to the turbot larvae. Gram et al. [17] recorded that iron could be a limiting factor for bacterial culture growth, a siderophore producing probiotic could deprive potential pathogens of iron as was tested using *P. fluorescens*, grown in iron free culture, inhibited growth of *V. anguillarum*, whereas the supernatant from iron-enriched cultures did not. The same finding was recorded by Smith and Davey [43] when studied the inhibitory action of *P. fluorescent* F19/3 toward *A. salmonicida* with and without iron enriched culture.

Digestion enhancement

Taking benefit from the experiences of non-aquaculture industries, and for safety reasons, some of the pre tested lactic acid bacteria and yeasts have been quickly accepted as probiotics in aquaculture. The most commonly used organisms in probiotic preparations are the lactic acid bacteria; these are found in large numbers in the gut of healthy animals, they are regarded as safe (GRAS status) in the words of the American Food and Drug Administration (FDA) [44].

The alimentary tract of fishes represents an interface between the external environment and the body. Its complex poly microbial ecology interacts with the internal and external environment and has an important influence on health and disease. The intestine is a complex multifunctional organ. In addition to digesting and absorbing feedstuff, it is critical for osmotic balance, endocrine regulation of digestion, metabolism and immunity. The fish alimentary microbiota is favored with a wide range of microbes with an increase in population, density, types and complexity of interactions, bacteria are among the most representative microbes [21]. The digestion processes of aquatic animals can be enhanced by addition of some microorganisms that may participate in the digestion processes, this can be done through production of extracellular enzymes, such as proteases, lipases, and/or have intended abilities for supplying necessary growth factors as fatty acids, vitamins and others [9,24]. Microbiota of adult penaeid shrimp (*Penaeus chinensis*) may serve as a supplementary source of vitamins, essential amino acids and enhance microbial activity in the digestive tract [45]. Lara et al. [46] observed a high activity for alkaline phosphatase in Nile tilapia (*Oreochromis niloticus*) when served probiotic in the diet, the result reflected the development of brush border membranes of enterocytes that were stimulated by probiotics, this can be an indicator of carbohydrate and lipid absorption and explain the higher weight gain and the best feed conversion rate. Wang et al. [45] recorded that microbiota may serve as a supplementary source of food, in addition, the microbial activity in the digestive tract may be a source of vitamins or essential amino acids. Lara flores et al. [47] recorded that the uses of lactic acid bacteria and yeast as probiotics in finfish have demonstrated beneficial effects on the growth performance and feed efficiency. These positive effects may be attributed to the capacity of the probiotic to stimulate and/or produce some enzymes on the intestinal tract. Haroun et al. [48] recorded that after the probiotic settlement in the intestine, it start to consume carbohydrates for self-growth and produce a range of digestive enzymes as amylase, protease and lipase which improve digestibility, in return a higher growth rates due to stimulation of a pre-digestion of secondary compounds and intestinal free disorders. Ziaei-Nead et al. [49] examined the effects of *Bacillus* spp. on *F. indicus* at different shrimp stages and recorded a significant difference in the growth rate in comparison with control groups. Tested shrimp ponds showed significantly higher activity of amylase, total protease, and lipase with a significantly higher apparent digestibility of some essential nutrients as phosphorus.

Growth in mucus

For bacteria to be a probiotic, it must be favored with the ability to fast growth, maintain in the gastro-intestinal tract and to compete for attachment sites, bacteria can only produce metabolites during the stationary growth phase [50], which may not occur in the gut due to constant flushing [51]. Any inability to compete for growth in the mucus of the gut wall suggests that these bacteria may not multiply sufficiently fast

to compensate for being flushed from the mucus during gut evacuation; hence it will not deliver true probiotic bacteria. The *in vitro* studies may create a false impression of the ability of probiotics to inhibit pathogens, the *in vivo* Screening for organisms with antagonistic abilities toward pathogens is an ultimate goal for scientists, Vine et al. [24] advised a an *in vitro* ranking index whereby candidate probionts grownup in the intestinal mucus samples were accordingly profiled to: lag-period and specific rate of growth. The strategy would vest the speedy screening of candidate probiotics, their results were debated by several authors as Sugita et al. and Robertson et al. [52,53] who conditioned the success of probiotics by testing its reactions both *in vivo* and *in vivo* and inspect its receptivity of excluding different pathogens.

Attachment to mucus

The probiotic concept has been widely applied for health promoting in farm animals, pets and aquatic animals guided by the success of probiotics in human's medicine. It appears that attachment and the production of antimicrobial compounds by lactic acid bacteria are the critical factors in excluding pathogens [54,55]. Attachment of lactic acid bacteria to the mucus layer may serve as the first barrier of defense against invading pathogenic bacteria [56], so it is therefore regarded as a prerequisite for colonization [57,58] and is important in the stimulation for the host's immune system [59–61]. The superior ability of bacterial pathogen to attach has been related to the virulence which is considered the first step of bacterial infection [62,63]. Research has been conducted on the ability of probiotics to attach to the intestinal mucus of fish [24,64,65]. Attachment ability is not necessarily host/probiont-species-specific but rather dependent on the bacterial strain [66]. Therefore, potential probionts should be tested for their ability to adhere to mucus *in vitro* and build on this result to move to the *in vivo* attempts, as the candidate probiotic may be transient *in vivo* and consequently not contribute to the health of the host organism.

The role of probiotics in growth enhancement

Among the various benefits of probiotics in aquaculture, the growth enhancement of the cultivated species is of premium importance. Typically this benefit is postulated to occur via the gut and is assumed to be as a result of bacterial species colonizing the gut of the host and bringing about a change in the bacterial composition of the gut that in some way benefits the health of the host [9]. There have been many speculations for this positive phenomena, probiotic products increase the appetite, improve digestibility [21]. Balcazar et al. [9] proved that probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time. Limiting factors control the colonization process from which body temperature, species genetic resistance, enzyme levels and water quality. Probiotic supplementation increase the absorbance efficiency of feeds [48], in this contest, several studies proved that the ability of the probiotic to compose proteases, amylases, and lipases, vitamins, fatty acids, and amino acids as a cofactor for the digestive process aid the improvement in the growth performance [9].

The use of probiotics as growth promoters in edible fishes has been reported. A probiotic *Streptococcus* strain was supplemented to the diet of Nile tilapia, *Oreochromis niloticus*, a significant increase in the content of crude protein and crude lipid was recorded, also fish weight has boosted from 0.154 g to 6.164 g in 9 weeks culture period [47]. In a study conducted by Standen et al. [67] *Pediococcus acidilactici* was evaluated as probiotic in a 6 weeks feeding trial on Nile tilapia, *Oreochromis niloticus* under a non-challenge conditions, results proved an improvement in intestinal health, growth performance and feed utilization and other zootechnical parameters in comparison with the control group ($P > 0.05$). In another study, Pirarat et al. [68] exploded the use of lactic acid bacteria from human origins as a probiotic supplementation in diet of tilapia (*Oreochromis niloticus*) on growth performance, gut mucosal, humoral and cellular immune response. The results showed that supplementation of *L. rhamnosus* reinforce both the intestinal structure through the increase in villous height in all parts of proximal and middle part of intestine, thus improving absorption, and the intestinal immune functions in tilapia. Jatoba et al. [69] assessed the dietary supplementation of the probiotic *Lactobacillus plantarum* in a polyculture system of Nile tilapia, *Oreochromis niloticus* and marine shrimp (*Litopenaeus vannamei*) for 12 weeks. Tilapia under experiment revealed higher values for feed utilization, net yield and final weight gain. The beneficial bacterial number represented as lactic acid bacteria was increased, whereas, viable heterotrophic bacteria counts were reduced in the gut of fish and shrimp fed the probiotic-supplemented diet. Zhou et al. [70] proved higher significant ($P < 0.05$) increases in final weight, daily weight gain, and specific growth rate of tilapia supplemented with *B. coagulans* B16 and *R. palustris* G06 as water additives in comparison with those fed with *B. subtilis* B10. Abd El-Rhman et al. [71] used the homologous strains *Micrococcus luteus* and *Pseudomonas* spp. isolated from isolated from gonads and intestine of Nile tilapia, *Oreochromis niloticus*, to evaluate its probiotic activities on growth-performance and survival rate. Results recommended using *M. luteus* as a probiotic *in vivo*.

In *Cyprinus carpio*, the dietary supplementation of chitosan oligosaccharides and *Bacillus coagulans*on in diet of *koi* (*Cyprinus carpio koi*) resulted in growth improvement [72]. The effect of baker's yeast (*Saccharomyces cerevisiae*), in the diet of the Indian major carps Rohu (*Labeo rohita*) was investigated using 4 groups which received four different diets for 8 weeks: a formulated diet as control diet and the same diets supplemented with 5%, 7.5% and 10% baker's yeast as an experimental diets. Growth parameters such as ADG, SGR, FCR and PER were evaluated during experimental trial. The results showed that, yeast cell wall feeding has a positive correlation with growth parameters. These results support the possible use of baker's yeast as growth promoters in common fish diets [73].

In diets of catfish, Abdelhamid et al. [74] evaluated the dietary beneficial effects of patent local probiotic T-Protphyt 2000 (consist of 5% dried fermentation products of *Aspergillus oryzae*) when added to the diet at graded levels (0, 1, 2, 3 g kg^{-1} diet). They found that diet containing 1 g kg^{-1} reflected the best feed utilization and in turn, growth parameters. Increasing the probiotic level increased fish carcass protein, fat and energy contents. Also, the aforementioned concentration led to improvement of most histometric characteristics

of the dorsal muscles of African catfish compared with the control and other treatments. An *in vivo* study was carried out by Dohail et al. [75] to evaluate the effects of *Lactobacillus acidophilus* on the growth performance in African catfish *Clarias gariepinus* fingerling. The results showed significant elevation in the growth performance parameters, specific growth rate, relative growth rate, protein efficiency ratio, feed conversion ratio and survival rates in comparison with the control.

In diets of catfish, Abdelhamid et al. [74] evaluated the dietary beneficial effects of commercial probiotic T-Protphyt 2000 (consist of 5% dried fermentation products of *Aspergillus oryzae*) when added to the diet at graded levels (0, 1, 2, 3 g kg^{-1} diet). They found that a concentration of 1 g kg^{-1} reflected the best growth and feeding efficiency parameters as well as increases in fish carcass protein, fat and energy contents. Also, the aforementioned concentration led to improvement of most histometric characteristics of the dorsal muscles of African catfish compared with the control and other treatments. An *in vivo* study was carried out by Dohail et al. [75] to evaluate the effects of *Lactobacillus acidophilus* on the growth performance in African catfish *Clarias gariepinus* fingerling. The results showed significant elevation in the growth performance parameters, specific growth rate, relative growth rate, protein efficiency ratio, feed conversion ratio and survival rates in comparison with the control. Queiroz and Boyd [76] applied Biostart, a commercial bacterial inoculums of *Bacillus* spp., into three channel catfish *Ictalurus punctatus* ponds, they aimed to study the effects of this product on fish survival, growth, production and improvement in water quality. There were significant increases in survival and net production and growth in ponds received the *Bacillus* spp. than in controls. The addition of product derived from the outer cell wall of *Saccharomyces cerevisiae* (Bio-Mos®), proved to have a positive influences on growth and survival rates of Channel Catfish Challenged with *Edwardsiella ictaluri* [77].

In marine fish species, the *bacillus* strains that make up the pre commercial Sanolife commercial products were selected for their ability to improve performance in the on growing marine species, a trial was carried out with Japanese flounder in a commercial recirculation system. Flounder received the *Bacillus* mixture in two separate methods, either by mixing with food or by adding it directly in water. Results revealed that the survival rate, FCR and weight gain were markedly improved each month in the 2 month experimental period [78]. Nikoskelainen et al. [79] investigated the potential probiotic properties designed for human medicine, six lactic acid bacteria (LAB) *Lactobacillus johnsonii* La1, *Bifidobacterium lactis* Bb12, *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus bulgaricus*, *Lactobacillus casei Shirota*, and *L. rhamnosus LC* 705, and one for animal use, *Enterococcus faecium Tehobak*, for use as a fish probiotic. The results encouraged the use of *L. rhamnosus* ATCC 53103 in fish culture as it evoked the premium results in growth performance, pathogen inhibition and mucosal adhesion characters. Lombardo et al. [80] investigated the effects of dietary probiotic administration on the marine *Fundulus heteroclitus* and the effects of such brood stock dietary treatment on the growth and survival of the new progeny. *Lactobacillus rhamnosus* IMC 501® was administered daily as a feed additive, at a final concentration of 10^6 cfu ml^{-1} for 8 days. The biometric parameters of broad stock (body weight, BW; total length, TL) and the survival rates of the larvae were measured in addition to other gonadal growth parameters. The results demonstrated the beneficial effects of probiotics on the mean BW and TL which were significantly higher only at 30 days post-hatching (dph) while no effects was recorded concerning larval studies. The authors recommend applying *L. rhamnosus* IMC 501® into marine fish diet. Additional investigations are needed to manipulate the use of probiotics as nutritional and immunological mediated factors on embryo and larval growth and development. The use of 0.5 g of *Bacillus cereus* strain in juvenile common dentex *Dentex dentex* L. food resulted in an increase in fish growth as a sequel of feed utilization improvement [81].

Yeasts are enchanted by a vast of probiotic characteristics, Yeasts do not seem to be plagued by antibiotics. This can be advantageous in probiotic preparations used for preventing disturbances within the self-microflora in presence of bactericide metabolites. Strains of yeast and *Debaryomyces hansenii* isolated from salmonids are shown to localize and grow in fish intestinal mucus. The probiotics yeast *Debaryomyces hansenii* HF1 are employed in larval culture of European bass, *Dicentrarchus labrax*. This probiotic has the flexability to provide spermine and spermidine, 2 polyamines concerned with the differentiation and maturation of the digestive tube in mammals. Additionally, *Debaryomyces hansenii* secretes digestive enzyme, amylase and trypsin that aid digestion and growth in ocean bass larvae [82]. On contrast to the previous results, Cerezuela et al. [83] studied the possible changes produced due to the use of administration of inulin and *Bacillus subtilis* as synbiotic in gilthead sea bream (*Sparus aurata* L.) intestinal morphology and microbiota. In an *in vivo* study, Gilthead sea bream were fed diet containing *B. subtilis* 10^7 cfu g^{-1} + inulin 10 g kg^{-1}, in addition to 2 more groups were solely fed on either *B. subtilis* 10^7 cfu g^{-1} or inulin 10 g kg^{-1} for 4 weeks. Significant differences in the signs of intestinal damage were detected by the morphometric study in the groups fed the synbiotics. All of the observed alterations were present only in the gut mucosa, the intestinal morphometric study revealed no effect of inulin or *B. subtilis* on the absorption region of the intestine. Furthermore, experimental diets caused a significant decrease in bacterial diversity resulted in important alterations in the intestinal microbiota, as demonstrated by the specific richness, Shannon, and range-weighted richness indices. The observed histological alterations manifested by different signs of gut edema and inflammation that could compromise their body homeostasis, In addition to the previous results, Cerezuela et al. [84] studied in a 4 weeks feeding trial the effects of dietary supplementation of *Tetraselmis chuii*, *Phaeodactylum tricornutum* microalgae and *Bacillus subtilis* probiotic single or combined on histology and microbial ecology in gilthead seabream (*Sparus aurata*) intestine. Results proved significant signs of intestinal damage, morphological alterations as viewed by light and electron microscopy, lowering in the number of goblet in addition to widening in the intercellular spaces and large vacuoles in enterocytes in all the tested groups. No effect was recorded on the intestinal absorptive area on using microalgae or *B. subtilis*. A significant reduction in microvilli height was recorded due to administration of diets containing *B. subtilis*. Moreover, the tested diets caused alterations in the intestinal microbiota by a significant decrease in bacterial diversity. More physio-functional studies are needed to correlate the nutritional and

immune aspects of fish gut. On genome level, six bacterial strains isolated from well-performing live food cultures were identified by sequencing fragments of their 16S rDNA genome to the genus level as *Roseobacter* spp., *Shewanella* spp., *Ruergeria* spp., *Paracoccus* spp., *Aeromonas* spp. and *Cytophaga* spp.

Numerous studies have shown that the application of probiotics can improve feed conversion, growth rates and weight gain of salmonids [85]. Application of *B. subtilis* and *B. licheniformis* resulted in significant improvement of rainbow trout fry feed conversion ratio (FCR), specific growth rate (SGR), weight gain and protein efficiency ratio (PER) after 2 months feeding trial [86]. Similar results were obtained using *Enterococcus faecium*, *B. subtilis* and *B. licheniformis*, when provided for 10 weeks in salmonids diet [87]. Barnes et al. [88,89] noted significant improvements in Rain bow trout, *Oncorhynchus mykiss* survival and growth when diets were incorporated with *S.cerevisiae*-based fermented yeast during the first months of feeding period.

In rainbow trout aquaculture, infectious diseases are the master constrain of economic losses. Probiotic supplementation was tested in respects to gut microbiota enhancement and improved growth of juvenile rainbow trout (*Oncorhynchus mykiss*). Ramos et al. [90] evaluated the dietary supplementation of multi-species (A: *Bacillus* spp., *Pediococcus* spp., *Enterococcus* spp., *Lactobacillus* spp.) and single-species probiotics (B: *Pediococcus acidilactici*) on growth performance and gut microbiota of rainbow trout (*Oncorhynchus mykiss*) in comparison with controls. Gut microbiol index was analyzed at the end of 96 days test days using 16S-DGGE. Differences in gut microbial profiles were assessed. Weight gain was significantly improved as well as changes in the gut microbial composition in fish fed diet containing *Bacillus* spp., *Pediococcus* spp., *Enterococcus* spp., *Lactobacillus* spp. for 56 days feeding relative to the controls. It was concluded that *Bacillus* spp., *Pediococcus* spp., *Enterococcus* spp., *Lactobacillus* spp. and *Pediococcus acidilactici* are a suitable probiotic candidate for growth of juvenile rainbow trout (*Oncorhynchus mykiss*). Another study was performed by Burbank et al. [91] who conducted an *in vitro* screening for 318 bacterial strains, isolated from the rainbow trout, *Oncorhynchus mykiss* (Walbaum) gastrointestinal (GI) tract. The strains were tested for their ability to inhibit growth of *Flavobacterium psychrophilum*, and to survive in rainbow trout bile. The result revealed a total of 16 bacterial isolates to be identified as probiotic candidates as it manage to survive the bile in the GIT and control *F. psychrophilum* as one of rainbow trout specific etiological agent.

Sole fish is a palatable highly demanded fish by consumers, although it is very difficult to farm, sole recently proved a continuous success in north marine water rearing system. A number of research papers handled the idea of raising sole fish under umbrella of probiotics. Chabrillon et al. [92] evaluated four bacterial families namely, members of the *Vibrionaceae* and *Pseudomonodaceae* and the genus *Micrococcus*, isolated from sea bream, for their adhesive ability to skin and intestinal mucus of farmed Senegalese sole, *Solea senegalensis*, as well as their antagonistic action to *Vibrio harveyi*. Interactions of the four isolates with *V. harveyi* in respect of adhesion to skin and intestinal mucus under exclusion, competition and displacement conditions were studied. The tested isolates showed higher adhesion ability to fish mucus than *V. harveyi*. The *in vivo* probiotic potential of the isolates was assessed by oral administration followed by challenge with the pathogenic *V. harveyi* strain Lg14/00. After challenge the mortality of the tested fish was significantly lower in comparison with control. This study demonstrate the ability of probiotic to interfere with attachment of pathogens, through the adhesion to host surfaces, are suitable criteria for selection of candidate probiotics for use in the culture of *Senegalese sole*.

In examples of growth improvement in ornamental fishes, in guppies, *P. sphenops*, *Poecilia reticulata*, and swordtail, *X. maculates*, *Xiphophorus helleri*, the incorporation of intestinal isolate of *Bacillus subtilis*, isolated from *Cirrhinus mrigala* into their diet for 50 and 90 days has been evaluated. The growth of the tested fish was increased as length and weight of the ornamental fishes was improved, the elevated specific activities of proteases and amylases in the digestive tract was reflected as a significant increases in growth and survival of *Xiphophorus* and *Poecilia* [93]. In Clownfish, a study was performed to explore if probiotic addition would improve larval development within the false percula clownfish, *Amphiprion ocellaris*, and to estimate any molecular responses following probiotic exposure. The *rhamnosus* IMC 501 was supplied from the onset of feeding post-hatch to clownfish larvae by live prey and into rearing water (group 1) and solely by live prey (group 2). The weight was duplicated in both larvae and juveniles of clownfish under test received the probiotic via live prey and in the rearing water. Additionally, development was accelerated with metamorphosis occurring 3 days earlier in fingerlings treated with probiotic. The molecular biomarkers tools supported the quicker growth observation. A significant increase in gene expression of growth factors (myostatin, peroxisome proliferator-activated receptors alpha and beta, insulin-like growth factors I and II, vitamin D receptor alpha, and retinoic acid receptor gamma) when probiotic was supplied with the aforementioned methods. The molecular tool marker allows understanding the mechanisms responsible for probiotic enhancement in fish development [94]. Probiotics also have been tested successfully in shellfish culture. Macey and Coyne [95] used 3 locally isolated probiotic strains (bacteria and yeast) from intestinal tract of abalone (*Haliotis midae*). A significant increases in the survival and growth rates were recorded in abalone supplemented with the isolated probiotics mixed diet in comparison to the controls. In addition, abalones nutritionally supplemented with probiotics had a significant resistance to pathogenic *Vibrio anguillarum* compared to untreated control.

In white shrimp *Litopenaeus vannamei* and *Fenneropenaeus indicus* vast strains of *Bacillus* have been tested as probiotics in order to improve dry matter digestibility, phosphorus, and crude protein. Consequences of *Bacillus* administration with a dose of $50\ g\ kg^{-1}$ feed revealed higher growth sizes [96]. Other research has suggested the importance of managing the probiotic in all ontogenetic stages of the shrimp to generate a constant effect on the production of digestive enzymes [97]. In *Macrobrachium rosenbergii* culture, *Lactobacillus sporogenes* was fed as bio-encapsulated probiotic via *Artemia*, A significant improvement in growth rate and feed efficiency ration of was recorded in the post-larvae stage [98]. In order to develop a potent endogenous probiotic from shrimp, screening of digestive canal bacteria of health *Litopenaeus vannamei* resulted in four species, they were identified as *Bacillus megaterium* BM1, *Bacillus firmus* BM2, *Actinobacillus* spp. BM3 and *Pseudomonas stutzeri* BM4. *B. megaterium* BM1 was the

ideal probiotic candidate for enhancing growth on *L. vannamei*, it resulted in production of digestive extra cellular enzymes and a premium value of steady growth rate. Concentration of 10^6 cells g^{-1} diet from *B. megaterium* BM1 in an *in vivo* study resulted in beneficial effects for the growth and feed utilization of *L. vannamei* [99].

Production of inhibitory substances

Probiotic microorganisms are favored with the ability to inhibit or even eliminate some potential pathogenic bacteria, this can be accomplished through production of inhibitory biological substances such as antibiotics, antibacterial substances, siderophores, bacteriolytic enzymes, proteases and protease inhibitor, lactic acid and other organic compounds like bacteriocins, hydrogen peroxide [100] and butyric acid production [101].

The production of antagonistic or inhibitory compounds

The production of antagonistic or inhibitory compounds against pathogenic or any other microflora is a proposed mode of action for probiotics. Although *in vitro* results of inhibition do not guarantee the *in vivo* results, due to a multifactor equation which can be summarized in host, pathogen, probiotic strain and environment factors [102–104]. Riquelme et al. [105] demonstrated that bacteria with antagonistic activity against other microorganisms were present in low quantities (2% of the total microflora) in the larval rearing environment of the Chilean scallop, *Argopecten purpuratus*, but may contribute up to 21% in microalgae monocultures Lodeiros et al. [106]. Once these bacteria enter the gastrointestinal tract, they dominate the digestive tract [107]. The probiotic *Pseudomonas fluorescens* AH2 retain effective antimicrobial products even after 7 days as recorded in an *in vitro* study [103].

Antagonism may not only be limited to other bacteria. Maeda et al. [108] isolated *Pseudoalteromonas undina*, VKM-124, which had vibrio-static activity and inhibited the cytopathic effect on *prawn epithelioma papillosum cyprini* cells. In addition, *P. undina* VKM-124 improved larval survival by giving the larvae a protection against *Baculo-like viruses*, *Irido virus* and *Sima-aji Neuro Necrosis Virus* (*SJNNV*) when added to prawn (*Penaeus* sp.) and sea bream (*Sparus aurata*) larval tanks. It is attainable that *in vivo* the probiotic activated the immune system of the exposed organism, thereby reducing the virus infection. More studies ought to be conducted to verify whether or not a decrease in infectious agent count is attributable to direct antagonism or via stimulation of the immune system.

Antimicrobial actions

Antibiotic production

There have been records for chemical components that are naturally isolated and exerted inhibitory activities against a wide array of Gram-positive bacteria. Trischman et al. [109] detected two new bicyclic peptides, Salinamides A and B, in a study on *Streptomyces* isolated from the surface of a jelly fish; these compounds have exhibit activity against an array of Gram-positive bacteria. Gierard et al. [110] recorded also the production of a novel cyclic deca-peptide antibiotic lotoatin-B from *Bacillus* spp. that was isolated from marine worm, this antibiotic inhibits the growth of methicillin-resistant *Staphylococcus aureus* and vancomycin resistant *enterococci*. Aotani et al. [111] produced lymphostin antibiotics from *Streptomyces* spp. which has the inhibitory action for other pathogenic bacteria. Ohtake et al. [112] found carbapenem as antibiotic product from different species of *Streptomyces*. Acebal et al. [113,114] detected large numbers of antibiotics from marine bacteria as lotoatins from *Bacillus* spp., agrochelin and sesbanimides from *Agro-bacterium*, 5-indomycinone and dihydrophenomycin methyl ester from *Streptomyces* spp. Rezanka and Dembitsky [115] recorded that antibiotic production has recently been found to be produced by a variety of organisms present in the marine surface environment as tunicates, sponge and bacteria.

Actinobacteria are treasured by thousands of biologically active secondary metabolites. *Streptomycetes* group are considered economically vital as 50–55% of antibiotics are created by this genus. The environmental and circumferential role of *Actinobacteria* in the marine ecosystem needs to be spotlighted as a probiotic in aquaculture [116].

Bacteriocins are proteins produced by certain types of bacteria that can antagonize other species which are related to the producer bacterium. Lactic acid bacteria and *Bacillus* are among the most common known to produce these compounds that may inhibit the growth of competing bacteria [117,118]. Bacteriocins are categorized into four classes: class I – antibiotics; class II – small hydrophobic, heat-stable peptides; class III – large heat-stable peptides; and class IV – complex bacteriocins: probiotics with lipid and/or carbohydrate [32]. Nisin is one of the famous bacteriocins, which is a ribosomally synthesized antimicrobial peptide produced by certain strains of *Lactococcus lactis* which has been proved to act against human *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and others [28]. Another counteracting finding was demonstrated by Vazquez et al. [119] who proposed that the inhibitory mechanism of LAB is due to lactic acid not to bacteriocin which cannot pass the plasmatic membrane of the Gram negative bacteria but only play a role in formation of transmembrane pores. On contrary lactic &acetic acid in undissociated form posses the ability to cross the membranes of micro-organisms to dissociate internally &to acidify the interior, promoting the expulsion of H^+ ions from the cells & causing uncoupling of Na–K (ATPase) pump. This finding widened the probiotic mode of action to include the lactic acid production.

Antiviral effects

Some probiotic bacteria have antiviral effects. Laboratory tests indicated that the inactivation of viruses can occur by chemical and biological substances, such as extracts from marine algae and the bacterial extracellular products. The production of antagonistic compounds may also be active against virus as documented by Balcazar et al. [28] who reported antiviral activity from *Vibrios* spp., *Pseudomonas* spp., *Aeromonas* spp. obtained from salmon hatcheries against *infectious hematopoietic necrosis virus* (*IHNV*). Also Balcazar et al. [28] isolated *Pseudoalteromonas undina* strain, which exerted antiviral effects by increasing survival in prawn (*Penaeus* sp.)

and sea bream (*Sparus aurata*) experimentally infected with *Sima-aji Neuro Necrosis Virus* (*SJNNV*), *Baculo-like viruses* and *Irido virus*. Gatesoupe [29] reported that *IHNV* and *Oncorhynchusmasou virus* (*OMV*) can be inhibited by the activity of two *Vibrio* strains isolated from a shrimp hatchery which showed promising results as antiviral agents. Harikrishnan et al. [120] studied the Effect of feeding two probiotics *Lactobacilli* and Sporolac, on *lymphocystis disease virus (LCDV)* infected *olive flounder*, *Paralichthys olivaceus*, they recorded desired effects in viral disease control.

Enzymes production

Some probiotic strains of marine origin have affinity to produce bacteriolytic enzymes against *V. parahaemolyticus* [121]. The isolated and characterized *Alteromonas* spp. Strain B-10-31 produces an alkaline protease inhibitor called (Monastatine) showed inhibitory activity against protease from *A. hydrophila* and thiol protease from *V. anguillarum* both pathogenic to fish [20].

Vitamin production

Vitamin products are among the valuable output of the probiotics. *In vitro* studies and humans trials have archived the capacity of some selected probiotic strains to compose Vitamin k [96], folic Acid [97] and B12 [122]. LeBlanc et al. [123] stated that certain lactic acid bacteria (LAB) have the privilege of synthesizing water-soluble vitamins such as the B-group (e.g. folates, riboflavin and vitamin B12). In addition, they also discussed the use of modern genetically modified strains to either increase vitamin production or design new vitamin-producing strains. Rossi et al. [124] specified Folate as an important and vital vitamin, not all the probiotic bacteria are apple to produce Folate, so they aimed to produce Folate-enriched fermented products and/or develop probiotic supplements that accomplish Folate biosynthesis *in vivo* within the colon. For this reason, *bifidobacteria* has been extensively studied for their capability to produce this vitamin which is generally required for growth and provide a substitution to Folate levels in the media. *Lactobacillus plantarum* constitutes an odd example among *lactobacilli*, since it is capable of *in vitro* Folate formation in presence of para-aminobenzoic acid (pABA), so it worth used in animal trials to validate its ability to produce the vitamin *in vivo*. Rats fed a Folate producing *bifidobacteria* probiotic revealed increased blood Folate level, confirming that formation and utilization of Folate *in vivo*. In human, the use of Folate-producing probiotic strains can be regarded as a new perspective in the specific use of probiotics. They aid in protection against inflammation and colon cancer.

Although Marine larviculture is labor and expensive, it is becoming increasingly popular. In marine species it is possible to manipulate the larval digestive system and health, this can be true through probiotic supplementation in the early stages of the life. Probiotics can exert its effects either through the culture water or via the live food. Vine et al. [24] stated that we can rely on the well-studied probiotics used in human medicine and terrestrial agriculture as it has proved to be successful in marine aquaculture, these findings lower the cost of the extensive biosafety trials. Technically, the selection of probiotics requires massive *in vitro* screening experiments, which assay for various benefits such production of vitamins, fatty acids and digestive enzymes. Further information regarding probiont host suitability must be addressed to guarantee safe interaction with live food and host pathogenicity. Finally, field *in vivo* tests need to be performed to calculate the cost-benefit ratio.

The systemic immunity of fish

The immune system is critical for survival and fitness of living organisms; it enables to distinguish between self, non-self (e.g., pathogens) and altered self. The immune system must be in a state of preparedness even in the absence of any antigenic challenge, it must be in strategic locations within the organism in order to sense and communicate information on invading foreign material, and it must be able to rapidly replenish immune cells [125].

Fishes are often considered to be of a primitive immune system in comparison with higher vertebrates, this fact may be related to two observations: First, while higher vertebrates have two separate compartments to generate myeloid and lymphoid immune cell types (lymphoid: lymph nodes, thymus, spleen; myeloid: bone marrow), fish do not possess bone marrow or lymph nodes, and produce lymphoid and myeloid cells in the same compartments. Second, the adaptive immune of fish usually shows a rather slow response to infective pathogens, taking weeks instead of days as in mammals [126]. Despite these "primitive" criteria, the fish immune system is efficient enough to support ecological success of fishes in a wide range of environments and against a plethora of infectious pathogens.

The immune system of fishes can be subdivided into broadly three categories which differ in the speed and specificity of response [127,128]. The first line of defense is presented by the external barriers separating the fish from its environment, i.e., the epithelia of skin, gills and alimentary canal. These epithelia work as mechanical barriers to invading pathogens, but they also contain chemical (antibodies, lysozyme, etc.) and cellular (immune cells) defenses. Inside the fish, the second immune category is formed by the innate immune system which enables a rapid response to invading pathogens. This system provides non-specific responses which are activated by pathogen associated molecular patterns (PAMP) that are common to many pathogens [129]. The main elements of the innate immune system of fishes include humoral factors such as lysozyme or complement factors, as well as phagocytic cells. The main functions of the phagocytic cells are to phagocytize tissue debris and microorganisms, to secrete immune response regulating factors and to bridge innate and adaptive immune responses.

The third line of immune defense is the adaptive or acquired immune system, a set of humoral and cellular components that enable a pathogen-specific response. Adaptive immunity provides organisms with a mechanism for deriving an almost limitless variation from very few genes [125].

Effect of probiotics on immune response enhancement

The ability of the administered probiotic to modulate the non-specific immune responses thus, increase disease resistance

during bacterial infections in aquatic animals was documented by several studies [9,29]. Recent studies have focused on the possible role of probiotics in immune system functions. Gatesoupe [29] reported that feed supplemented by selected bacterial probiotics caused an increase in some cellular and humoral parameters. Villamil et al. [130] found that *Lactococcus lactis* caused the higher increases in immune functions of turbot (*S. maximus*). Later, Villamil et al. [25] proved that the whole cell, fractions whole cell and the extra cellular products of LAB such as nisin act as Immunomodulator in turbot (*Scophthalmus maximus*), the increase was in chemiluminescence's and nitric oxide production in a dose and time dependant manner. In shrimp, Balcazar et al. [131] increased the resistance of shrimp, *Litopenaeus vannamei*, against *Vibrio harveyi* and white spot syndrome by administration of a mixture of *Bacillus* and *Vibrio* spp. Chiu et al. [132] reported increases in activities of superoxide dismutase (SOD), phenoloxidase (PO), respiratory burst as well as the clearance efficiency of *Vibrio alginolyticus*, in addition, a recorded increase in the mRNA transcription of prophenoloxidase (proPO), and peroxinectin (PE) as immune profile factors in white shrimp, *Litopenaeus vannamei*, when treated with *Lactobacillus plantarum* supplemented food. Liu et al. [133] proved that *B. subtilis* was able to survive in grouper, *Epinephelus coioides*, posterior intestines during the feeding period; the relative survival percentages of fish challenged with *Streptococcus* spp. and *iridovirus* were increased in time and dose dependent manner. Significant increases in respiratory bursts, phagocytic activity, superoxide dismutase (SOD) level of leukocytes and serum alternative complement activity (ACH 50) when compared with controls.

Activating the immune system is costly operation [134]. In teleosts, probiotics can positively stimulate various immunohematological parameters such as mononuclear phagocytic cells (monocytes, macrophages) and polymorphonuclear leukocytes (neutrophils) and NK cells [131]. Probiotics actively stimulate the proliferation of B lymphocytes, thus elevation of immunoglobulin level in both *in vitro* and *in vivo* conditions, Elevation of immunoglobulin level by probiotics supplementation is reported in many animals and fish [68,135,136].

Probiotics can effectively stimulate phagocytosis through alarming of the pahgocytic cells, the later is accountable for early intervention through activation of inflammatory responses before antibody production and plays a crucial role in antibacterial defenses in numerous fish and shellfish species [137–150].

Respiratory burst activity is an important innate defense mechanism of fish. The findings of respiratory burst activity following probiotics treatment in fish are typically contradictory. Whereas some studies indicate probiotics do not have important impact on this non-specific defense reaction of fish [135,151,152]. Many *in vitro* and *in vivo* studies showed important increase in Respiratory burst activity by numerous probiotics in several aquatic animals as well as fish [153–159].

Lysozyme is one of the important bactericidal enzymes of innate immunity is an indispensable tool of fish to fight against infectious agents [160]. Lysozymes can be found in serum, mucosal membranes of skin and intestine. Probiotics either single or in combination are found to trigger the lysozyme level in teleosts. The enhancement of lysozyme level was recorded by various types of probiotics [24,29,136,161,162].

The peroxidase is an important enzyme that utilizes oxidative radicals to kill pathogens. Dietary supplement of probiotic like *B. subtilis* alone or together with *L. delbrueckii* ssp. *lactis* for 3 weeks end with high serum protease activity, however it did not enhance the oxidase activity of head kidney leukocytes of *S. aurata* [163].

Regarding Complement Activity, in teleosts, complement system, a component of the non-specific immune response, plays a key role in adaptive immune responses, involved in chemotaxis, opsonization, phagocytosis and degradation of pathogens and has effector mechanisms like direct killing of microorganisms by lysis [164]. Probiotics can enhance natural complement activity of fish [164,165]. Dietary as well as water treatment by many probiotics are often reported to stimulate the piscine complement components [156,166].

Cytokines are protein mediators produced by immune cells and contribute to cell growth, differentiation and defense mechanisms of the host [167]. Available literatures indicate that a number of probiotics can effectively modulate the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-12, tumor necrosis factor α (TNF-α), and gamma interferon (IFN-γ) and anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGF-β) in many animals [168–170].

Cerezuela et al. [138] studied the combined or individual effects of two microalgae (*Phaeodactylum tricornutum and Tetraselmis chuii*) and *Bacillus subtilis* on immunity, expression of genes, and competence to challenge with *Photobacterium damselae* subsp. *piscicida* of gilthead sea bream. To test the capacity of *B. subtilis* to grow employing the microalgae polysaccharides as energy and carbon source, an *in vitro* assay demonstrated that the digestion product of microalgae, mainly *P. tricornutum*, aid in the growth of *B. subtilis*. In addition, the outcome of the *in vivo* study recorded the capability of *B. subtilis*, *T. chuii*, and *P. tricornutum*, as feed supply singly or in combination, to exhibit up-regulating effects on gilthead sea bream immune parameters. *P. tricornutum* offered the elevated Immunostimulatory action. The results were of even significant between combination feeding and feeding ingredients separately. Another feeding experiment was conducted to determine effects of *Hanseniaspora opuntiae* C21 on immune response and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumbers *Apostichopus japonicus*. Different concentrations of C21 containing diets were tested for 30–50 days. Results indicated that C21 significantly improved and enhanced the phagocytic activity, lysozyme, phenoloxidase activity, total nitric oxide synthase, superoxide dismutase, alkaline phosphatases, and acid phosphatase activities in coelomocytes and coelomic fluid of sea cucumbers. Incidence and mortality rates against *V. splendidus* were lowered as results of feeding C21 supplemented ration [171].

Effect of probiotics on gut immunity

The gut is the organ where probiotics not only establish but also execute their functions including immunostimulaory activity. The immune system of the gut is referred to as gut associated lymphoid tissue (GALT) and the piscine gut immune system is quite different from mammals. Unlike mammals, fish lack Peyer's patches, secretory IgA and antigen-transporting M cells in the gut [172]. However, many diffusely organized

lymphoid cells, macrophages, granulocytes and mucus IgM found in the intestine of fish constitutes the immune function.

There was a masking for the effect of probiotics on local gut immunity in fish species due to lack of suitable tools which facilitate the access and investigate the gut immune response following probiotics treatment. Few conducted studies indicated that probiotics can stimulate the piscine gut immune system with marked increase in the number of Ig^+ cells and acidophilic granulocytes (AGs) [119,173–175]. Recent studies get the privilege of the recent techniques and extensively studied the correlation between the improvement of the gut immunity and the probiotic supply [82,176–182].

Probiotics can also lead to a significant increase in T-cells in fish. In a study, Picchietti et al. [175] recorded increased T lymphocytes in gut without any change in CD4 and CD8α transcript in sea bass (*D. labrax*) by *L. delbrueckii* ssp. *delbrueckii* supplemented through live carriers like artemia and rotifers. Enhancement of gut mucosal lysozyme by *C. maltaromaticum* and *C. divergens* [160] and phagocytic activity of mucosal leukocytes by LAB group of probiotics such as *L. lactis* spp. *L. mesenteroides* and *L. sakei* are also reported in *O. mykiss* [176]. Clownfish (*Amphiprion percula*) has been a source for probiotics as some beneficial strains was isolated from its gastrointestinal tract. Probiotic strains have the ability to generate antimicrobial metabolites and have been used to inactivate several pathogens such as *Vibrio alginolyticus* and *Aeromonas hydrophila*. The isolated bacteria have the potential to colonize the intestinal mucus and therefore can be used as prophylactic agent and/or therapeutic [184,185]. In addition, concentrations of 10^6–10^8 cells g^{-1} of probiotic boost the generation of intestinal healthy bacteria and diminish the amount of heterotrophic microorganisms of ornamental fishes from the genera *Xiphophorus* and *Poecilia* [186].

Influence on water quality

There is considerable interest in use of probiotics to improve conditions for production in pond aquaculture. The mechanism of actions to the positive influence on water quality is still in infancy. In aquaculture, to improve water quality, fish raisers my relay on removal of toxic materials from water. Li et al. [183] performed a study to configure the possible role of probiotic bacteria in improving the shrimp water culture, they found that the addition of photosynthetic bacteria into the water resulted in elimination of a number of toxic metabolic and toxic products thus enhance water quality. The heterotrophic probiotic bacteria may catalyst some important chemical actions such as nitrogen fixation, oxidation, nitrification, denitrification and sulphurication. Addition of such bacteria to farm water aids in decomposing the various sources of organic material such as the remaining food materials, extra plankton to in organic salts as phosphate, CO_2 and nitrate. These inorganic salts products aid in nutrition and abundance of micro algae, the photosynthetic bacteria dominate in the water and inhibit the growth of other pathogenic microorganisms. The formed micro algae provide suitable media for both the serviceable bacteria and cultured animals [187,188].

It has been presumed that among the major role of the beneficial heterotrophic bacteria, the acceleration of organic matter decomposition by establishing the Nitrogen:Carbon ratio as a management tools [189,190]. The regular use of probiotics enhances the hegemony of heterotrophic bacteria in the environment. Bacteria from the genus *Bacillus*, are known to convert organic matter to CO_2 thus acquired additional character for becoming a probiotic [30]. During the production cycle of juvenile *Penaeus monodon*, addition of high levels of Gram-positive bacteria as *Bacillus* spp. can minimize the accumulation of organic carbon which is responsible for the final black sludge formation after harvest [29]. Liao et al. [191] isolated a new aerobic denitrifying strain X0412 named *Stenetrophomonas maltophilia* from shrimp ponds. The identified strain found to produce the nitrite reductase gene. Wang et al. [192] recorded that by the 16S rDNA sequence analysis technique, a total of 27 bacterial strains belonged to 11 genera were identified as denitrifying bacterial strains capable of both nitrate and nitrite reduction, hence improving the fish pond water characters. In conclusion, addition of probiotics to aquaculture exert multiple advantages as reduction in nitrogen and phosphorus concentrations; enhanced decomposition of organic matter, increase algal growth, abundance of dissolved oxygen, decrease in toxic algae (blue-green cyanobacteria), control of toxic metabolites and finally profit shrimp and fish production.

Interaction with harmful phytoplankton

Aquatic cultured species are hindered with the development of harmful algae in water, adding controlling agents to antagonize such undesirable growths is appreciated in aquaculture farms. Some probiotic bacteria have a selective ability to antagonize the development of the harmful algae during aquaculture production cycles. Fukami et al. [193] demonstrated that some probiotic bacterial strains may have significant algicidal effect on many toxic micro algae particularly of red tide plankton, they recorded the algicidal ability of seawater origin *Flavobacterium* spp. and the control of *Gymnodinuim mikimotoi* algal blooms.

Interaction with live food

Early stages of marine larval development require live food as many do not accept artificial diets. Phytoplankton (microalgae) and rotifers are the first bite up live feeds for most cultured marine fish species [194,195], due to its nutrient-producing photosynthetic ability, in most cases higher organisms are unable to synthesize such is the case of polyunsaturated fatty acids and vitamins. Also it was used as a delivery system for biological materials such as vaccines, probiotics and therapeutics [9]. There must be a cautious selection for probiotic bacteria administered during larval rearing where unicellular algae are added as food in the green water technique as the main source of food. Probiotic bacteria with antagonistic action toward algae would be undesirable in such larval rearing feeding regimes, as their possible interaction with these unicellular algae must be taken into consideration when the mode of action is being investigated.

Central diatoms as *Chaetoceros* spp., are within groups of microalgae proven to be a good live food used in aquaculture, however, production has limitations due to the complexity of their nutritional requirements [196]. Gomez et al. [197] assessed the growth of *Vibrio alginolyticus C7b* probiotic in the presence of the microalgae *Chaetoceros muelleri*, it was

proved that these organisms can be grown together to achieve high fed density for shrimp.

Rotifers are small size, more accessible larval food substrate, it can be exampled with the nauplii of brine shrimp, which is a very common marine live feed. Planas et al. [198] used lactic acid, *Pediococcus acidilactici*, *Lactococcus casei* spp. *casei*, and *Lactobacillus lactis* spp. *lactis* to increase the growth of the rotifer *Brachionus plicatilis* and obtained the best results. The bacterial flora of rotifers is approximately 5×10^3 bacteria per individual [199]. Attempts to load rotifers with a considerably higher bacterial count to turbot larvae feeding have proven unsuccessful [200]. The amount of probiotic cells that adhere to the live food depends on the probiont, duration of exposure and the state (dead or alive) of the live food organism [201]. As the live food's bacterial load increases it may reach levels that negatively affect the health of the host larvae. For example, Olsen et al. [202] found that bacterial overloading of 4-day-old *Artemia* fed to halibut larvae resulted in poorer larval growth.

It must be noticed that any change in the selected diet will affect the different loaded bacterial community characters. In Arctic charr (*S. alpinus*), alteration of dietary fatty acids resulted in a major change in contributions of the lactic acid bacterial flora [203–205]. Large numbers of *Vibrio* spp. in the rearing water and larval intestine are usually attributed to the presence of *Artemia* [202,204–206], which diminish as the fish are weaned onto a formulated diet [207]. Live feeding of rotifers or *Artemia* can be manipulated to act as a vector for probiotics. [200,208,209]. In addition, a positive effect of probiotics on live food cultures has been documented [25,209] as has the transfer of these bacteria into larval interior [209–211].

The *in vitro* studies for the delivery methods to the larvae should advance the large scale *in vivo* applications. Some probiotics may be able to attach to live food. If probiotics can be administered via live food, their application in marine fish larviculture could be expanded [212].

Probiotics and reproduction

Aquaculture is of high economic yield projects, if managed properly. Reproduction process constitutes the backbone for any production yield, thus the financial outcome from aquaculture projects. Reproductive process is regulated by many elements, fish species, nutrition and environment are the master leading elements. Nutrition is closely intermingled with the timed reproductive consequences, from gametes through puberty to adults in both sexes. Recent researches focused on the possible role of probiotic in reproductive process and new progeny with special emphasis to the marine species. Probiotic bacteria used as dietary additives seem to offer an attractive choice inducing overall health benefits to the host organism.

Ghosh et al. [213] tested the incorporation of *B.* subtilis isolated from intestine of *Cirrhinus mrigala*, in diets of four species of ornamental fishes in a 1-year feeding experiment. The results showed an increase in the gonadosomatic index, fecundity, viability, and production of fry from the females of all tested species. They suggested that the vitamins B synthesized by the probiotic, especially vitamin B1 and B12, contribute in lowering the number of dead or deformed alevins. Abasali and Mohamad [214] recorded an increase in the gonadosomatic index and the production of fingerlings of females in reproductive age and the relative fecundity in *X. helleri* spp. supplemented with commercial probiotic (Primalac) containing 4 species lactic acid producing bacteria. Lombardo et al. [80] investigated the effects of dietary administration of *Lactobacillus rhamnosus* IMC 501® on the growth and survival of the new progeny of obtained from the marine teleost *Fundulus heteroclitus* brood stock fed probiotic-supplemented diets. They recorded an improvement in gonadal growth (gonadosomatic index, GSI), fecundity, embryo survival and hatching rate of the tested larvae. On the contrary, no effect on the hatching rate was shown. A scientific explanation ought to be given for the mechanisms of action of probiotic on the reproductive axis as well as the nutritional-/immunological-mediated maternal interactions and profiles on fertilization, larval development and growth.

In Zebrafish, Carnevali et al. [215] reviewed the reproductive effects of *Lactobacillus rhamnosus*, as a diet supplement on zibrafish *Danio rerio* as a fish model. They reported that long term administration of *L. rhamnosus* may accelerate the larval growth by acting on the growth promoting factors as insulin-like growth factors-I and II (igfI), α and β receptors of peroxisome proliferators (ppar α,β), vitamin D receptor-α (vdrα) and retinoic acid receptor-γ (rarγ). In addition, physiology of reproductive system was positively altered as gonadal differentiation was foreseeable at 6 weeks with a higher expression of gnrh3 at the larval stage. Moreover, brood stock fixed with *L. rhamnosus*-supplemented diet revealed better reproductive performances in picture of increase in ovulated oocytes quantification and in embryos quality. On molecular bases, The observations were correlated with the hormones and reproduction gene expression as the aromatase cytochrome p 19 (cyp19a), the vitellogenin (vtg) and the α isoform of the E2 receptor (erα), luteinizing hormone receptor (lhr), 20-β hydroxysteroid dehydrogenase (20β-hsd), membrane progesterone receptors α and β, cyclin B, activinβA1, smad2, transforming growth factor β1 (tgfβ1), growth differentiation factor9 (gdf9) and bone morphogenetic protein15 (bmp15).

Avella et al. [216] hypothesized that a continuous administration of an exogenous probiotic might influence the host's development. In Zebrafish model, a 2-months treatment study using *L. rhamnosus* was conducted, the tested period represented from birth to sexual maturation. They monitored the presence of *L. rhamnosus* in zebrafish during the entire treatment. The fish at the early 6 days post-fertilization (dpf) expressed elevated gene expression levels for Insulin-like growth factors-I and -II, Peroxisome proliferator activated receptors-α and -β, VDR-α and RAR-γ. Higher GnRH3 expression was found at different intervals from *L. rhamnosus* treatment. The resultant larvae exhibited earlier maturation and development in bone calcification and gonads.

Molecular techniques for characterization and evaluation of probiotics

Although conventional methods for microbial characterization rely on phenotypic characterization, growth, sugar fermentation index, serology studies and biochemical reactions have been proven useful and accredit for many years, yet they are time consuming, insufficient for detailed identification and load inherit imperfection in level of subspecies identification. In addition, the health and legislative authorities,

manufacturers and consumer call for sensitive, easy, fast and reliable methods to identify and characterize the microbial content of probiotics [217]. Knowledge of the molecular base of host–microbe interactions is advanced day after day, the molecular approach provides a more complete picture about bacterial community composition than do cultured-based methods. Various molecular techniques, using different genetic markers, have proven useful in sub-species discrimination or strain differentiation. Molecular methods aid in recovery and analysis of the bacterial DNA directly from field samples have been proven useful for studying less cultivable microbial populations, in addition it skip the laborious and time consuming purification procedures. Recently, authorities depend on both results from findings from conventional culture-based methods detailed by molecular identification techniques that are based on the 16S rDNA gene to reach a final judgment for microbial profiles [218,219]. The following paragraphs review the most popular molecular used methods in fish probiotic studies.

Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and thermal gradient gel electrophoresis (TGGE)

The (PCR-DGGE/TGGE) methods are reliable, rapid, sensitive and easy to study microbial diversity [220–222]. Molecular methods enable characterization and quantification of the intestinal microbiota, while also providing a classification scheme to predict phylogenetic relationships. It improved understanding microbe–microbe and host–microbe interactions in health and disease, and the potential for manipulation of the fish microbiota by nutritional and environmental factors [223]. Profiling the 16Sr RNA population by DGGE/TGGE enable the rapid estimation of the presence and relative abundance of microorganisms in a sample [224]. The general principles of DGGE/TGGE are the separation of fragments of the individual rRNA genes based on differences in chemical stability or melting temperature of these genes. After more than a decade of application in microbial population studies, the DGGE/TGGE techniques gradually reaches maturity. The *Bacillus halotolerance* (SHPB) probiotic was characterized using the PCR and 16Sr DNA gene amplification [225]. The identification of SHBP probiotic confirmed as *Bacillus halotolerance*. The modes of action of *bacillus* include the production of bacteriocin-like compounds [226]. Bacteriocins are antibacterial proteins produced by bacteria to kill or inhibit the other bacterial growth [227]. The bacterium produces an amplicon of approximately 1500 bp and for the bacteriocin gene a 1000 bp amplicon Cultures. Further researches are required to specify the exact type of bacteriocin produced by the probiotic *B. halotolerance* [147]. In a study performed by Muñoz-Atienza et al. [204] to detect the antibiotic resistance genes, the non-enterococcal strains showing antibiotic resistances were fully identified using PCR to investigate the presence of the respective antibiotic resistance genes.

Avella et al. [216] evaluated the effect of *L. rhamnosus* beneficial bacteria on gene expression modulation for growth-related factors in clownfish. Alteration in molecular biomarkers detected by real time PCR supported the faster growth observation. On molecular bases, the increase in growth rate was explained by the significant increase in gene expression of growth stimulation factors as vitamin D receptor α, myostatin, peroxisome proliferator-activated receptors α and β, insulin-like growth factors I and II, and retinoic acid receptor γ). Moreover, probiotic treatment lessened the severity of the general stress response as exhibited by lower levels of glucocorticoid receptor and 70-kDa heat shock protein gene expression.

An investigated study was performed by Carnevali et al. [228] on *Dicentrarchus labrax* (European sea bass) juveniles fed Lactic Acid Bacteria (LAB) strain, *L. delbrueckii delbrueckii* , for a short (25 days) and a long (59 days) time, the expression of two antagonistic genes involved in muscular growth (IGF-I and myostatin (MSTN) was analyzed through real-time PCR. An increase in IGF-I transcription was observed in fish treated with LAB, being IGF-I mRNA levels six times higher in both treated groups with respect to the control. On the contrary, MSTN mRNA transcription was significantly inhibited in treated groups. These results are in agreement with the increase in body weight recorded in this study. Fish fed on LAB showed 81% higher body weight in long treated group and 28% in short treated one with respect to control.

Fluorescence in situ hybridization (FISH) technique

Fluorescence in situ hybridization (FISH) has been increasingly used to analyze GIT bacterial communities [229]. Although PCR-based fingerprinting is the most sensitive technique to detect low concentrations sequences in the samples, many factors can influence the amplification reaction and the fingerprinting techniques, thus no sufficient quantitative data well result [230]. FISH with rRNA target probes has been developed for the in situ identification of single Microbial cells and is the most commonly applied among the non-PCR-based molecular techniques [231]. This method is based on the hybridization of synthetic oligonucleotide probes to specific regions within the bacterial ribosome and does not require cultivation. The FISH technique can be applied for the in situ detection of probiotic *Lactobacillus* cells in fecal and biopsy samples. The potential of FISH has recently been demonstrated for *Bifidobacteria* in fecal samples [232]. Due to its speed and sensitivity, this technique is considered a powerful tool for phylogenetic, ecological, diagnostic and environmental studies in microbiology [233].

In a study performed by Denev et al. [223] the FISH technique was applied to characterize a probiotic photosynthetic bacteria mixture used in aquaculture. Through the use of group or species-specific probes, it is possible to identify different bacterial groups in complex probiotics mixtures, thus providing quantitative information for the understanding of the probiotics mixture and the possible inter species interaction. PCR-DGGE with FISH technique are proven effective, sensitive, flexibile and inexpensive and therefore can widely be applied in probiotics studies [223].The subtype of the *Saccharomyces cerevisiae* yeast species known as *S. cerevisiae Hansen* CBS 5926 was formerly believed to be a separate species, *Saccharomyces boulardii*. It is widely considered non-pathogenic and is used as a probiotic agent for treatment and prevention of diarrhea. The biological properties of *Saccharomyces* spp. show considerable intra-species difference from the beneficial properties of yeast probiotic. Septicemia and fungemia caused by *S. boulardii* have recently been

Table 1 The potential Gram positive bacteria used as probiotic.

Probiotic agents Bacterial probiotics	Fish species	Conducted study	Nature of study	References
Gram positive bacteria				
Bacillus species	*Bacillus* spp. is a Gram-positive, non-pathogenic, spore-forming organism [244] recently exerted an immunostimulatory effects in human, animals against a variety of diseases Green et al. [16] and fish			
Bacillus spp.	Common snook larvae, *Centropomus undecimalis* (Bloch)	Survival rate of larvae, food absorption by detection of protease levels, estimation for number of suspected pathogenic bacteria in the gut	*In vitro*	Irianto and Austin [20].
B. subtilis and *B. licheniformis* (Bioplus2B)®	Rainbow trout, *Oncorhynchus mykiss*	Ressistance for *Y. ruckeri*	*In vitro*	Raida et al. [245]
B. sublitis BT23	*P. monodon*	Antagonistic effect for pathogenic *Vibrios* and reduction in accumulated mortality	*In vitro*	Vaseeharan and Ramasamy [246]
B. subtilis	*Penaeus japonicus* post-larvae	Study the level of survival in response to bacterial challenge	*In vitro*	Dakar and Gohar [247]
B. licheniformis and *B. subtilis*, (Biogen®)	*Oreochromis niloticus*	Improve fish digestibility, stability in the intestine and use a large number of sugars (carbohydrates) for their growth and produce range of relevant digestive enzymes (amylase, protease and lipase)	*In vitro*	Haroun et al. [48]
B. subtilis	Indian major carp, *Labeo rohita*	Survival and growth performance and fish immunity	*In vitro*	Kumar et al. [248]
B. megaterium	*Litopenaeus vannamei* (Boone)	Growth and feed utilization	*In vitro*	Yuniarti et al. [99]
Bacillus spp. *mixture* Sanolife, INVE®	Gilthead sea bream (*Sparus aurata*)	Direct inhibition for fish pathogen, *Vibrio* spp. Mortality and survival rate	*In vivo*	Decamp et al. [78]
	Juveniles and larvae of Japanese flounder (*Paralichthys olivaceus*) and Southern flounder (*P. lethostigma*)	Direct inhibition for fish pathogen Mortality and survival rate Weight gain and growth performance	*In vivo*	
	Senegalese sole (*Solea sengalensis*)	Mortality and survival rate	*In vivo*	
	Turbot, *Scophthalmus nraximus*	Mortality and survival rate	*In vivo*	
Lactic Acid Bacteria (LAB)				
Lactobacillus spp.	Lactic acid bacteria are Gram-positive bacteria. They have no mobility and are non-sporulating bacteria that produce lactic acid. Some members of this group contain both rods (*lactobacilli and carnobacteria*) and cocci (*streptococci*) [58]. Different species of lactic acid bacteria (such as *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Enterococcus*, *Vagococcus*, *Lactobacillus*, *Carnobacterium*) have adapted to grow under widely different environmental conditions. They are found in the gastrointestinal tract of various endothermic animals, in milk and dairy products, seafood products, and on some plant surfaces [249]			
Heat-killed lactic acid bacteria probiotics isolated from the Mongolian dairy products namely, *Lactobacillus paracasei* spp. *paracasei* (strain 06TCa22) *L. plantarum* (strain 06CC2)	Japanese pufferfish (*Takifugu rubripes*) head kidney (HK) cells	Immunostimulant response to fish assayed by multiplex RT-PCR analysis	*In vitro*	Biswas et al. [18].
Lactic Acid Bacteria of aquatic origin used as probiotics in aquaculture	Laboratory study	Antimicrobial activity, antibiotic susceptibility and virulence factors	*In vitro*	Muñoz-Atienza et al. [250]
Human probiotic, *Lactobacillus rhamnosus* ATCC 53101	Rainbow trout, Oncorhynchus mykiss	Dose estimation, Reduced mortalities,, growth performance and challenge with Aeromonas salmonicida.	*In vitro*	Nikoskelainen et al. [251]

L. lactis, *Leu. mesenteroides*, *L. sakei*	Rainbow trout, *Oncorhynchus mykiss*	Disease resistance, gut microbiota (inclusive of probiont colonization), immunological/hematological response	*In vitro*	Balcazar et al. [252]
L. plantarum, *L. salivarius*, *L. rhamnosus*	Blue swimming crab, *Portunus pelagicus* larvae	Enhance survival rates	*In vitro*	Talpur et al. [253]
L. rhamnosus	Rainbow trout, *Oncorhynchus mykiss*	Gut microbiota (inclusive of probiont colonization), immunological/ hematological	*In vitro*	Nikoskelainen et al. [254]
L. rhamnosus	Rainbow trout, *Oncorhynchus mykiss*	Gut microbiota (inclusive of probiont colonization), immunological/ hematological	*In vitro*	Panigrahi and Azad [255]
L. rhamnosus	Rainbow trout, *Oncorhynchus mykiss*	Gut microbiota (inclusive of probiont colonization), immunological/ hematological	*In vitro*	Panigrahi et al. [136]
L. rhamnosus, *B. subtilis*, *E. faecium*	Rainbow trout, *Oncorhynchus mykiss*	Gut microbiota (inclusive of probiont colonization), immunological/ hematological	*In vitro*	Panigrahi et al. [165]
L. acidophillus and *L. sporogenes*	*Macrobrachium rosenbergii*	Growth rate and inhibition of Gram negative bacteria in the gut	*In vitro*	Himabindu et al. [256]
Lactobacilli				Vazquez et al. [119]
Viable or heat-killed *Lactococcus lactis*	Turbot, *Scophthalmus nraximus* macrophages	Immune response of head kidney macrophage chemiluminescent (CL) Nitric oxide (NO) and the antibacterial effect of the extracellular products against *V. anguiltarum*	*In vitro* and *In vivo*	Villamil et al. [130]
Streptococcus spp. (*S. faecium*)	Nile tilapia, *O. nilotics*	Growth performance and feed efficiency	*In vitro*	Lara-Flores et al. [47]
Enterococcus spp. *Enterococcus faciurn*	Sheat fish, *Silurus glanis*	Improving growth	*In vitro*	Bogut et al. [257]
Enterococcus faecium SF68 (commercial products)	European Eel, *Anguilla anguilla*	Reduce Edwardsiellosis	*In vitro*	Chang and Liu [258]
Vagococcus fluvialis	Leukocytes from head kidney of Gilthead sea bream (*Sparus aurata*) European sea bass (*Dicentrarchus labrax*)	Phagocytic and respiratory burst activity and the peroxidase content of leukocytes	*In vitro*	Román et al. [137]
Carnobacterium inhibens K1	Salmonids	Enhanced appetite and feeding efficiency and antagonism against *A. salmonicida*, *V. ordalli* and *Y. ruckeri*	*In vitro*	Robertson et al. [53]
Weissella hellenica DS-12 from intestinal contents of farmed flounder, *Paralichthys alivaceus*	Laboratory plate study	Antagonistic to some bacterial fish pathogens	*In vitro*	Byun et al. [259] Vine et al. [260]
Micrococcus luteus	Rainbow trout, *Oncorhynchus mykiss*	Combat *A. salmonicida* infection	*In vitro*	Irianto and Austin [146]

Table 2 The potential Gram negative bacteria, algae, yeast and Bacteriophages in aquaculture.

Potential probiotics	Host	Pathogen tested and study conducted	Nature of study	References
Gram negative bacteria				
Pseudomonas fluorescens	Finfish culture	Inhibit *A. salmonicida* and *Saprolegnia* sp.	*In vivo*	Smith and Davey [43]
P. fluorescens AH2, isolated from *Lates niloticus*	Rainbow trout, *Oncorhynchus mykiss*	Reduced mortality following challenge with *V. anguillarum*	*In vitro*	Gram et al. [17]
Pseudomonas	Rainbow trout	Survival rates and Inhibitory to *V. anguillarurn* in disk diffusion assay	*In vitro*	Spanggard et al. [240]
Vibrio alginoliticus	Juveniles and larvae of Japanese flounder (*Paralichthys olivaceus*) intestinal bacteria isolate	Antibacterial abilities of *Vibrio* spp. inhibited the growth of *Pasteurella piscicida*	*In vivo*	Sugita et al. [261]
Aeromonas spp. (strain A199)	Eels (*Anguilla australis Richardson*)	Antagonistic activity against *Saprolegnia* spp.	*In vitro*	Lategan and Gibson [262]
A. hydrophila A3-51	Rainbow trout, *Oncorhynchus mykiss*	Controlling infections by *A. salmonicida*	*In vitro*	Irianto and Austin [146]
Bdellovibrio	Sturgeon	Anti-bacterial action against *Aeromonas hydrophila* infections in sturgeons	*In vitro*	Cao et al. [263]
Microalgae				
Tetrasehnis suecica	Penaeids, Salmonids	Reduction in bacterial diseases due to antimicrobial compounds in the algal cells	*In vitro*	Austin and Day [264]
Blue green algae *Spirulina platensis* (*Arthrospira platensis*)	*O. niloticus*	Growth performance, nutrient utilization, innate immune response and challenge infection	*In vitro*	Ibrahem et al.[158]
		The role of Spirulina as chemoprotective agent through estimation of P53 expression level	*In vitro*	Ibrahem and Ibrahim [265]
Yeast probiotics	Yeast is promising candidates as probiotics, because of its abilities to produce polyamines that participate in numerous biological processes Bardócz et al. [266], including cell replication and differentiation, biosynthesis of nucleic acids and proteins Tovar et al. [267]. In addition yeast can adhere and grow in the intestinal mucus of fish			
Active or inactive yeast	*O. niloticus*	Growth performance and nutrient utilization	*In vitro*	Abd El-halim et al. [268]
Saccharomyces cerevisiae	*Trout* spp.	Protein source substituting	*In vivo*	Rumsey et al. [269] Rumsey et al. [270]
Cell wall of yeast (β-GIucan, mannoprotein and chitin)	Gilthead sea bream (*Sparus aurata* L.)	Innate immune response and challenge infection	*In vitro*	Esteban et al. [271]
Cell wall of yeast, zymoferment®	*O. niloticus*	The growth, health and immunity	*In vitro*	Nashwa et al. [272]
Live yeast *Debrayomyces hansenii* CBS 8339	European sea bass, *Dicentrarchus labrax* larvae	Functions of intestinal enzymes, alkaline phosphatase, arninopeptidase N	*In vitro*	Tovar-Ramirez et al. [273]
S. cerevisiae (Diamond V®)	Catfish, *Clarias gariepinus*	Effects of dietary supplementation of on growth performance, liver and kidney functions and digestive enzymes	*In vitro*	Mansour et al. [274]
	Catfish, *Clarias gariepinus*	Hematological and immunomodulatory effects	*In vitro*	Ibrahem et al. [159]
B-(1, 3) (1, 6)-D-glucan	*Cyprinus carpio* L.	Growth performance and intestinal immunity	*In vitro*	Kuhlwein et al. [275]
Bacteriophages				
	ayu Plecoglossus altivelis	Control of *Pseudomonas plecoglossicida* infection	*In vivo*	Park et al.[276], Nakai and Park [277], Park and Nakai [278]

Table 3 The studies on the current status of using probiotics in aquaculture in Egypt.

Potential probiotics	Host	Pathogen tested and study conducted	Nature of study	References
Micrococcus luteus, *Pseudomonas* species isolated from the gonads and intestine of *Oreochromis niloticus*	*O. niloticus*	Their efficacy on the growth-performance and survival rate, besides some blood-parameters and chemistry. Antagonize *Aeromonas hydrophila* infection	*In vitro*: *Pseudomonas* spp. *In vivo*: *M. luteus*	Abd El-Rhman et al. [71]
Bacillus subtilis, *Lactobacillus acidophilus*	*O. niloticus*	Effect on the immune response of Nile tilapia (*Oreochromis niloticus*), beside its protective effect against challenge infections	*In vitro* and *In vivo*	Aly et al. [282]
Saccharomyces cerevisiae, beta-glucans and laminaran	*O. niloticus*	Effect on the immune response of Nile tilapia (*Oreochromis niloticus*), beside its protective effect against challenge infections Study the probiotic action under immune depressive stressful condition and the resistance to diseases	*In vitro* and *In vivo*	El-Boshy ea al. [283].
Aspergillus oryzae	African catfish (*Clarias gariepins*)	Fish Performance and Quality, Blood Parameters, Assessment of Antibacterial Activity of the Probiotic		Abd elhamid et al.[284]
Bacillus subtilis and Biogen®) with spices	*O. niloticus*	Growth performance	*In vivo*	Soltan and El-Laithy [285]
Dead *Saccharomyces cerevisae* yeast (group 1) *Bacillussubtilis* and *Saccharomyces cerevisae* (group 2)	*O. niloticus*	Effects on non-specific immune response, phagocytic activity test. Histological profile Resistance to the challenged pathogenic microorganisms	*In vitro*	Marzouk et al. [286]
Saccharomyces cerevisae yeast (first group) Live *Bacillus subtilis* and *Saccharomyces cerevisae* (second group)	*O. niloticus*	Effect on growth performance parameters	*In vitro*	Marzouk et al. [287]
Commercial probiotics (Premalac and Biogen®)	Nile tilapia fingerlings	Growth performance, immune response	*In vitro*	Ali et al. [288]
Probiotic (EMMH®)	Nile tilapia (*Oreochromis niloticus*) fingerlings	Evaluation of as a growth promoter	*In vivo*	Abo-State et al. [289]
	Mono sex Nile tilapia (*Oreochromis niloticus*) fingerlings	Used as growth promoters in commercial diets	*In vivo*	Eid and Mohamed [290]
Brewer's yeast	African catfish *Clarias gariepinus*	Effects on the performance and welfare	*In vivo*	Essa et al.[291]
Biogen®	Nile tilapia *Oreochromis niloticus*	Studies on physiological changes and growth performance	*In vivo*	Khattab et al. [292]
Commercial live bakers' yeast, Saccharomyces cerevisiae as	Nile tilapia, *Oreochromis niloticus* (L.) Fry	Growth and immunity promoter, the challenge in situ with *Aeromonas hydrophila*	*In vivo*	Abdel-Tawwab et al. [293]
Blue green algae Spirulina platensis (*Arthrospira platensis*)	*O. niloticus*	Growth performance, nutrient utilization, innate immune response and challenge infection		Ibrahem et al.[185]
		The role of Spirulina as chemoprotective agent through estimation of P53 expression level		Ibrahem and Ibrahim [256]
Active or inactive yeast	*O. niloticus*	Growth performance and nutrient utilization	*In vitro*	Abd Elhalim et al. [268]
Cell wall of yeast, zymoferment®	*O. niloticus*	The growth, health and immunity	*In vitro*	Nashwa et al. [272]
Sacc.cerevisiae (Diamond V®)	Catfish, *Clarias gariepinus*	Effects of dietary supplementation of on growth performance, liver and kidney functions and digestive enzymes	*In vitro*	Mansour et al. [274]
	Catfish *Clarias gariepinus*	Hematological and immunomodulatory effects	*In vitro*	Ibrahem et al. [159]

P. = Pseudomonas, A. = Aeromonas, V. = Vibrio, Pa. = Pasteurella, Ed. = Edwardsiella, Y. = Yersinia, Ent. = Enterococcus, E. = Escherichia, M = Micrococcus, L. = Lactobacillus, P. = Photobacterium, Str. = Streptococcus, Sacc. = Saccharomyces, B. = Bacillus, O = Oreochromis.

described in immune deficient patients receiving this yeast as biocontrol agent. It cannot be distinguished from other *S. cerevisiae* strains by ordinary phenotypic criteria, so identification of these infections requires molecular typing, in an comparative study to determine the accurate molecular diagnostic tool, the yeast was identified using different molecular methods, PCR-restriction enzyme analysis, sequencing of rDNA spacer regions, microsatellite polymorphism analysis of the *S. cerevisiae* genes YKL139w and YLR177w, and the last based on hybridization analysis with Ty917. The results suggest that micro-satellite polymorphism analysis of the YKL139w and YLR177w genes, as well as the analysis by Ty917 hybridization were the ultimate tool for efficient and complete identification of *S. boulardii* strains [234]. In sum, the application of molecular methodologies to bacterial analysis should facilitate the development of detailed knowledge of the target biota which is critical to reach accurate characterization and validation for probiotic strains for fish welfare.

Monitoring of commercial probiotic production

Commercial probiotic production should take into account beneficial traits of strain useful during industrial processing. To overcome the problem of inactivation during the manufacturing process, aquaculture industries try to improve the technology by screening for more resistant strains or alternatively by protecting the probiont through micro–bio encapsulation. By monitoring probiotics and the microbial community structure and dynamics in the manufacture process and *in vivo* culture system, nucleic acid–based techniques have been used. Highly discriminative molecular methods as previously mentioned can be used for accurate probiotic species labeling, which is important for responsible quality control efforts, to build consumer confidence in product labeling, and for safety considerations. The reliable identification of probiotics requires molecular methods with a high taxonomic resolution that are linked to up-to-date identification libraries [235].

The safety profile of a probiotic strain is of critical importance in the selection process, as it should determinate the antibiotic resistance strains and subsequent confirmation for the non-transmission of drug resistance genes or virulence plasmids, upon selection of a safe probiotic strain [236]. Evaluation should also take the end-product formulation into consideration because this can induce adverse effects in some subjects or negate the positive effects altogether.

Quality control of probiotics in aquaculture is an important topic. With the increased use of molecular methods for the definitive analysis of the bacterial components of probiotic products and for *in vivo* validation, it is expected that both the probiotics quality and functional properties can significantly be improved to meet the demands of aquaculture [235,237]. *Lactobacilli* and *bifidobacteria* have traditionally been recognized as potential health-promoting microbes in the human gastrointestinal tract. The adding knowledge of the bacterial genomics together with the advanced post-genomic mammalian host response analyses, clarification of the molecular interactions and mechanisms that deal with the host-health effects observed are beginning to be Taken together, to elevate the standards expected from a probiotic formula [238]. Recent years have seen an evolution in the development and application of molecular tools for identifying and analyzing microbal community and activity. These tools are increasingly applied to strains of lactic acid bacteria (LAB), used as probiotics, for identification and analysis of their activity. Additional aspects of probiotic LAB include their viability and vitality during processing and analysis of their actions in the gastrointestinal tract [239].

Probiotic selection criteria

The microorganisms intended for use as probiotics in aquaculture should exert antimicrobial activity and be regarded as safe not only for the aquatic hosts but also for their surrounding environments and humans [55].

Several previous reviews have proposed favorable characteristics for the selection of potential probionts for applications with fish species [9,24,240–242]. Following on these papers Merrifield et al. [22] propose an extended list of criteria for potential probiotic, some of which are essential (E) and some considered as merely favorable (F). The more of these characteristics that are fulfilled by a candidate probiotic species, the more appropriate that species shall be considered and thus more likely to be an effective fish probiotic.

As it is unlikely to find a candidate that will fulfill all of these characteristics we should begin to further explore the possibilities of simultaneously using several probiotics or the use of probiotics with prebiotics (termed synbiotics) [243]. Through the combined application of multiple favorable probiotic candidates it may be possible to produce greater benefits (and satisfy more of the previously suggested characteristics) than the application of individual probiotic.

Probiotics groups

A wide range of probiotics groups examined for use in aquaculture has been investigated; these groups can be categorized into living bacteria of both Gram-positive and Gram-negative reactions, unicellular algae, bacteriophages and yeasts. A highlight for the recent research outcome for the last 15 years is summarized in Tables 1 and 2.

Probiotics in aquaculture of Egypt: Current state

Egypt is one of the major contributors to the world aquaculture projects. Production from both wild fishing and aquaculture are of premium importance on fresh and marine continents [279]. Aquaculture development has accelerated throughout the country, since 1982, it has accounted for more than 70% of the country's aquatic production, making Egypt the largest producer of aquatic products in Africa and in high rank production in the world [280]. As fast growing sector, the desire for more and efficient production with minimal hindrances forced the producers to seek for health strategies that medley both fish and consumers. Globally, aquaculture is expanding into new intense and diverse directions. With the increasing of production manipulation, production obstacles appear among which, disease problems are of premium importance. Diseases not only lower the net production, produce low quality products, but also aid in transmission of the various etiological agents to other hosts and in some cases humans in contact, hence impeding both economic and social

development in many countries [281]. Strategic planning, etiological expectations by early warning and diseases anticipation are back stones for effective management and control [2].

Probiotics, which control pathogens through a variety of mechanisms, is increasingly studied in Egypt. The goal of this section is to tabulate the studies on the current status of using probiotics in aquaculture in Egypt Table 3.

Conclusion remark and recent prospects

Aquaculture is presented as a valuable solution to meet the growing demand for fish and shellfish needs, to meet the ongoing globalization of food shortage, improving aquaculture practices by new technological innovations for food production is a difficult assignment for scientists and biologists.

The use of probiotics offers viable alternatives for new generation of a higher-quality live product in terms of size, health, safety, production time and needs. Based on the aforementioned research results on probiotics, it is obvious that the use of probiotic agents in aquaculture is needed. At present, the probiotics are widely applied around the world with interesting results. Probiotics are pioneered by many advantages and benefits that can possibly improve the quality and quantity of the aquaculture yield. The application of probiotics will become a major field in the development of aquaculture in the future, based on the massive advantages of its application. However, there is still a need to focus on several points including: The probiotic mechanisms on both gastrointestinal and health action. Questions about differences among microbial strains in adhesion, adhesion receptors, and competitive exclusion of pathogens, and importance of microbial viability for health effects also require further study. The Scientific data emphasizes that scientific documentation is available to direct efforts to specific microbial strains and specific target subpopulations. However, characterization of novel selection criteria for new strains is needed to allow further probiotic development.

Although, next-generation sequencing methodologies offer great potential for phylogenetic identification of probiotic microorganisms without using conventional cultivation techniques, further studies and grants should be afforded to the development of molecular techniques such as PCR, FISH, DGGE and generation of genomic libraries to unveil the diversity present in aquaculture systems. Further studies and attention must be under taken to the composition of microbial communities and the administered probiotic, as it can be altered by husbandry practices and environmental conditions that stimulate the proliferation of selected bacterial species. A careful evaluation for time, type, frequency and dose of probiotic application and to assess the duration of the desired action for example as growth promoter or of immunostimulant and to make the application cost-effective need to be evaluated before any practical use in aquaculture. The administration of probiotic to food fish during harvest time must be telescoped for human health hazards and possible microbial interaction especially in live probiotic product. Also dissemination of the probiotic agents to the natural water and subsequently to the wide ecosystem must be studied to evaluate its potential effects on the microbial ecosystem balance.

Conflict of Interest

The author has declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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