Evaluation of the impact of probiotics usage in aquaculture: A systematic review

David Elisha Henry
M S Ramaiah University of Applied Sciences
Renuka Basavaiah (renukabs@gmail.com)
Promic Svasthya Private Limited

Research Article

Keywords: Aquaculture, Probiotic, Resistance, Systemic review

Posted Date: June 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1737405/v1

Abstract

In aquaculture, probiotics are widely used for preventing the diseases. Probiotics are live beneficial bacteria that are ingested into the gastrointestinal tract through food or water or any dietary supplement to boost immunity and internal microbial resistance. Several experimental studies have been undertaken on the effect of probiotics on the immune system of diverse aquatic animals, as well as the mechanism involved to control the harmful pathogens. As a result, the current study aims to conduct a systematic review to update the findings related to specific immunological markers. Relevant studies from PubMed, EMBASE, and Google Scholar were systematically searched until the latest issue. Experimental studies written in the English language were included in the review. The main outcome was to identify the insightful outcome about the impact of probiotics**.** Systemic review was performed for 12 studies. The SYRCL'S risk of bias tool for the animal study was also used to evaluate the quality of the included studies. In conclusion, the results of the current review study summarize that probiotic could boost the immune system of aquatic animals by increasing plasma immunoglobulin, cytokines, plasma lysozyme, respiratory burst, peroxidase, phagocytosis, and alternative complement activities.

Introduction

Aquaculture, one of the fastest-growing food-producing sectors, accounts for 90% to global production [1]. However, when aquatic animals are exposed to stressful conditions, diseases develop, the environment deteriorates, and substantial economic losses [1]. Broad-spectrum antibiotic and chemicals destroys both harmful and beneficial bacteria in the pond, causing the issues for aquatic organisms [1, 2]. Furthermore, the utility of antimicrobial agents results in the evolution of antimicrobial resistance among pathogenic bacteria [3]. Therefore, an alternative approach has been emerged i.e., probiotics to control only the potential pathogens in the water [4].

Probiotics are live beneficial bacteria that are given into the gastrointestinal tract via food or water or any dietary supplement that boost immunity and internal microbial resistance [1, 5]. Probiotics increase the number of beneficial bacteria, which compete with harmful microorganisms and limit their growth [3]. Furthermore, probiotics play an essential role in the degradation of organic matter, which helps to decrease the production of sludge and slime [1]. Therefore, water quality would improve by reducing the disease incidence, increasing zooplankton numbers, decreasing odors, and ultimately improving aquacultural productivity [6]. Additionally, probiotics are utilized in aquaculture to stimulate growth, nutrient absorption, and stress reduction in aquatic organisms to boost the overall production [7].

Experimental studies confirmed that commercial probiotic products prepared from bacterial species such as *Bacillus* sp., *Lactobacillus* sp., *Clostridium* spp., *Enterococcus* sp., and *Staphylococcus* sp., stimulated innate immunity and growth in aquatic animals [1, 5]. Hence, we performed a systemic review to update the knowledge regarding bacterial probiotic sources, probiotic use in fish diets against pathogenic bacteria, probiotic mode of action, and beneficial aspects of probiotics in fish. Furthermore, this review study provides valuable information for for future research for scientists, researchers, pharmacists, nutritionists, pharmaceutical industries, agricultural engineers, and veterinarians.

Methods

Search strategy and study selection

A systematic literature search using PubMed, EMBASE, and Google Scholar yielded relevant studies. The articles were screened from the databases using MeSH terms with boolean operators such as ("fish" OR "fish*" OR "aquaculture" OR "fish culture" OR "Fish farming") AND ("probiotics" OR "bacteria"). Only the experimental studies written in the English language were included in the review. Other studies such as case reports, laboratory studies, and uncontrolled trials were excluded. The publication time of the retrieved articles is from the available date of inception until the latest issue. Reference lists from relevant articles selected were hand-searched to screen potentially eligible studies (Figure 1).

Type of studies and outcome

Only experimental studies were included in the review. The following comparisons were considered: placebo and any other control except probiotics. The main outcome of the study was any insightful outcome about the effects of probiotics***.***

Data extraction and collection

A total of 481 articles were found in the literature search, with 45 duplicates were removed. The remaining 436 records were subjected to a title/abstract level screening, with 380 articles being excluded. A total of 56 full-text articles were then reviewed for eligibility based on the inclusion criteria. However, 44 articles were excluded as they were not relevant to the study. Hence, a total of 12 studies were included for systemic review and discussion. Using a pre-designed data collection form, two reviewers independently extracted relevant data from the included studies, and any discrepancies encountered were resolved through discussion between the reviewers. The main contents of the data collection form include basic information of studies (study ID, title, authors, aim, methods); reporting quality; intervention (specific information of fishes, probiotic); control, results (changes after the probiotic supplementation).

Assessment of risk of bias and relevance in included studies

Risk of bias and studies included were assessed by reviewers independently. SYRCLE's risk of bias tool was used to assess the quality of animal studies [8].

Results And Discussion

Assessment of study risk of bias

Table 1 shows the results of SYRCLE's risk of bias tool. Overall, there is a high level of certainty in the quality of studies as critical details about the study designs were included in studies [9]. In terms of selection bias, none of the studies (n = 12) provided a complete description of sequence generation process. In terms of animals' characteristics, all the studies found that the animals were similar to one another. Information about the allocation concealment was not clearly reported in few studies (n = 3). All the studies reported random housing and random outcome assessment. However, none of the studies described the blinding. None of the studies reported blinding of personnel. Incomplete outcome data were addressed in all the studies. Selective reporting and other important sources of bias were absent in all the studies included (Table 1).

Table 1: Cochrane Collaboration's tool for assessing the risk of bias

Studies	**Random sequence generation**	**Baseline characteristics**	**Allocation concealment**	**Random housing**	**Blinding**	**Random outcome assessment**	**Blinding**	**Incomplete outcome**	**Selective outcome reporting**	**C s b**
	Was the allocation sequence adequately generated and applied?	Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	Were animals selected at random for outcome assessment?	Was the outcome assessor blinded?	Were incomplete outcome data adequately addressed?	Are reports of the study free of selective outcome reporting?	V s a f c p t r h c
Standen BT 2013[10]	No	Yes	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Y
Fuchs VI_2017[11]	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Y
Salinas I_2018[12]	No	Yes	Yes	Yes	No	Yes	Unclear	Yes	Yes	Y
Gupta A_2014[13]	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Y
Pirarat N_2011[14]	No	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Y
Balcázar JL_2007[15]	No	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Y
Ljubobratovic U_2017[16]	No	Yes	Yes	Yes	No	Yes	Unclear	Yes	Yes	Y
Nayak SK_2007[17]	No	Yes	Yes	Yes	No	Yes	Unclear	Yes	Yes	Y
Selim KM_2015[18]	No	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Y
Cerezuela R_2013[19]	No	Yes	Yes	Yes	No	Yes	Unclear	Yes	Yes	Y
Liu W_2013[20]	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Y
Xing CF_2013[21]	No	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Y

Table 2 summarises the included studies. The studies were categorised with respect to fish type/breed, intervention and control arms, and main outcome.

Table 2. : The studies were categorised according to fish type/breed, intervention and control arms, and main outcome.

Studies	Fish type or breed	Intervention arm (n)	Control arm (n)	Outcomes
Standen BT 2013 [10]	Genetically male red Nile tilapia *O. niloticus* (GMT, Fishgen UK)	*P. acidilactic*	o iso-nitrogenous and iso-lipidic diets without probiotics	Probiotic treatment potentiated the immune-responsiveness of the intestine as upregulation of the gene expression of the pro-inflammatory cytokine TNFa was observed in the probiotic fed fish (P<0.05). Intraepithelial leucocyte levels (IELs) also elevated in the intestine of *P. acidilactici* fed *tilapia* after six weeks (P<0.05) of feeding and a trend towards elevated goblet cells was also observed after six weeks (P=0.08). Concomitantly at week six, along with elevated IELs and elevated TNFa mRNA levels in the intestine, an increased abundance of circulating neutrophils and monocytes were observed in fish fed the probiotic supplemented diet (P<0.05).
Fuchs VI_2017 [11]	Turbots (Scophthalmus maximus)	Four diets: a) Saccharomyces cerevisiae, b) alginic acid product of brown algal extracts c) purified yeast nucleotides (cytidine-5V-monophosphate (CMP), disodium uridine-5V-monophosphate (UMP), adenosine-5V-monophosphate (AMP), disodium inosine-5V-monophosphate (IMP), disodium guanidine-5V-monophosphate (GMP)) and ribosomal RNA, c) probiotic product of bacteria *strains B. subtilis* and *B. licheniformis*	FM-based diet (C-HF; 585 g FM protein kg−1 feed) and a FM reduced diet (C-LF; 320 g FM kg−1 feed)	Plasma lysozyme activity, neutrophil reactive oxygen species production, and total plasma protein levels did not significantly differ between treatment groups; however, plasma cholesterol increased significantly in fish fed β-glucan/MOS (GM), alginic acid (AC), nucleotides/RNA (NR) and C-HF compared to C-LF (I). Plasma glucose and triglyceride levels significantly increased with GM and NR treatments, while glucose levels were significantly higher in C-HF compared to C-LF. Immunostimulant-supplemented diets exhibited significantly lower cortisol levels compared to controls C-LF (at 0.5 h) and C-HF (at 1 h) post stress, respectively (II). FM substitution did not modulate the innate immune response but was associated with reduced levels of cholesterol.
Salinas I_2008 [12]	Seawater teleost Sparus aurata L. (a protandrous hermaphrodite species) obtained from Culmarex S.A. (Murcia, Spain)	Heat-inactivated 1 X 10^7 CFU g ^-1 *Lactobacillus*; (III) diet supplemented with heat-inactivated 1 X 10^7 CFU g ^-1 *Bacillus;* (IV) diet supplemented with heat-inactivated 0.5 X 10^7 CFU g ^-1 *Lactobacillus* and 0.5 X 10^7 CFU g ^-1 *Bacillus*	Non-supplemented commercial pelleted diet	Feeding the mixture of the two killed bacteria species significantly increased natural complement, serum peroxidase and phagocytic activities compared with controls.
Gupta A_2014 [13]	Cyprinus carpio	Three bacterial strains viz. *Bacillus coagulans* (B1), *B. licheniformis* (B2) and *Paenibacillus polymyxa* (B3)	Without probiotic	Fish fry were fed these diets and growth performance, various non-specific immune parameters and disease resistance study were conducted at 80 days post-feeding. The antagonism study showed inhibition zone against *Aeromonas hydrophila* and *Vibrio harveyi*. The growth-promoting influences of probiotic supplemented dietary treatments were observed with fish fry and the optimum survival, growth and feed utilization were obtained with *P. polymyxa* (B3) supplemented diet. Study of different non-specific innate immunological parameters viz. lysozyme activity, respiratory burst assay and myeloperoxidase content showed significant ($p < 0.05$) higher values in fish fry fed B3 diet at 109 CFU/g. Dietary supplementation of B. *coagulans*, *B. licheniformis* and *P. polymyxa* significantly ($p < 0.05$) enhanced the resistance of fish fry against bacterial challenge
Pirarat N_2011 [14]	tilapia (Oreochromis niloticus)	*L. rhamonsus* GG (ATCC 53103), was cultured in MRS broth at 30C for 48 h, centrifuged and washed with sterile PBS three times	wihtout probiotic	Supplementation of *L. rhamnosus* promoted the intestinal structure and the mucosal immunity of tilapia. Probiotic fish had a greater villous height in all parts of the intestines and, significantly, in the proximal and middle part. The population of intraepithelial lymphocytes was significantly higher in the probiotic group than in the control group in all parts of the intestines. The population of acidophilic granulocyte in the probiotic group was significantly higher at the proximal and distal parts when compared with the control group. The higher serum complement activity as well as the enhanced phagocytosis and killing ability of the head kidney leukocytes in the probiotic supplemented fish corresponded with the higher level of TNF alpha and IL-1 gene expression, suggested the induction of IL-1 and TNF alpha cytokines by *L. rhamnosus* indicating an important regulator of gut associated immune systems
Balcázar JL_2007 [15]	brown trout (Salmo trutta)	Three bacterial strains, *Lc. lactis* ssp. *lactis* CLFP 100, *Leu. mesenteroides* CLFP 196 and *Lb. sakei* CLFP 202, isolated from salmonids and genetically identified by 16S rRNA gene sequencing, were selected from a pool of 246 strains obtained from the intestinal content of healthy salmonids.	Unsupplemented food	Only *Lb. lactis* ssp. *lactis* and *Leu. mesenteroides* were detected at levels above 1 £ 102 colony-forming units/g at the end of the fourth week. In comparison to untreated control fish, the alternative complement activity in the serum was found to be significantly greater in all LAB groups at the alternative complement activity in the serum the end of the second week. Groups supplemented with *Lc. lactis* ssp. *lactis* and *Leu. mesenteroides* exhibited an elevated level of lysozyme activity at the end of the third week, but the group supplemented with Lb. sakei did not exhibit any significant change in lysozyme activity. Serum immunoglobulin levels were higher compared with the

				control group, but there was no significant difference between the LAB and control groups.
Ljubobratovic U_2017 [16]	Juvenile pike-perch (Sander lucioperca)	Two groups received the combination of OTOHIME and nauplii enriched either with *Lactobacillus paracasei* BGHN14+ *Lactobacillus rhamnosus* BGT10 or with *Lactobacillus reuteri* BGGO6−55 +*Lactobacillus salivarius* BGHO1, and one group received OTOHIME hydrolyzed by BGHN14 +BGT10 and non-enriched *nauplii*	Control group received non-enriched nauplii and non-hydrolyzed OTOHIME	There was positive effect of Artemia enriched with BGGO6−55 +BGHO1 on fish growth, skeletal development and trypsin to chymotrypsin activity ratio (T/C), as an indicator of protein digestibility. Hydrolysis of OTOHIME was also associated with better skeletal development, higher T/C values and lower levels of *Aeromonas* and M*ycobacterium* spp., which are important fish pathogens
Nayak SK_2007 [17]	Rohu (Labeo rohita Ham.)	*B. subtilis* isolated from the gastrointestine, a vitamin C treated feed contained ascorbyl polyphosphate (Hi-Media, India) at 100 mg/kg diet. A probiotic and vitamin C combined feed was prepared by mixing both *B. subtili* at 108 CFU/g and ascorbyl polyphosphate at 100 mg/kg diet	no additional supplement	The total serum protein and globulin content was significantly higher ($p < 0.05$) in probiotic (*B. subtilis* at 108 CFU/g of the feed) fed group while the respiratory burst activity of blood neutrophils was significantly high in vitamin C fed group. The antibody level was significantly high in *Bacillus subtilis* treated group followed by the probiotic (*B. subtilis* at 108 CFU/g of the feed) and ascorbyl polyphosphate combined group. The least percentage of mortality was recorded in *B. subtilis* treated group (25%) followed by 35 and 40% in ascorbyl polyphosphate treated and *B. subtilis* and ascorbyl polyphosphate combined groups, respectively
Selim KM_2015 [18]	Nile Tilapia, Oreochromis niloticus	*Bacillus amyloliquefaciens* was added to the basal diets at levels of 1×104 CFU/g (G2) and 1×10^6 CFU/g (G3)	-	After 15d of treatment, the serum killing percentages and phagocytic activities were significantly higher in G3 than in G1 and G2, whereas the same parameters had significantly higher values in G3 and G2 than in G1 after 30 d. After both 15d and 30d, the lysozyme activities and nitric oxide assay results (mmo/l) were significantly higher in G3 than G2, and the lowest values were observed in G1. The percentage of serum killing, serum nitric oxide and serum lysozyme activity were significantly increased by the time of *Bacillus amyloliquefaciens* administration independently of the probiotic dose, and the phagocytic activity percentage was significantly decreased at the end.
Cerezuela R_2013 [19]	Seawater teleost gilthead sea bream (S. aurata L.), obtained from CULMAREX S.A. (Murcia, Spain),	*B. subtilis* (CECT 35, Valencia, Spain) were grown in nutrient agar (pH 6.8; 30 degree C) plates for 2-3 days. Colonies recovered from plates were then inoculated in 750 ml nutrient broth in continuous gentle agitation for 15 h	Without probiotic	Intestinal morphometric study revealed no effect of inulin or *B. subtilis* on the intestinal absorptive area. Experimental diets cause important alterations in the intestinal microbiota by significantly decreasing bacterial diversity, as demonstrated by the specific richness, Shannon, and range-weighted richness indices. The observed alterations demonstrate that fish fed experimental diets had different signs of gut oedema and inflammation that could compromise their body homeostasis, which is mainly maintained by the epithelial lining of the gastrointestinal tract.
Liu W_2013 [20]	juvenile hybrid tilapia	The LALB and HALB strains were used. The pathogenic strain A. hydrophila NJ-1 was used in the challenge test.	Basal diet without probiotic	The adhesive gut bacterial communities were altered in the fish fed either the HALB or the LALB, but the response was more rapid and substantial with the adhesive strain. The two strains induced similar changes in the patterns (upregulation or downregulation) of intestinal, splenic or kidney cytokine expression, but they differed in the degree of response for these genes. Changes in intestinal HSP70 expression levels coincided with changes in the similarity coefficient of the adhesive gut bacterial communities between the probiotic treatments. The highest dose of the HALB appeared to protect against the toxic effects of immersion in A. hydrophila ($P < 0.05$)
Xing CF_2013 [21]	cobia (Rachycentron canadum)	Pdp strain P40, cultured in brain heart infusion (BHI) broth with 20C & salinity at 28C. The LAB 4012 was isolated from the intestine of a 3-day fasted adult cobia, and cultured in MRS broth (Fluka) at 37C, and was proved to be able to resist the treatments of gastric acid (pH 2.0) and 0.3% bile salt	LAB unfed group	After a 2-week feeding of LAB 4012, the growth rate of the fed cobia was 12% higher than that of the non-fed group, and the relative percentage of survival of the fed cobia was found to be 74.4 in Pdp immersion challenge. Respiratory burst of peripheral blood leukocytes in the LAB 4012-fed group was significantly higher than that of the non-fed group. LAB 4012 did not improve specific antibody response in cobia after immunization with Pdp vaccine, it still significantly raised the survival rate by 22% over that of the non-fed group after Pdp immersion challenge

Probiotics modulate immune responses in aquatic animals by increasing lysozyme and complement activity, inducing proinflammatory cytokines, increasing mucosal and systemic antibody production, increasing respiratory burst activity, stimulating natural killer cell activity and activating phagocytic activity. The present review study covers the effects of various probiotics on innate immune responses in various fish strains.

Probiotic impact on serum lysozyme activity

Lysozyme, a bactericidal cationic enzyme is produced by leucocytes (mainly neutrophils and macrophages) and reacts against gram-positive and few gram-negative bacteria [18, 22]. When infected with microorganisms, it attacks the peptidoglycan layers in the cell walls of bacteria, lyzes them, and increases the sera of fish [22]. In Selim et al. study, *Bacillus amyloliquefaciens* activated serum lysozyme in tilapia when supplemented at 104 and 106 CFU/g [18]. In rainbow trout, *olive flounder*, and *paralichthys olivaceus*, *Bacillus* strains supplementation boosted lysozyme activity [23–26]. These results imply that probiotics enhances the innate immune system in aquatic amimals via serum lysozyme activity.

Probiotic impact on plasma lysozyme activity

Tretament of Nile tilapia with probiotic bacteria, *B. subtilis,* at a concentration of 5×10^{6} CFU g feed^{-1} promoted the innate immune system by increasing the mean corpuscular hemoglobin and improving macrophages plasma lysozyme activity [26]. Supplementation of commercial bacillus probiotic significantly increased the plasma lysozyme activity in rainbow trout [27]. A few former studies have found that administration of probiotic diet for 8 weeks controlled multiple bacterial diseases via plasma lysozyme activity [28,29]. In a meta-analysis review study, probiotics had no effect on plasma lysozyme activity, and the effect slightly declined with an increase in duration of the experiment [22].

Probiotic impact on plasma immunoglobulin

Probiotics interact with immune cells such as monocytes, macrophages, neutrophils, and natural killers cells, and enhance their natural immune responses in different fish.[17] Immunoglobulins (Ig) are known to have a protective function against disease in humans and animals. Administration of three bacterial strains of *Lc. lactis ssp*. (lactis CLFP 100, *Leu. mesenteroides* CLFP 196 and *Lb. sakei* CLFP) at 106 CFU/g feed for two weeks elevated the serum immunoglobulin levels in brown trout (*Salmo trutta*), but not at a significant level [15]. Similarly, probiotics tretament boosted the plasma Ig levels in rainbow trout, however, the levels decreased as increase in duration of the experiments [22]. Other similar study conducted on rainbow trout found that probiotics increased the Ig levels in a transient manner and the levels declined after stopping the probiotics [30].

Probiotic impact on cytokine

Cytokines, including chemokines, interferons, interleukins, lymphokines, and tumor necrosis factor, are released by cells and influence the behavior of other cells and even sometimes produces other cells [31]. *Lb. plantarum* supplementation upregulated the expression of IL-1b, IL-10, and TNF in the head kidney [32]. In another study, feeding to Nile tilapia with *Pediococcus acidilactici* upregulated the expression of proinflammatory cytokine TNF-a [10]. In a study by Kim et al., after feeding with *Lactococcus lactis* upregulated the IL-1, and IFN- c expression in olive flounder (*Paralichthys olivaceus*) [33]. This indicates diverse microorganisms can be used as probiotics in aquaculture to stimulate or modulate immune responses.

Probiotic impact on peroxidase activity

The peroxidase enzyme, which produces hypochlorous acid using oxidizing radicals kills pathogens. The peroxidase is released from the azurophilic granules of neutrophils during the oxidative respiratory burst. Supplementation of probiotics such as *B. subtilis*, alone or in combination with *N. delbrueckii* spp. Lactis for three weeks stimulated the serum protease activity but did not increases the peroxidase activity of head kidney leukocytes from *S. aurata* [12]. Similarly, in a Wang et al.study., treatment of *E. faecium* at a concentration of 107 CFU/Ml for every 4-40 days significantly increased the serum peroxidase activity in *O. niloticus* [4].

Probiotic impact on serum alternative hemolytic complement pathway

The activity of alternative hemolytic complement pathway activity in fish is another most crucial component of the innate immune system. The bacterial strains of *Lc. lactis* ssp. at 106 CFU/g feed for 2 weeks increased the serum alternative complement activity in all the LAB groups when compared to control [15]. Meta-analysis also demonstrated that probiotics could increase the immunity of rainbow trout via stimulating serum alternative hemolytic complement pathway [22]. While other studies reported that the results may varies concerning the type of bacteria [34] and duration of experiment [35].

Probiotic impact on serum total protein

Blood protein levels have been found to be a good indicator of fish health. A meta-analysis review study also reported that probiotics increase the serum total protein in rainbow trout [22]. Several previous studies have found that probiotics have increasing effects on serum total protein in fish [17,36]. Other studies reported probiotic treatment had no effect on serum total protein [11,37]. However, high levels of serum total protein, on the other hand, seems to stimulate an innate immunological response in fish.

Probiotic impact on serum complement activity

The complement system is another essential serum compound that play a vital role in cellular defense mechanism of fish by directly killing microorganisms either through lysis, phagocytosis, chemical adsorption and activation of leukocytes, and other immune compounds or modulation of specific antibody responses by localization of antigens for B lymphocytes [38,39]. A recent meta-analysis study demonstrated that probiotic treatment in rainbow trout increased the activity of the complement system and the activity elevated in response to higher concentrations of probiotics [22]. In Pirarat et al., treatment to *tilapia* with *L. rhamnosus* improved the serum complement activity [14].

Probiotic impact on phagocyte activity

Phagocytes are white blood cells (neutrophils and macrophages), which engulf foreign bacteria and kill them via the production of reactive oxygen species such as hydrogen peroxide, superoxide anion, and hydroxyl radicals during respiratory burst [31]. In a Salinas et al., heat inactivated *lactobacillus* bacteria significantly increased the phagocytic activities in seawater teleost *Sparus aurata L* when compared with controls [12]. Similarly in a meta-analysis review study, probiotics increased the phagocytic activity in rainbow trout [22]. In a comparable study conducted by Pirarat et al., a two weeks supplementation of *L. rhamnosus* in *tilapia* (*Oreochromis niloticus*) significantly stimulated the phagocytosis activity [40].

Probiotic impact on respiratory burst

Respiratory burst activity is the other strong defense mechanism of fish against pathogens, where the probiotics improve the respiratory burst of phagocytic cells and protect the cells [31]. Xing et al. study, demonstrated that treatment with lactic acid bacteria (LAB 4012) for two weeks significantly improved the respiratory burst of peripheral blood lymphocytes and stimulated the protection of Cobia (*Rachycentron canadum*) from photobacteriosis challenge [21]. Rahimi et al. reported that probiotics had no affect the respiratory burst activity [22]. Furthermore, an increase in the duration of experiment and the growth of fish reduced the respiratory burst activity [22]. Therefore, further studies are needed to reach a definite conclusion on the effects of the probiotics on respiratory burst activity.

Despite the fact that systematic reviews are the gold standard and preferred technique for conducting experimental studies, they are not exclusive. Scoping reviews, which are commonly used to uncover knowledge gaps, are allowed. We recongnize that the number of studies included was inadequate when compared to a broad topic. Furthermore, the inclusion of only a few databases might have overlooked other publications that are not included in these databases. Furthermore, although we did not assign a time frame to the publications obtained in this study; the earliest article published was in the year 2007 in the included studies. Hence, older articles that did not use the searched term in the titles or abstracts may be overlooked. Another limitation encountered is the exclusion of journals published in languages other than English. Neverthless the low risk of bias identified in most studies is the main strength of this review.

In conclusion, the current review summarizes probiotics could improve the immune system of aquatic animals by increasing plasma immunoglobulin, cytokine activity, plasma lysozyme activity, respiratory burst activity, peroxidase activity, phagocytosis activity, and alternative complement activity. However, additional high-quality, large-scale trials might require to be performed to substantiate the view of the study.

Declarations

Acknowledgments

All the authors have contributed equally to the development of the review article. Dr. David Elisha Henry would like to thank the vice-chancellor, M S Ramaiah University of Applied Sciences for the support and for providing the seed grant.

References

1. Sahu M, Swarnakumar N, Sivakumar K, T. T, Kannan L. 2008. Probiotics in aquaculture: Importance and future perspectives. Indian J Microbiol 48: 299–308.
2. Ninawe AS, Selvin J. 2009. Probiotics in shrimp aquaculture: Avenues and challenges. Crit Rev Microbiol 35: 43–66.
3. Fernandes S, Kerkar S. Chapter 14 - Bacterial probiotics over antibiotics: A boon to aquaculture. In: Meena SN, Naik MM, editors. Advances in Biological Science Research [Internet]. Academic Press; 2019 [cited 2022 Apr 12]. p. 215–32. Available from: https://www.sciencedirect.com/science/article/pii/B9780128174975000148
4. Wang YB, Li JR, Lin J. 2008. Probiotics in aquaculture: Challenges and outlook. Aquaculture 281: 1–4.
5. El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MAO, et al. 2021. The functionality of probiotics in aquaculture: An overview. Fish Shellfish Immunol 117: 36–52.
6. Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC. 2012. Use of probiotics in aquaculture. *ISRN Microbiol* 2012: 916845.
7. Probiotics in Aquaculture | Probiotics To Reduce Pathogens-invivo solution [Internet]. invivo. 2020 [cited 2022 Apr 12]. Available from: https://invivo.solutions/probiotics-in-aquaculture/
8. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. 2014. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14: 43.
9. Ryan EA, Imes S, Wallace C, Jones S. 2000 Herbal tea in the treatment of diabetes mellitus. Clin Investig Med Med Clin Exp 23: 311–7.
10. Standen BT, Rawling MD, Davies SJ, Castex M, Foey A, Gioacchini G, et al. 2013. Probiotic Pediococcus acidilactici modulates both localised intestinal- and peripheral-immunity in tilapia (Oreochromis niloticus). Fish Shellfish Immunol 35: 1097–104.
11. Fuchs VI, Schmidt J, Slater MJ, Buck BH, Steinhagen D. 2017. Influence of immunostimulant polysaccharides, nucleic acids, and Bacillus strains on the innate immune and acute stress response in turbots (Scophthalmus maximus) fed soy bean- and wheat-based diets. Fish Physiol Biochem 43: 1501–15.
12. Salinas I, Abelli L, Bertoni F, Picchietti S, Roque A, Furones D, et al. 2008. Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol 25: 114–23.
13. Gupta A, Gupta P, Dhawan A. 2014. Dietary supplementation of probiotics affects growth, immune response and disease resistance of Cyprinus carpio fry. Fish Shellfish Immunol 41: 113–9.
14. Pirarat N, Pinpimai K, Endo M, Katagiri T, Ponpornpisit A, Chansue N, et al. 2011. Modulation of intestinal morphology and immunity in nile tilapia (Oreochromis niloticus) by Lactobacillus rhamnosus GG. Res Vet Sci 91: e92–7.

15. Balcázar JL, de Blas I, Ruiz-Zarzuela I, Vendrell D, Calvo AC, Márquez I, et al. 2007. Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (Salmo trutta). Br J Nutr 97: 522–7.
16. Ljubobratovic U, Kosanovic D, Vukotic G, Molnar Z, Stanisavljevic N, Ristovic T, et al. 2017. Supplementation of lactobacilli improves growth, regulates microbiota composition and suppresses skeletal anomalies in juvenile pike-perch (Sander lucioperca) reared in recirculating aquaculture system (RAS): A pilot study. Res Vet Sci 115: 451–62.
17. Nayak SK. 2010. Probiotics and immunity: a fish perspective. Fish Shellfish Immunol 29:2–14.
18. Selim KM, Reda RM. 2015. Improvement of immunity and disease resistance in the Nile tilapia, Oreochromis niloticus, by dietary supplementation with Bacillus amyloliquefaciens. Fish Shellfish Immunol 44: 496–503.
19. Cerezuela R, Fumanal M, Tapia-Paniagua ST, Meseguer J, Moriñigo MÁ, Esteban MÁ. 2013. Changes in intestinal morphology and microbiota caused by dietary administration of inulin and Bacillus subtilis in gilthead sea bream (Sparus aurata L.) specimens. Fish Shellfish Immunol 34: 1063–70.
20. Liu W, Ren P, He S, Xu L, Yang Y, Gu Z, et al. 2013. Comparison of adhesive gut bacteria composition, immunity, and disease resistance in juvenile hybrid tilapia fed two different Lactobacillus strains. Fish Shellfish Immunol 35: 54–62.
21. Xing CF, Hu HH, Huang JB, Fang HC, Kai YH, Wu YC, et al. 2013. Diet supplementation of Pediococcus pentosaceus in cobia (Rachycentron canadum) enhances growth rate, respiratory burst and resistance against photobacteriosis. Fish Shellfish Immunol 35: 1122–8.
22. Rahimi R, Mirahmadi SA, Hajirezaee S, Fallah AA. 2022. How probiotics impact on immunological parameters in rainbow trout (Oncorhynchus mykiss)? A systematic review and meta-analysis. Rev Aquac 14: 27–53.
23. Liu CH, Chiu CH, Wang SW, Cheng W. 2012. Dietary administration of the probiotic, Bacillus subtilis E20, enhances the growth, innate immune responses, and disease resistance of the grouper, Epinephelus coioides. Fish Shellfish Immunol 33: 699–706.
24. Harikrishnan R, Balasundaram C, Heo MS. 2010. Lactobacillus sakei BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, Epinephelus bruneus. Fish Shellfish Immunol 29: 1037–43.
25. Sharifuzzaman SM, Austin B. 2010. Development of protection in rainbow trout (Oncorhynchus mykiss, Walbaum) to Vibrio anguillarum following use of the probiotic Kocuria SM1. Fish Shellfish Immunol 29: 212–6.
26. Telli GS, Ranzani-Paiva MJT, Dias D de C, Sussel FR, Ishikawa CM, Tachibana L. 2014. Dietary administration of Bacillus subtilis on hematology and non-specific immunity of Nile tilapia Oreochromis niloticus raised at different stocking densities. Fish Shellfish Immunol 39: 305–11.
27. Ramos MA, Gonçalves JFM, Costas B, Batista S, Lochmann R, Pires MA, et al. Commercial Bacillus probiotic supplementation of rainbow trout (Oncorhynchys mykiss) and brown trout (Salmo trutta): growth, immune responses and intestinal morphology. 2017 [cited 2022 Apr 11]; Available from: https://pubag.nal.usda.gov/catalog/5878596
28. Brunt J, Newaj-Fyzul A, Austin B. 2007. The development of probiotics for the control of multiple bacterial diseases of rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis 30: 573–9.
29. Merrifield D l., Dimitroglou A, Bradley G, Baker R t. m., Davies S j. 2010. Probiotic applications for rainbow trout (Oncorhynchus mykiss Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. *Aquac Nutr* 16: 504–10.
30. Andani HRR, Tukmechi A, Meshkini S, Sheikhzadeh N. 2012. Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (Oncorhynchus mykiss). J Appl Ichthyol 28: 728–34.
31. Akhter N, Wu B, Memon AM, Mohsin M. 2015. Probiotics and prebiotics associated with aquaculture: A review. Fish Shellfish Immunol 45: 733–41.
32. Pérez-Sánchez T, Balcázar JL, Merrifield DL, Carnevali O, Gioacchini G, de Blas I, et al. 2011. Expression of immune-related genes in rainbow trout (Oncorhynchus mykiss) induced by probiotic bacteria during Lactococcus garvieae infection. Fish Shellfish Immunol 31: 196–201.
33. Kim D, Beck BR, Heo SB, Kim J, Kim HD, Lee SM, et al. 2013. Lactococcus lactis BFE920 activates the innate immune system of olive flounder (Paralichthys olivaceus), resulting in protection against Streptococcus iniae infection and enhancing feed efficiency and weight gain in large-scale field studies. Fish Shellfish Immunol 35: 1585–90.
34. Sun YZ, Yang HL, Ma RL, Song K, Li JS. 2012. Effect of Lactococcus lactis and Enterococcus faecium on growth performance, digestive enzymes and immune response of grouper Epinephelus coioides. Aquac Nutr 18: 281–9.
35. Korkmaz OA, Sumlu E, Koca HB, Pektas MB, Kocabas A, Sadi G, et al. 2019. Effects of Lactobacillus Plantarum and Lactobacillus Helveticus on Renal Insulin Signaling, Inflammatory Markers, and Glucose Transporters in High-Fructose-Fed Rats. Med Kaunas Lith 55: E207.
36. Ramesh D, Souissi S. 2018. Effects of potential probiotic Bacillus subtilis KADR1 and its subcellular components on immune responses and disease resistance in Labeo rohita. Aquac Res 49: 367–77.
37. Vazirzadeh A, Roosta H, Masoumi H, Farhadi A, Jeffs A. 2020. Long-term effects of three probiotics, singular or combined, on serum innate immune parameters and expressions of cytokine genes in rainbow trout during grow-out. Fish Shellfish Immunol 98: 748–57.
38. Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S, Sugita H. 2004. Immune responses in rainbow trout Oncorhynchus mykiss induced by a potential probiotic bacteria Lactobacillus rhamnosus JCM 1136. Vet Immunol Immunopathol 102: 379–88.
39. [Effects of bactocell probiotic and manan peribiotic on growth and immunity in rainbow trout [Oncorhynchus mykiss] | Virtual Health Sciences Library [Internet]. 2019 [cited 2022 Apr 11]. Available from: https://vlibrary.emro.who.int/imemr/effects-of-bactocell-probiotic-and-manan-peribiotic-on-growth-and-immunity-in-rainbow-trout-oncorhynchus-mykiss-2/
40. Pirarat N, Kobayashi T, Katagiri T, Maita M, Endo M. 2006. Protective effects and mechanisms of a probiotic bacterium Lactobacillus rhamnosus against experimental Edwardsiella tarda infection in tilapia (Oreochromis niloticus). Vet Immunol Immunopathol 113: 339–47.

Figures

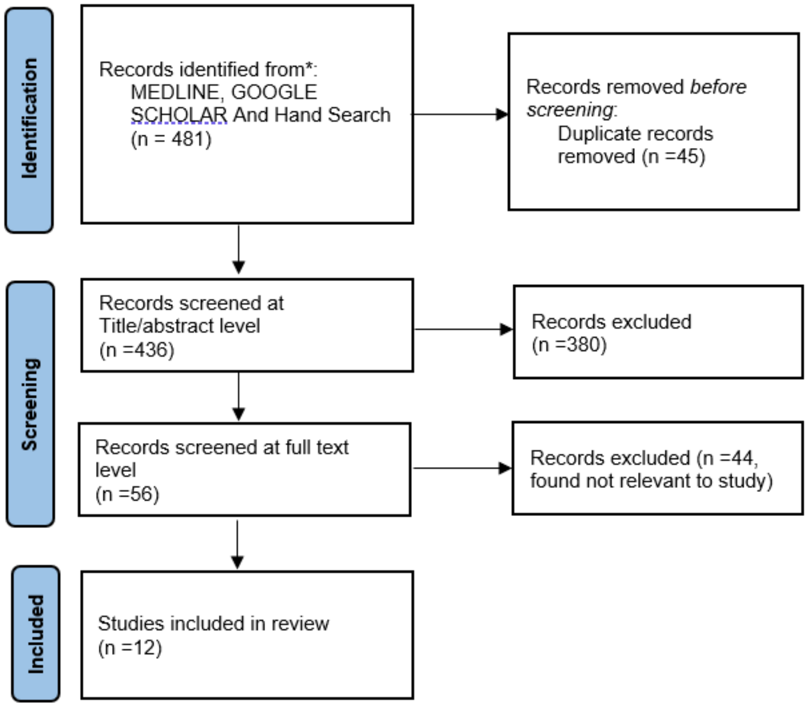

Figure 1

PRISMA flow diagram for the systematic review which included searches of databases