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The potential use of *Pediococcus* spp. probiotic in aquaculture: A review

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SUMMARY

Aquaculture production has significantly increased over the previous few decades. However, antibiotics have been implemented regularly and extensively to overcome outbreaks of pathogens in aquaculture to cover the human needs for animal protein, leading to the appearance of resistant strains that may cause serious damages in the environment and also human health. In the last few years, the implementation of probiotics as an alternative technique to antibiotics use in fish production has achieved promising results in aquaculture due to their beneficial impact on fish health and growth performance. Among different types of probiotics, the Pediococcus spp. bacteria stand out as a promising probiotic for their beneficial impact to aquaculture. Thus, the current study has been conducted to give an overview about the interactions between Pediococcus spp. and aquaculture. In addition, this review highlights the role of Pediococcus spp in promoting growth performance, improving feed conversion ratios and the intestinal architecture, enhancing the immune response and inhibiting fish pathogens, thereby preventing or at least reducing the use of antibiotics. Practical use of Pediococcus spp. probiotic in aquaculture as feed additives through selected case studies is also considered.

Keywords: *Probiotics; Pediococcus spp.; growth performance; gut microbiota; aquaculture; disease resistance*

INTRODUCTION

According to FAO (2024a), the aquaculture industry continues to be one of the major source of food for millions of people around the globe. By returning to the fishery yearbook (2021), around 89% of overall fish production was used directly for human consumption (FAO, 2024b). Bacterial pathogens, which affect fish production are routinely occurred and, these pathogens are include mainly Gram negative bacteria, such as *Aeromonas salmonicida, A. hydrophila*, *Vibrio harveyi*, *V. anguillarum, Flavobacterium psychrophilum*, *Pseudomonas fluorescens*, *Yersinia ruckeri,* and *Citrobacter freundii*; rarely, Gram positive bacteria, such as *Streptococcus* spp., that may cause fish health related problems (Lewbart, 2001). These pathogens and unfavorable environmental factors continually effect productivity, resulting in significant losses for farmers. Poor fish management practices such as excessive stock densities, overfeeding, and water contamination also contribute to the occurrence and spread of pathogens in aquaculture systems (Abarike et al., 2018 and Hasan et al., 2020).

Antibiotics are extensively applied in aquaculture to overcome diseases. As a result, bacteria with the potential confer antibiotic resistance and then negatively affect aquaculture production, fish consumers' health and the environment, may appear (Watts et al., 2017; Elsabagh et al., 2018; Won et al., 2020). The continuous use of antibiotics to overcome pathogens in aquaculture has led to the development of selective pressure on the microbial community and it has led to development of antibiotic-resistant bacteria that can spread widely (Gao et al., 2012). Alternatively, probiotics are being employed as an ecofriendly feed supplements for achieving sustainability in aquaculture industry (Zibiene and Zibas, 2019).

According to Gismondo et al. (1999), the probiotic term comes from two Greek words "pro" meaning "for" and "bios" meaning "life"; however, the original probiotic definition was proposed by Parker (1974), who defined probiotic as microorganisms and substances contributing to intestinal microbial balance. Several definitions of probiotics have been proposed whenever new findings emerged. According to Fuller (1989), probiotics are live microbial feed supplements or cultured products, which beneficially affect the host by enhancing the microbial balance. A probiotic is a single or mixed culture of live microorganisms used to improve the indigenous gut microbiota (Havenaar et al., 1992). According to Skjermo and Vadstein (1999) probiotics are live beneficial bacteria that enhance the viability of their host. Furthermore, probiotics are defined as 'live micro-organisms which when administered in adequate quantity produce health benefits to the host' (FAO/WHO 2001). A probiotic can also be a live, dead or cell component of microbes, which is consumed feed or water additives, to benefit the host by enhancing health status, increasing disease resistance, improving growth parameters and feed utilization, stress response or general health, all these factors are achieved via improving the hosts microbial balance or the microbial balance of the aquatic environment (Merrifield et al., 2010). Many researches have proven that probiotics are useful in a wide range of fish and aquatic organisms (Opiyo et al., 2019;

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Nargesi et al., 2020; Kari et al., 2022). Probiotics offer benefits to their hosts via different ways such as enhancing growth parameters (Boonthai et al., 2011; Kumar et al., 2006; Silva et al., 2013), or decreasing the disease incidence (Silva et al., 2013; Newaj-Fyzul et al., 2007). Moreover, probiotics have been demonstrated to be effective in a range of aquatic environments, including freshwater (Rahiman et al., 2010), marine and brackish water (Vijayan et al., 2005). The majority of probiotics in use are members of the lactic acid bacteria (LAB) group, including genera such as *Leuconostoc, Pediococcus, Lactococcus, Enterococcus,* and *Oenococcus* (Ouwehand et al., 2002).

Among a wide range of probiotics, *Pediococcus* spp. belong to the family Lactobacillaceae, which is a key member of LAB. *Pediococcus* spp. stand out as effective probiotics for their strong benefits to aquaculture industry. Positive correlations between *Pediococcus* spp. and fish performance from different species such as Nile tilapia (*Oreochromis niloticus*) (Hendam et al., 2023), European sea bass (*Dicentrarchus labrax* L.) (Eissa et al., 2021), zebrafish (*Danio rerio*) (Ahmadifar et al., 2023), rainbow trout (*Oncorhynchus mykiss*) (Kousha et al., 2020), shrimp (*Litopenaeus vannamei*) (Adel et al., 2017) were recently recorded. To the best of our knowledge, numerous review articles have been published on the beneficial effects of probiotics in aquaculture. However, there is a no published review on the beneficial use of *Pediococcus* spp. alone in aquaculture. Consequently, the objective of the present review is to provide a collection of notes on the functionality and mode of action of the probiotic *Pediococcus* spp. in aquaculture, based on the findings of recently published studies.

METHODOLOGY

In this review, the literature were searched from two databases including: PubMed and Web of Science (WoS) databases. Original research articles were processed, which were published in last decade and clearly provided the impact of *Pediococcus* spp. supplemented diets in aquaculture based on growth parameters, feed utilization efficiency, intestinal architecture and integrity, diseases resistance, gut microbiome, and blood parameters. The extracted data that were closely relevant are considered, viewed, summarized and discussed.

RESULTS AND DISCUSSION

Effect of *Pediococcus* spp. probiotic on growth performance, feed utilization and survival of fish

Several research studies have indicated that the dietary supplementation with *P. acidilactici* could lead to improved specific growth rates (SGR %/day), feed conversion ratios (FCR) and survival rate (%) in fish (*Figure 1* and *Table 1*). For instance, Eissa et al. (2021) noticed that growth performance (SGR, weight gain rate %) of European sea bass (*Dicentrarchus labrax* L.) at initial body weight (9 ± 0.2 g) was significantly improved when fish received a diet supplemented with *P. acidilactici* for 8 weeks. Similarly, a study about shrimp (*Penaeus vannamei*) showed that final body weight, survival, FCR, and weight gain (%) were significantly improved at *P. acidilactici* supplementation for 60 days (Wu et al., 2022). Furthermore, Valipour et al. (2018) noticed that the weight gains and FCR of Caspian kutum (*Rutilus kutum*) could be improved when fish received diet supplement with *P. acidilactici* for 60 days. Very recently, Nile tilapia (*Oreochromis niloticus*) showed a remarkable improvement in final body weight, FCR, SGR, and weight gain when fish were fed with a diet supplemented with *P. acidilactici* for 56 days.

Another method of enhancing growth parameters is the ability of the probiotic *Pediococcus* spp to activate digestive enzymes secretion. This probiotic genus has been demonstrated to stimulate the production of protease, amylase, and lipase in the gastrointestinal tract (Arani et al., 2019). These enzymes play an important role in the breakdown of feed components, thereby facilitating nutrient absorption. A recent study on zebrafish demonstrated that dietary supplementation with the probiotic *P. acidilactici* not only improved growth parameters and feed efficiency but also enhanced the activity of digestive enzymes, leading to more efficient nutrient utilization (Ahmadifar et al., 2023). This enzymatic enhancement has particular benefit in aquaculture, where optimal nutrient absorption is crucial for growth performance.

Effect of *Pediococcus* spp probiotic on diseases resistance and immunity parameters of fish

Pediococcus spp. showed various beneficial impact on aquatic animal species, including enhanced immune parameters and increased resistance against pathogenic bacteria (*Figure 1* and *Table 2*). Huang et al. (2014) who isolated the probiotic *P. pentosaceus* (4012) from the intestine of cobia (*Rachycentron canadum*), noted that antibacterial activity against *Vibrio anguillarum,* a pathogenic bacterium under *in vitro* conditions was relatively high. In addition, dietary supplementation by *P. pentosaceus* 4012 remarkably reduced the mortality rate (%) of groupers (Epinephelinae) after challenging test with *V. anguillarum*. Diets enriched with the probiotic *P. acidilactici* could increase the rate of resistance of rainbow trout to symptoms associated with vertebral compression syndrome (Aubin et al., 2005). In addition, the administration of a mixture containing galacto-oligosaccharides and *P. acidilactici* for 60 days could improve the immune related parameters and resistance against the pathogen *Streptococcus iniae* in fry of rainbow trout. Numerous previous studies revealed an increasing attention about using of *P. acidilactici* as an effective aquaculture probiotic. *P. acidilactici* in combination with fructo-oligosaccharides was fed to Atlantic salmon, *Salmo salar* for two months and led to enhanced lysozyme activity and a higher infiltration of epithelial leucocytes in the intestine (Abid et al., 2013). The quantitative PCR showed that the upregulation of immune related genes such as of IL1b, TLR3, TNFa, MX-1and IL8 were detected in the anterior and posterior parts of intestine (Abid et al., 2013).

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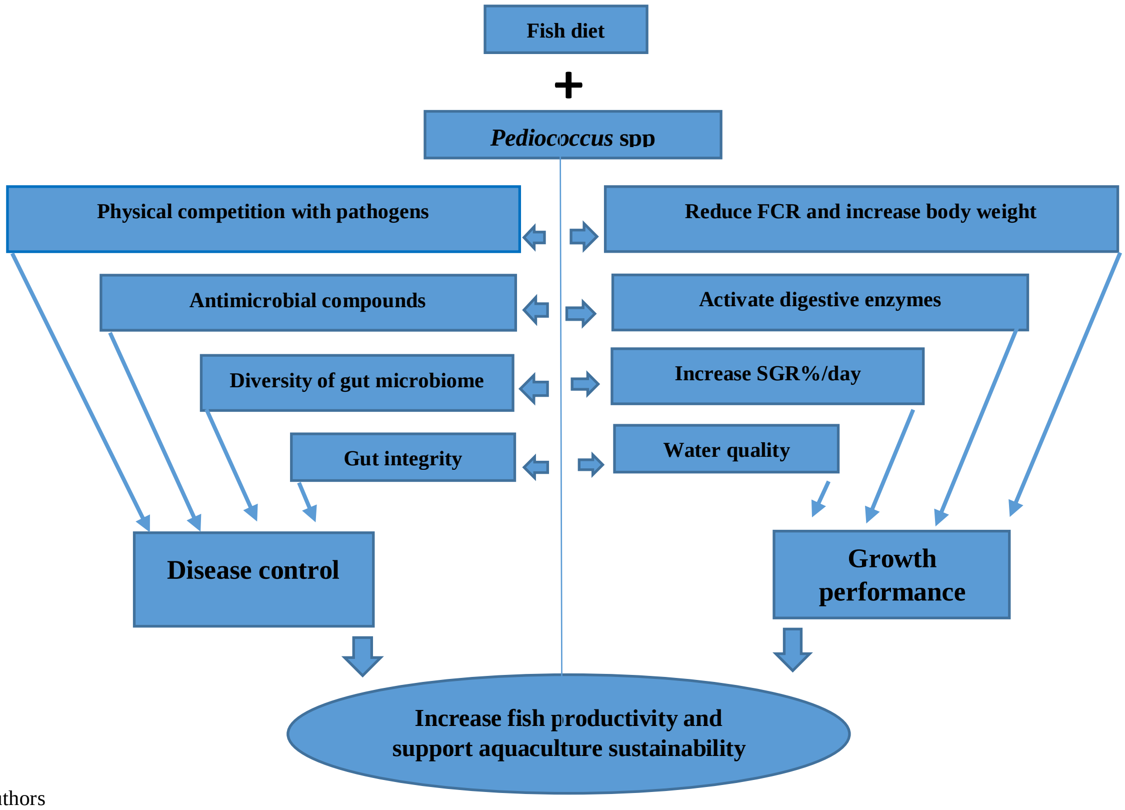
Figure 1. **The interaction between *Pediococcus* spp probiotic and their hosts in aquaculture**



source: authors

It seems that *Pediococcus* species can be considered as an efficient disease protection tool in fish aquaculture, because several studies approved the ability of *Pediococcus* to control various types of fish pathogens. For example, *P. pentosaceus* improved the tolerance of white shrimp (*Litopenaeus vannamei*) against pathogenic bacterium, *Vibrio anguillarum* (ATCC12486) in a 14 days lasting experiment (Adel et al., 2017). Recently, Hendam et al. (2023) noticed that resistance of Nile tilapia against *Aspergillus flavus* can be increased when fish received diet supplemented with *P. acidilactici* for 56 days. In addition, the probiotic's culture of the microencapsulated *P. acidilactici* exhibited strong antimicrobial activity against pathogenic *Escherichia coli* (APEC) under in vitro condition (Mitsuwan et al., 2023).

One of the key mechanisms through which *Pediococcus* spp. enhances disease resistance is by modulating the immune response of aquatic organisms. Research has shown that dietary supplementation with *P. acidilactici* can lead to increased levels of immune-related parameters, such as lysozyme activity and immunoglobulin production (Newaj-Fyzul and Austin, 2014). For example, *Pediococcus* spp. can produce antimicrobial compounds such as bacteriocins against pathogenic bacteria, which are antimicrobial peptides produced by lactic acid bacteria (Mitsuwan et al., 2023). These bacteriocins can reduce the growth of harmful bacteria, e.g. *Vibrio* species, which cause diseases in aquaculture (Pinoargote and Ravishankar, 2018).

Effect of *Pediococcus* spp. probiotic on gut microbiome and intestinal architectures of aquatic organisms

Modulation of gut microbiota

One of the key mechanisms by which *Pediococcus* spp. exert their beneficial effects is through the enhancement of the diversity and stability of gut microbiota. *P. acidilactici*, has been noticed to promote the growth of beneficial bacteria while inhibiting pathogenic strains that cause dysbiosis. For instance, *P. acidilactici* has been associated with increased populations of beneficial lactic acid bacteria in the gut, which can enhance the overall microbial balance and functionality of the intestinal microbiome (Standen et al., 2013; Standen et al., 2015). This shift in microbial composition is crucial for maintaining gut health, as a diverse microbiome is often linked to improved nutrient absorption and immune function (Hines et al., 2022).

In addition, *Pediococcus* spp. can also regulate the abundance of LAB which produce short-chain fatty acids (SCFAs) through fermentation processes. SCFAs are known to play a significant role in gut health by serving as an energy source for colonocytes and having anti-inflammatory properties (Sullam et al., 2012). Also, the fermentation of dietary fibers by *Pediococcus* spp. can lead to increased SCFAs production, which in turn supports gut health and enhances the overall metabolic profile of the host (Ma et al., 2022).

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Table 1. **Effect of *Pediococcus* spp. on growth parameters and survival of different fish species based on selected literature**

Host x *Pediococcus* spp.	Period of the experiment (days)	Dosage	Effect	Reference
Red tilapia (***Oreochromis niloticus***) x *P. acidilactici*	32	10^7 CFU g^{-1}	Growth rate (%) **NE*** Survival ↑	Ferguson et al. (2010)
Nile tilapia (***Oreochromis niloticus***) x *P. acidilactici*	56	0.1 to 0.3 g/m^3 water	Growth rate ↑ Survival ↑ Feed conversion ratio (FCR) ↓ Specific growth rate SGR%/day ↑	Hendam et al. (2023)
Nile Tilapia (*Oreochromis niloticus*) x *P. acidilactici*	56	2.0, 2.5, and 3.0 g/kg diet	Final weight ↑ FCR ↓ SGR (%/day) ↑ Weight gain % ↑	Eissa et al. (2023)
Nile Tilapia (*Oreochromis niloticus*) x *P. acidilactici*	45	2.81×10^6 CFU g^{-1}	Weight gain rate (%) NE SGR (%/day) NE Final weight (g) NE	Standen et al. (2013)
Atlantic salmon (*Salmo salar*) x *P. acidilactici*	63	–	weight gain rate (%) ↑ SGR%/day ↑	Gong et al. (2019)
European sea bass (*Dicentrarchus labrax* L.) x *P. acidilactici*	60	2.0, 2.5, and 3.0 g/kg diet	Weight gain rate (%)↑ SGR (%/day) ↑ Final weight (g) ↑	Eissa et al. (2022)
Common carp (*Cyprinus carpio*) x *P. pentosaceus*	45	10^8 CFU g^{-1}	Weight gain rate (%)↑ SGR (%/day) ↑ Final weight (g) ↑	Ahmadifar et al. (2019)
Common carp (*Cyprinus carpio*) x *P. acidilactici*	42	1 g/kg	Weight gain **NE** FCR **NE** SGR (%/day) **NE** Survival **NE**	Unpublished work
Shrimp (*Penaeus vannamei*) x *P. acidilactici*	60	10^3, 10^5, and 10^7 CFU /g	Final weight (g) ↑ Survival (%) ↑ FCR ↓ Weight gain % ↑	Wu et al. (2022)
Shrimp (*Litopenaeus vannamei*) x *P. pentosaceus*	60	10^6, 10^7 and 10^8 CFU/g	Body weight (g) ↑ Weight gain (cm) ↑ Final length (cm) ↑ Survival rate (%) ↑ Protease and amylase ↑	Adel et al. (2017)
Zebrafish (*Danio rerio*) x *P. acidilactici*	56	10^7 CFU/g	Final weight ↑ FCR ↓ Weight gain ↑	Ahmadifar et al. (2023)
Caspian kutum (*Rutilus kutum*) x *P. acidilactici*	60	1×10^9, 2 :1×10^9, 3×10^9 CFU kg^{-1}	Weight gain↑ FCR ↓ SGR (%/day) ↑ Survival ↑	Valipour et al. (2018)

"↓": Decrease, "↑": increase, "NE": no effect

Improvements of intestinal architectures

The presence of *Pediococcus* spp. can lead to structural improvements in the intestinal architecture. Studies have indicated that probiotics can increase the number of goblet cells and intestinal villi, which are essential for nutrient absorption and barrier function (Standen et al., 2013; Standen et al., 2015). The enhancement of these structural features can improve the gut's ability to resist pathogen invasions and maintain homeostasis. For example, *P. acidilactici* has been shown to increase the levels of intestinal epithelial cells, which play a critical role in forming a protective barrier against pathogens (Standen et al., 2013; Standen et al., 2015). In addition, the intestinal histology of European sea bass (*Dicentrarchus labrax* L.) showed a significant increase of the muscular layer thickness

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comparing to the control. Correspondingly, the average villi height can significantly ($p<0.001$) improved compared to the control (Eissa et al., 2022 and 2023). Very recently, *P. acidilactici* has also showed a remarkable improvement of intestinal structures (villi width, villus surface area and crypt depth) of the common carp (*Cyprinus carpio*) that received a diet supplemented with, *P. acidilactici* for 42 days in recirculating aquaculture system (unpublished results, University of Debrecen, Hungary).

Table 2. **Effect of *Pediococcus* spp. on disease resistance, immunity parameters and gut microbiome of different fish species based on selected literature**

Host x *Pediococcus* spp.	**Period of the experiment (days)**	**Dosage**	**Outcomes**	**Reference**
Red tilapia (*Oreochromis niloticus*) x *P. acidilactici*	32	10^7 CFU g^{-1}	Gut integrity, erythrocytes and leucocyte levels **NE*** Haematocrit (PCV%) ↑ Leucocytes ↑ Species diversity ↓ LAB levels ↑	Ferguson et al. (2010)
Nile tilapia (*Oreochromis niloticus*) x *P. acidilactici*	56	0.1 to 0.3 g/m³ water	Blood performance (BP) ↑ PCV% and RBCs ↑ WBCs ↑ Total protein ↑ Against *Aspergillus flavus* ↑	Hendam et al. (2023)
Nile Tilapia (*Oreochromis niloticus*) x *P. acidilactici*	45	2.81×10^6 CFU g^{-1}	Cultivable intestinal bacteria ↑	Standen et al. (2013)
Common carp (*Cyprinus carpio*) x *P. acidilactici*	42	1 g/kg	Width of villi ↑ Villus surface area ↑ Crypt depth ↑	Unpublished work
Common carp (*Cyprinus carpio*) x *P. pentosaceus*	45	10^8 CFU g^{-1}	RBCs ↑ WBCs ↑ Hemoglobin ↑ Hematocrit ↑	Ahmadifar et al. (2019)
Orange-spotted grouper (*Epinephelus coioides*) x *P. pentosaceus*	21	–	Mortality caused by *V. anguillarum* ↓	Huang et al. (2014)
European sea bass (*Dicentrarchus labrax*) x *P. acidilactici*	60	2.0, 2.5, and 3.0 g/kg diet	Villi height ↑ Muscular layer thickness ↑ Crude lipid ↑ Total protein ↓ Albumin and glucose ↓	Eissa et al. (2022)
Atlantic salmon (*Salmo salar*) x *P. acidilactici*	63	3.5 g kg^{-1} and 7 g kg^{-1}	IL1b, IL8, TNFa a, MX-1 and TLR3 ↑	Abid et al. (2013)
Rainbow trout (*Oncorhynchus mykiss*) x *P. acidilactici*	60	0, 7 and 9 log CFU/g	LAB levels ↑ Counts of cultivable bacteria **NE** Lysozyme (U/ml) ↑	Kousha et al. (2020)
Shrimp x *P. pentosaceus*	-	–	White spot syndrome virus ↓	Ghosh, 2023
Shrimp (*Litopenaeus vannamei*) x *P. pentosaceus*	60	10^6, 10^7 and 10^8 CFU/g	*lactobacillus* sp. count ↑ *Bacillus* sp. count ↑ Total haemocyte counts ↑ Lysozyme activity ↑ Abnormal behaviour ↑ Clinical signs ↑	Adel et al. (2017)

"↓": Decrease, "↑": increase, "NE": no effect, "-":value is not available

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The improvement of intestinal architectures such as villus length, width, and surface area is an important factor for fish performance and health status. It is suggested that the relative increase in villus length is positively correlated to the increase in the absorptive area of the intestinal tissue which would enhance the digestibility, feed utilization and growth performance. The exact mechanism by which how *Pediococcus* spp probiotics could improve intestinal absorption is not fully understood. However, the cells on the tip of the villus are continuously sloughed off and the renewing rates of the intestinal epithelium are reported to be extraordinary high in order to replace these cells (Aubin et al., 2005).

CONCLUSIONS

Pediococcus spp. which belong to the Lactobacillaceae family, are considered one of the most effective probiotic bacteria in aquaculture. they can interact with their host through different ways and may have various positive impacts to aquaculture industries. The key mechanisms by which *Pediococcus* spp provide its beneficial effect to their host is through enhancement of digestive enzymes, survival and growth parameters, diversity of gut microbiota, diseases resistance and immunity parameters, intestinal architectures, gut integrity, and overall fish performance. *Pediococcus* spp seems to be a promising and alternative agent to antibiotics for sustainable aquaculture production.

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