



Multi-Strain Probiotics and Synbiotics for the Control of Acute Hepatopancreatic Necrosis Disease (AHPND) in Whiteleg Shrimp (*Litopenaeus vannamei*)

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Abstract

Acute Hepatopancreatic Necrosis Disease (AHPND) is one of the most severe bacterial diseases affecting global aquaculture of whiteleg shrimp (*Litopenaeus vannamei*). The disease is primarily associated with specific strains of *Vibrio parahaemolyticus* carrying the PirA and PirB toxin genes, and can result in mortality rates approaching 100% in affected farms. The increasing concern over antibiotic resistance and environmental impacts of antimicrobial misuse has driven interest in probiotics as a sustainable alternative strategy. Recent studies have indicated that multi-strain probiotics and synbiotics may provide superior protective effects compared with single-strain formulations in controlling AHPND. This review compiles and critically analyzes published research on the efficacy of multi-strain probiotics and synbiotics in reducing AHPND incidence and severity in *L. vannamei*. The findings suggest that microbial consortia and synbiotic formulations may enhance disease resistance through improved gut microbiota balance, pathogen inhibition, and immune modulation in shrimp. Overall, multi-strain probiotic and synbiotic strategies represent promising sustainable approaches for the management of AHPND in shrimp aquaculture systems.

Keywords: AHPND; Multi-strain probiotics; Synbiotics; *Litopenaeus vannamei*; *Vibrio parahaemolyticus*; shrimp aquaculture.

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Introduction

The farming industry of the western white shrimp (*L. vannamei*) has grown significantly in recent decades and plays an important role in ensuring food security and creating employment in developing countries (Ghaednia *et al.*, 2024). However, the intensive development of this industry has faced the serious challenge of infectious disease outbreaks, particularly bacterial diseases (Shen *et al.*, 2025). Among bacterial pathogens, specific strains of *V. parahaemolyticus* carrying a plasmid containing *PirA* and *PirB* toxin genes are the causative agents of Acute Hepatopancreatic Necrosis Disease (AHPND) (Tran *et al.*, 2013).

AHPND, also known as Early Mortality Syndrome (EMS), was first reported in 2009 in southern China and then rapidly spread to Vietnam, Thailand, Malaysia, Mexico, and other shrimp-producing countries (Nimlamai, 2025). The disease is characterized by severe hepatopancreatic atrophy, sloughing of epithelial cells from the tubules, and mortality of up to 100% within 20 to 30 days after initial infection (Kumar *et al.*, 2021). Global economic losses due to this disease are estimated to be in the billions of dollars. Traditionally, AHPND control has involved the use of antibiotics. However, the indiscriminate use of antibiotics has led to the emergence of resistant strains, accumulation of drug residues in shrimp meat, and damage to beneficial gut microbiota (Galaviz-Silva *et al.*, 2025). This situation has made the search for safe, effective, and

environmentally friendly alternatives a fundamental priority (Situmorang *et al.*, 2025).

Probiotics have emerged as one of the most promising antibiotic alternatives (Chin *et al.*, 2024). In aquaculture, probiotics act through mechanisms such as producing antibacterial compounds, competing with pathogens for adhesion and nutrients, and modulating the host's immune system (Galaviz-Silva *et al.*, 2025). Although early research mainly focused on single-strain probiotics, recent scientific evidence suggests that multi-strain probiotics and synbiotics may offer greater advantages than single-strain formulations (Shen *et al.*, 2025). However, a recent meta-analysis study showed that under specific conditions, single-strain probiotics might be more effective than multi-strain probiotics in reducing AHPND-related mortality (Ghaednia *et al.*, 2024). This contradictory finding indicates the need for a more detailed investigation and systematic comparison of the effectiveness of these two approaches.

AHPND: Causal agent, mechanism, and epidemiology

Causal agent

The main causative agent of AHPND is specific strains of the bacterium *V. parahaemolyticus* containing the pVA1 plasmid of approximately 69 kilobases (Tran *et al.*, 2013). This plasmid carries genes encoding *PirA* and *PirB* toxins (Photobacterium insect-related toxins), which exert their toxicity by creating pores in the membrane of hepatopancreatic epithelial cells (Han et

al., 2015). Although *V. parahaemolyticus* is the most common known agent, there are reports of other *Vibrio* species, such as *Vibrio harveyi*, *Vibrio owensii*, and *Vibrio campbellii*, causing similar AHPND symptoms (Tuán *et al.*, 2018).

Clinical signs and histopathology

Shrimp infected with AHPND exhibit several clinical signs, including lethargy,

unusual swimming, reduced appetite, empty or semi-empty gut, and pallor or whitening of the hepatopancreas (Kumar *et al.*, 2021). Histopathological examination reveals severe sloughing of epithelial cells from the hepatopancreatic tubules, tubule dilation, cell necrosis, and hemocyte infiltration (Galaviz-Silva *et al.*, 2025) (Figs. 1 and 2).

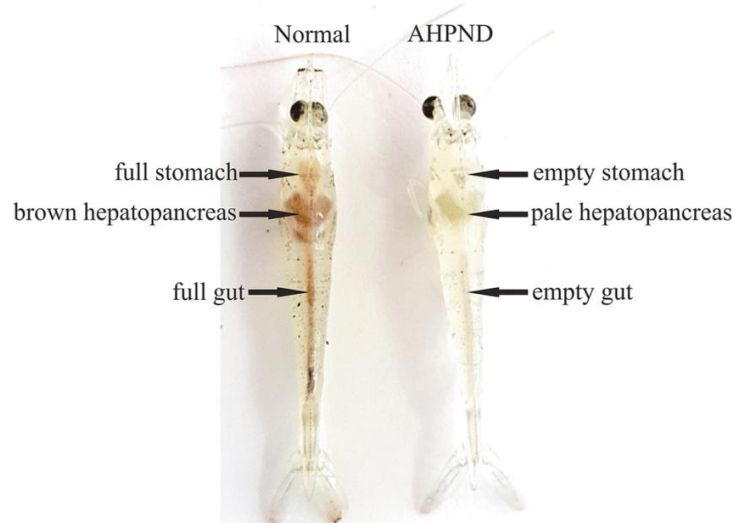


Figure 1: Severe clinical sign of Acute Hepatopancreatic Necrosis Disease (AHPND) infection in *Litopenaeus vannamei*: As seen in healthy shrimp, the hepatopancreas, stomach, and midgut are brown and normal, whereas all these organs appear pale in shrimp infected with AHPND.

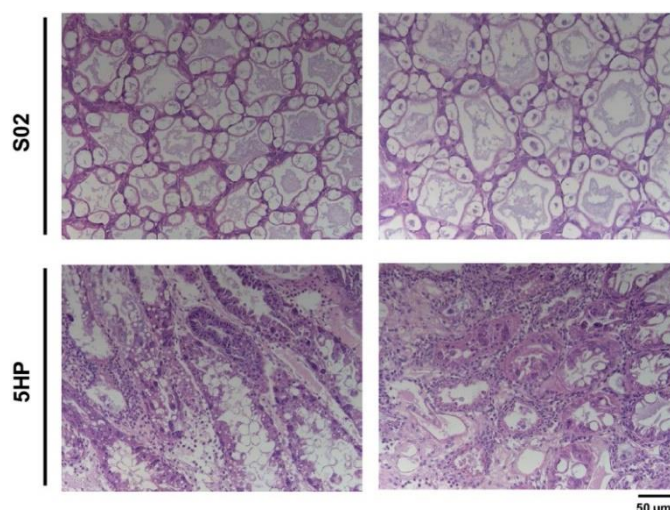


Figure 2: Histopathology of Acute Hepatopancreatic Necrosis Disease (AHPND). Hematoxylin and eosin-stained hepatopancreas from shrimp infected with non-AHPND-causing *V. parahaemolyticus* (S02) and AHPND-causing (5HP) *V. parahaemolyticus*. As observed, the S02 group contains normal tubules in the hepatopancreas, whereas the hepatopancreas infected with 5HP shows characteristic signs of AHPND, including sloughed epithelial cells, necrosis, and hemocyte infiltration. Scale bar: 50 μm.

Geographical spread

AHPND was first reported in 2009 in Hainan Province, China, and then rapidly spread to other Southeast Asian countries including Vietnam (2010), Thailand (2011), Malaysia (2011), and the Philippines (2015). In 2013, the disease reached the American continent, particularly Mexico, and was subsequently reported in the United States (2017), South Korea (2018), and Japan (2020) (Escobedo-Bonilla *et al.*, 2025). This rapid spread has caused serious global concern.

Probiotics: From single-strain to multi-strain

Definition and mechanisms of action

In aquaculture, probiotics are defined as live microorganisms that, through improving the host-associated or environmental microbiota, increase disease resistance, improve water quality, and enhance nutrition (Chin *et al.*, 2024).

The main mechanisms of action of probiotics include: production of antibacterial compounds including bacteriocins, surfactin, fengycin, and other antimicrobial peptides (Tuán *et al.*, 2018); competition for adhesion and nutrients to prevent pathogen colonization in the digestive tract; immune modulation by stimulating the innate immunity of shrimp through increased activity of enzymes such as phenoloxidase (PO), lysozyme, and superoxide dismutase (Galaviz-Silva *et al.*, 2025); and production of digestive enzymes to improve nutrient digestion and absorption (Situmorang *et al.*, 2025).

Effective probiotic strains for AHPND control

Numerous studies have demonstrated the effectiveness of various bacterial genera in controlling AHPND. Table 1 summarizes the most important probiotic strains and their characteristics.

Table 1: Effective probiotic strains in controlling AHPND in western white shrimp.

Genus/Species	Isolation Source	Main Mechanism of Action	Survival Rate Post-Challenge	Reference
<i>Bacillus subtilis</i> K3	Stomach of AHPND-surviving shrimp	AMP production (srfAA+, bacA+)	80-95%	Proespraiwong <i>et al.</i> , 2023
<i>Bacillus pumilus</i> Sonora	Marine sediments	Surfactin, fengycin, bacilysin production	88.9%	Soto-Marfileño <i>et al.</i> , 2024
<i>Bacillus sp. BV1</i>	Natural resources	Bacteriocin production	85-90%	Tuán <i>et al.</i> , 2018
<i>Lactobacillus plantarum</i>	Various sources	Immune stimulation, organic acid production	Significant increase in survival	Chin <i>et al.</i> , 2024
<i>Halomonas alkaliphila</i>	Biofloc system	Improved humoral immunity, AMPs	70%	Situmorang <i>et al.</i> , 2025

Single-strain vs. multi-strain probiotics

There is a significant debate in the scientific literature regarding the

superiority of single-strain versus multi-strain probiotics. Some studies have shown that multi-strain probiotics have greater advantages due to synergistic effects, greater stability under different environmental conditions, and broader coverage of antibacterial mechanisms (Shen *et al.*, 2025).

However, a comprehensive meta-analysis published recently showed that single-strain probiotics were more

effective in reducing AHPND-related mortality than multi-strain probiotics (Ghaednia *et al.*, 2024). This contradictory finding suggests that the choice of probiotic type should be based on specific culture conditions, pathogen strain, and the exact composition of the consortium. Table 2 provides a more detailed comparison between these two approaches.

Table 2: Comparison of single-strain and multi-strain probiotics in controlling AHPND.

Feature	Single-Strain Probiotic (SSP)	Multi-Strain Probiotic (MSP)	Reference
Effectiveness under controlled conditions	+++	++	Ghaednia <i>et al.</i> , 2024
Effectiveness under farm conditions	+	+++	Shen <i>et al.</i> , 2025
Synergistic effect	No	Yes	Shen <i>et al.</i> , 2025
Spectrum of antibacterial activity	Limited	Broad	Tuán <i>et al.</i> , 2018
Production cost	Low	Medium to High	Situmorang <i>et al.</i> , 2025
Risk of secondary resistance	Higher	Lower	Galaviz-Silva <i>et al.</i> , 2025

Synthetic microbial communities (SynComs): The new generation of probiotics

Concept and construction method

Synthetic Microbial Communities (SynComs) are a new generation of multi-strain probiotics designed purposefully and based on microbial ecology knowledge (Shen *et al.*, 2025). Unlike traditional approaches that use single strains or random combinations, SynComs consist of selected groups of native strains that naturally interact positively. A pioneering study by Shen and colleagues (2025) presented an innovative method for constructing SynComs. This method includes three main stages: enrichment of gut microbial

communities using selective culture media (e.g., R2A+Kan, R2A, GM1); identification of key strains using next-generation sequencing and bioinformatic analyses; and reconstruction and validation of the SynCom under in vivo conditions.

Effectiveness of SynComs in controlling AHPND

Results from the Shen *et al.* (2025) study showed that consortia derived from R2A+Kan, R2A, and GM1 culture media increased shrimp resistance against *V. parahaemolyticus* by over 68%. In particular, the designed SynComs (SynComR2A+Kan and SynComAll) increased shrimp survival

by 68.1% and 66.6%, respectively, compared to the control group. Notably, the combined administration method (IM+MF), adding the probiotic to both

water and feed, yielded the best result, improving survival by 80.1% post-challenge (Shen *et al.*, 2025) (Table 3).

Table 3: Effectiveness of synthetic microbial communities in controlling AHPND.

SynCom Name	Strain Composition	Administration Method	Survival Increase	Reference
SynComR2A+Kan	6 strains (<i>Tenacibaculum</i> , <i>Bacillus</i> , <i>Ruegeria</i> , <i>Paracoccus</i> , <i>Microbacterium</i> , <i>Exiguobacterium</i>)	IM+MF	68.1%	Shen <i>et al.</i> , 2025
SynComAll	8 strains (6 above + <i>Demequina</i> + <i>Tritonibacter</i>)	IM+MF	66.6%	Shen <i>et al.</i> , 2025
R2A+Kan consortium	Enriched community (wide range of bacteria)	IM+MF	>68%	Shen <i>et al.</i> , 2025

R2A: Reasoner's 2A Agar (low-nutrient medium); Kan: Kanamycin; GM1: Medium containing yeast extract and other substances; IM: In-water administration; MF: Medicated feed administration; IM+MF: Combined administration (adding probiotic to both water and feed).

Synbiotics: A synergistic approach of probiotics and prebiotics

Definition and fundamentals

Synbiotics are synergistic combinations of probiotics and prebiotics. Prebiotics are non-digestible compounds that selectively stimulate the growth and activity of probiotics in the digestive tract (Situmorang *et al.*, 2025). The use of synbiotics can offer several benefits, including increased probiotic establishment in the gut, improved host immunity, and reduced production costs.

Application of synbiotics in controlling AHPND

A comprehensive study by Situmorang *et al.* (2025) investigated the effectiveness of a synbiotic containing the probiotic *H. alkaliphila*, the red alga *Kappaphycus alvarezii* prebiotic, and the microalga *Spirulina* sp. in a biofloc system. Results showed that this combined approach increased shrimp

survival post-challenge with *V. parahaemolyticus* causing AHPND to 70% (compared to 73% in the group receiving the antibiotic enrofloxacin), upregulated the expression of humoral immune genes including ALF-a, lysozyme, and proPO, improved water quality, and enhanced growth parameters.

Mechanisms of action of synbiotics

The main mechanisms of action of synbiotics in controlling AHPND include: providing a specific substrate for probiotic growth in the gut (Situmorang *et al.*, 2025); production of short-chain fatty acids (SCFAs) which have immunomodulatory effects (Galaviz-Silva *et al.*, 2025); direct competition with pathogens for adhesion and nutrients (Chin *et al.*, 2024); and induction of antimicrobial peptide production by probiotics (Tuán *et al.*, 2018) (Table 4).

Table 4: Effective synbiotic formulations for controlling AHPND.

Probiotic	Prebiotic	Ratio/Dose	Main Effect	Reference
<i>H. alkaliphila</i>	<i>K. alvarezii</i> + <i>Spirulina</i> sp.	10 ⁹ CFU/kg + 0.5%	70% survival post-challenge	Situmorang <i>et al.</i> , 2025
<i>B. subtilis</i>	Red algae extract	10 ⁸ CFU/g	68% mortality reduction	Lim <i>et al.</i> , 2020
<i>L. plantarum</i>	Honey (natural prebiotic)	10 ⁸ CFU/g	Increased microbiota diversity	Chin <i>et al.</i> , 2024

Role of gut microbiota in resistance to AHPND

Microbiota changes under AHPND conditions

Metagenomic studies have shown that infection with *V. parahaemolyticus* causing AHPND induces severe dysbiosis in the shrimp gut microbiota. These changes include reduced microbial diversity, increased abundance of pathogenic genera (e.g., *Vibrio* and *Photobacterium*), and decreased populations of beneficial bacteria (e.g., *Bacillus* and *Lactobacillus*) (Galaviz-Silva *et al.*, 2025). A 2025 study by Galaviz-Silva and colleagues showed that shrimp with high survival post-AHPND infection had greater microbial diversity and their microbiota contained higher abundances of *Bacillus*, *Lactobacillus*, and *Pseudomonas* genera.

Discussion

Aquaculture, as one of the fastest-growing food production industries globally, plays a vital role in ensuring food security (Ghaednia *et al.*, 2024). However, the outbreak of infectious diseases, especially Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *V. parahaemolyticus*, is one of the most

serious threats to the farming industry of the western white shrimp (*L. vannamei*) (Tran *et al.*, 2013). This disease, first reported in China in 2009, rapidly spread to Southeast Asian countries, Latin America, and other regions, causing economic losses amounting to billions of dollars (Tang *et al.*, 2024).

Traditionally, AHPND control has involved the use of antibiotics. However, the indiscriminate use of antibiotics has led to the emergence of strains resistant to multiple antibiotic classes (Galaviz-Silva *et al.*, 2025). For example, in Asia, 87% of shrimp production operations pose significant risks regarding antimicrobial resistance development (Situmorang *et al.*, 2025). This alarming situation reveals the urgent need for sustainable and effective alternatives (Chin *et al.*, 2024). In this regard, probiotics have emerged as one of the most promising alternatives to antibiotics. Scientific evidence shows that probiotics can increase shrimp resistance against AHPND through various mechanisms, including the production of antibacterial compounds, competition with pathogens for adhesion and nutrients, modulation of the host's immune system, and improvement of water quality (Tuán *et al.*, 2018; Shen *et al.*, 2025).

Advantages of multi-Strain probiotics compared to single-strain

In recent years, researchers' attention has focused on multi-strain probiotics and Synthetic Microbial Communities (SynComs). These approaches offer significant advantages over single-strain probiotics due to synergistic effects, greater stability under different environmental conditions, and broader coverage of antibacterial mechanisms. A pioneering study by Shen *et al.* (2025) showed that consortia derived from specific culture media (R2A+Kan, R2A, GM1) increased shrimp resistance against *V. parahaemolyticus* by over 68%. This research showed that key strains including *Tenacibaculum*, *Bacillus*, *Ruegeria*, *Paracoccus*, *Microbacterium*, and *Exiguobacterium* play an important role in this protection. Specifically, the designed SynComs (SynComR2A+Kan and SynComAll) increased shrimp survival by 68.1% and 66.6%, respectively, compared to the control group. Notably, the combined administration method (IM+MF) yielded the best result, improving survival by 80.1% post-challenge (Shen *et al.*, 2025).

In another study, Kewcharoen and Srisapoom (2019) showed that *B. subtilis* AQAHS001 isolated from Thai coastal shrimp farms could inhibit a wide range of *V. parahaemolyticus* causing AHPND strains. Dietary administration of this probiotic at 10^9 CFU/kg diet for 5 weeks significantly increased weight gain, improved feed conversion ratio, and increased the expression of immune genes including

prophenoloxidase, lysozyme, and anti-lipoplysaccharide factor. This study also showed that the mentioned probiotic increases the thickness of the intestinal wall and microvilli in western white shrimp, indicating improved nutrient absorption and strengthening of the mucosal immune barrier (Galaviz-Silva *et al.*, 2025).

Role of synbiotics in controlling AHPND

Synbiotics are synergistic combinations of probiotics and prebiotics. A comprehensive study investigated the effectiveness of a synbiotic containing the probiotic *H. alkaliphila*, the red alga *Kappaphycus alvarezii* prebiotic, and the microalga *Spirulina* sp. in a biofloc system. The results showed that this combined approach increased shrimp survival post-challenge with *V. parahaemolyticus* causing AHPND to 70%, comparable to the group receiving the antibiotic enrofloxacin (73%) (Situmorang *et al.*, 2025).

Furthermore, gene expression analysis showed that the synbiotic increased the expression of antimicrobial peptides (ALF-a, lyz) and enzymes related to melanization (proPO), indicating the strengthening of humoral immune responses (Galaviz-Silva *et al.*, 2025).

In another study, Kewcharoen and Srisapoom (2019) investigated the effects of a synbiotic containing a *Bacillus* mixture (including *Bacillus amyloliquefaciens*, *B. pumilus*, and *B. subtilis*) and chitosan on western white shrimp. Results showed that the ASC synbiotic treatment (containing *B.*

subtilis and *B. amyloliquefaciens* with chitosan) provided the best resistance against *V. parahaemolyticus* causing AHPND, showing a survival rate of 52.8% at 120 hours post-exposure, which was significantly higher than the control group (22.2%) (Chin *et al.*, 2024). Additionally, all synbiotic treatments caused a significant increase in the expression of growth-related genes and immune genes (ALF and proPO) in the shrimp's hepatopancreas and gut (Galaviz-Silva *et al.*, 2025).

Role of gut microbiota and dysbiosis

Metagenomic studies have shown that infection with *V. parahaemolyticus* causing AHPND induces severe dysbiosis in the shrimp gut microbiota. These changes include reduced microbial diversity, increased abundance of pathogenic genera (e.g., *Vibrio* and *Photobacterium*), and decreased populations of beneficial bacteria (e.g., *Bacillus* and *Lactobacillus*). The concept of microbiota resilience refers to the ability of the microbial ecosystem to return to a balanced state after a disturbance (Galaviz-Silva *et al.*, 2025). Probiotics can help restore this resilience by restoring beneficial microbiota and suppressing pathogens (Shen *et al.*, 2025). Galaviz-Silva and colleagues (2025) showed that shrimp with high survival post-AHPND infection had greater microbial diversity and their microbiota contained higher abundances of beneficial bacteria. In this study, the common microbiome core in groups receiving effective probiotics included

the families Rhodobacteraceae, *Celeribacter indicus*, *Ruegeria atlantica*, and *Thalassobius mediterraneus*, indicating the protective role of these bacteria against *V. parahaemolyticus* causing AHPND. In contrast, shrimp treated with ineffective probiotics showed lower microbial diversity and a higher abundance of *Vibrio* spp. (Galaviz-Silva *et al.*, 2025).

Role of Bacillus genus bacteria as top probiotic candidates

Among the studied probiotics, bacteria of the genus *Bacillus* are considered the most promising candidates due to their ability to produce a wide range of antimicrobial peptides, resistance to adverse environmental conditions, and ability to form spores (Tang *et al.*, 2025). Kewcharoen and Srisapoom (2019) showed that *B. subtilis* AQAHS001 isolated from farms could effectively reduce *Vibrio* populations in water and shrimp bodies and increase the expression of immune genes. Furthermore, this strain can tolerate high temperatures and adverse conditions, which is very important for the commercial feed production process. Also, by sequencing the genome of *Bacillus pumilus* Sonora, identified 28 biosynthetic gene clusters responsible for producing antimicrobial metabolites including surfactin, fengycin, and bacilysin. This strain showed strong inhibitory activity with an inhibition zone diameter of 35 mm against *V. parahaemolyticus* causing AHPND (Soto-Marfileño *et al.*, 2024). In a recent study, the *Bacillus safensis* BS22LVI

strain isolated from the gut of healthy shrimp showed a broad antimicrobial spectrum against several *Vibrio* species, including *V. parahaemolyticus* causing AHPND, *V. campbellii* causing AHPND, *V. harveyi*, *V. alginolyticus*, and *V. owensii* (Lee *et al.*, 2025). Dietary administration of this probiotic at 10^9 CFU/g caused a significant reduction in mortality (22.2% compared to 100% in the control group), a reduction in *pirA* toxin gene copies in the hepatopancreas, and an increase in total hemocyte count (THC) (Lee *et al.*, 2025).

Molecular mechanisms of probiotic action

Studies have shown that probiotics can exert their protective effects through several molecular mechanisms (Shen *et al.*, 2025):

1. **Quorum Sensing (QS) Interference:** The study by Stianingrum *et al.* (2025) showed that the combination of biofloc and the probiotic *Pseudoalteromonas piscicida* 1Ub can reduce the expression of *V. parahaemolyticus* virulence genes by disrupting this system. This combined approach also increases shrimp immune responses, including increased total hemocyte count, respiratory burst, phenoloxidase activity, and phagocytosis (Galaviz-Silva *et al.*, 2025).
2. **Humoral Immunity Enhancement:** Probiotics strengthen the innate immune system of shrimp (which lacks adaptive immunity) by increasing the expression of

genes encoding antimicrobial peptides like ALF-a and enzymes involved in the melanization pathway like proPO (Galaviz-Silva *et al.*, 2025). Kewcharoen and Srisapoome (2019) showed that administration of *B. subtilis* AQAHBS001 significantly increased phagocytic activity and clearance efficiency in western white shrimp.

3. **Improving Gut Integrity:** Studies have shown that probiotics can strengthen the mucosal immune barrier and improve nutrient absorption by increasing the thickness of the intestinal wall and the height of microvilli (Galaviz-Silva *et al.*, 2025).

Key findings:

1. Probiotics of the genus *Bacillus* are the most promising candidates for controlling AHPND due to their ability to produce a wide range of antimicrobial metabolites and resistance to adverse environmental conditions. The *B. subtilis* AQAHBS001 strain significantly increased shrimp resistance by enhancing immune gene expression and improving gut integrity.
2. Multi-strain probiotics and synbiotics offer significant advantages over single-strain probiotics due to synergistic effects and greater stability under real farm conditions. A mixture of three *Bacillus* strains increased survival by up to 40%, while designed SynComs improved

- survival by up to 68% (Shen *et al.*, 2025). Furthermore, the ASC synbiotic treatment (*B. subtilis* and *B. amyloliquefaciens* with chitosan) increased shrimp survival to 52.8% post-challenge with *V. parahaemolyticus* causing AHPND (Chin *et al.*, 2024).
3. The combined administration method (IM+MF), which involves adding the probiotic to both water and feed, yields the best results, improving survival by up to 80.1% (Shen *et al.*, 2025).
 4. Synbiotics, by providing a specific substrate for probiotics, increase their establishment in the gut and enhance immunomodulatory effects, and can offer survival comparable to antibiotics (70% vs. 73%) (Situmorang *et al.*, 2025).
 5. The biofloc system combined with synbiotics can provide survival comparable to the antibiotic enrofloxacin while preventing the development of antibiotic resistance (Situmorang *et al.*, 2025). This system also stabilizes water quality and increases total shrimp biomass (Galaviz-Silva *et al.*, 2025).
 6. Microbiota analysis indicates that the presence of specific genera such as Rhodobacteraceae, *Celeribacter indicus*, *Ruegeria atlantica*, and *Thalassobius mediterraneus* is associated with high shrimp survival upon AHPND challenge, and effective probiotics can promote this beneficial microbiota (Galaviz-Silva *et al.*, 2025).
 7. Quorum Sensing inhibition has been identified as a key mechanism in reducing the pathogenicity of *V. parahaemolyticus*. The combination of biofloc and the probiotic *P. piscicida* 1Ub can reduce bacterial virulence gene expression and enhance the shrimp's immune response (Galaviz-Silva *et al.*, 2025).
- Recommendations for future research:*
1. Conduct standardized comparative studies between single-strain and multi-strain probiotics under identical farm conditions (Ghaednia *et al.*, 2024).
 2. Investigate the molecular mechanisms of the synergistic effect in multi-strain consortia, particularly in Quorum Sensing inhibition and toxin degradation (Shen *et al.*, 2025).
 3. Develop rapid screening methods based on next-generation sequencing to identify effective native strains (Tuán *et al.*, 2018).
 4. Optimize synbiotic formulations using low-cost, accessible prebiotics such as chitosan and seaweed extracts (Chin *et al.*, 2024; Situmorang *et al.*, 2025).
 5. Conduct field-scale commercial studies to assess the economic viability and practicality of these approaches.

Conclusion

Multi-strain probiotics, synbiotics, and biofloc systems are promising as innovative and sustainable strategies for controlling AHPND in western white shrimp (Ghaednia *et al.*, 2024; Shen *et*

al., 2025; Situmorang *et al.*, 2025). Careful selection of strains considering complementary mechanisms, optimization of formulation, and appropriate administration method are essential to achieve maximum effectiveness. Given the prevalence of AHPND in shrimp farms in southern Iran and the emergence of antibiotic-resistant strains, the development and localization of these technologies could be an important step towards reducing losses, increasing production, enhancing biosecurity, and reducing dependence on antibiotics in the country's shrimp industry (Ghaednia *et al.*, 2024). The approach of using native multi-strain consortia and synbiotics, while maintaining the ecological balance of culture environments, will significantly contribute to achieving sustainable development goals in aquaculture. Considering that the western white shrimp (*L. vannamei*) is the main target species for farming in Iran, investing in applied research on native probiotics for this species will yield the highest economic return for the country's fisheries industry (Thadajarassiri *et al.*, 2023; López Reyes *et al.*, 2024).

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