

A REVIEW TO ASSESS THE EFFECTIVENESS OF PROBIOTIC SPECIES TO CONTROL AQUATIC MORTALITY

SurvathJabeen S

Department of Biotechnology, School of Life Science, Vels institute of science technology and Advanced Studies, Chennai

Suganthi. M

Department of Biotechnology, School of Life Science, Vels institute of science technology and Advanced Studies, Chennai

Ramprasath C

Eukpro Biotech Private limited, Chrompet, Chennai - 600 044, Tamil Nadu, India

Manjuladevi K

Department of Pharmacology, school of Pharmaceutical Science, Vels institute of science technology and Advanced Studies, Chennai

Abirami G

Department of Biotechnology, School of Life Science, Vels institute of science technology and Advanced Studies, Chennai

Abstract

Probiotics have received a lot of interest recently in aquaculture as a substitute for the use of antibiotics in the control of aquatic illness. However, bacterial cells are mainly reliant on the aquaculture where they develop, and the usage of those probiotic species has given significant results. Therefore, it is preferable to isolate bacteria from aquaculture where they proliferate better. The genera *Vibrio* and *Bacillus* as well as the species *Thalassobacter* utilize contain bacteria that have been effectively employed as probiotics. The majority of studies have identified these strains from various penaeid species. The majority of studies have identified the bacterial species which act as probiotic strains gut of shrimp. Two of the most promising preventative strategies created in the last few years in the battle against illnesses due to the presence of micro-organisms which act as probiotics that will act as an immune suppressant and the main mechanism by which they exhibit their mode of action is by mean of competitive inhibition. Additionally, probiotic bacteria were shown to be able to manufacture certain digestive enzymes, which may enhance shrimp digestion and boost their capacity to withstand stress and general health. However, the use of probiotics in aquatic environments continues to be debatable since there is a lack of reliable evidence and in-person examples of the efficacy mainly in the surrounding environment. The present review will highlight the perspective of the usage of shrimp gut microbiota as an approach for the enhancement of aquaculture species

Keywords: *Aquaculture, Probiotics, Shrimp gut microbiota, Competitive inhibition.*

INTRODUCTION

Viral and bacterial epizootics act as major organism in limiting the development of aquaculture production. Aquaculture has emerged as a profitable food industry to meet the increasing need for a diet high in nutrients and proteins which acts as a supplement to the continuously growing population [1]. Due to the high protein content, fish product consumption is rising significantly in both developed and developing countries. Bacterial

pathogens from the groups of *Pseudomonas*, *Aeromonas*, *Vibrio*, and *Streptococcus* are responsible for the majority of illness outbreaks [2]. The usage of chemotherapeutics has been limited due to its accumulation in aquatic habitats and besides changing the microbiota of aquatic environments. Thus the application of probiotics is an ideal option as it is involved in boosting the immunity of host organisms by acting as a growth promoter [4].

Gut proliferating micro-organisms are regarded as significant regulators of a variety of host metabolic processes. As a result, the characterization and manipulation of this gut microbiota will be a better option in gut microbiome research. The diverse bacterial gut microbiota plays crucial functions in an organism's health and development. They are shaped in such a way that it performs a probabilistic approach when they acted as probiotic supplements. These processes are typically influenced by biological and environmental conditions. The main factors determining in shaping of gut microbiota include the host immune system, gut pH, and food content [5]. Still, now, several studies have been conducted to identify the potential of gut microbiota on various species have been done. Thus, using gut microbiome have been limited to a certain extent as the properties of the bacterial strains depend on the environment

in which they thrive. So the need for an ideal approach which is creating probiotics from shrimp gut where they proliferate steadily is essential. Major probiotic organisms include genus of *Vibrio*, *Bacillus*, and species of *Thalassobacter utilis*. Most probiotic bacteria are isolated from the gut of penaeid species which uses competitive exclusion as a preventive strategy in fighting against viral infection. Despite all these advantages, probiotics isolated from shrimp gut lack evidential demonstration in their mechanism of action *in vivo*. This paper will mainly cover the possible source of probiotics from shrimp gut microbe and their mechanism of action in shrimp aquaculture.

MARINESHRIMP MICROFLORA:

One of the most crucial species in aquatic aquaculture is the shrimp. Although shrimp farming has grown significantly in recent years, concerns from numerous environmental issues, such as pathogen infection, limit the industry's ability to operate sustainably on a global scale. Proteobacteria was the predominant phylum in the majority of investigation studies conducted worldwide. Proteobacteria, Cyanobacteria, Actinobacteria, and Fusobacteria are majorly found in the intestinal gut of shrimp. Proteobacteria are commonly present in the gut microbiome of aquatic shrimps. The class Proteobacteria exhibits a wide range of physiology, morphology, and genetic characteristics features which lacks in most other crustacea. Most of the species in this category are facultative anaerobes, and they are Gram-negative bacteria. The biggest class in this phylum is, gamma-proteobacteria, which are commonly discussed as the most abundantly available bacterium in the intestine of *P. shrimp* and Pacific white shrimp. Despite this, *Vibriospp* is frequently referred to be the dominating genus in the shrimp, and many of

them coexist peacefully with the host. This is a crucial qualification given that medicinal remedies are frequently created to target the vibriogenes. Shrimp usually gets affected with WSSV infection which leads to white spot syndrome. This leads to death of the organism within 7 to 10 days.

Probiotics can enhance digestive function by synthesizing vitamins, and cofactors thus increasing enzymatic activity. These characteristics may help with weight gain, better digestion, or food absorption. Although probiotics may have the ability to increase nutritional quality, their primary goal is to maximize their advantages by limiting the growth of harmful bacteria in shrimp production systems. The management and prevention of disease outbreaks in aquaculture greatly depend on the continual monitoring of ponds which includes pH, oxygen, temperature, salinity and turbidity conditions [6].

NEED FOR PROBIOTICS IN SHRIMP AQUACULTURE:

Early mortality syndrome has been reported since 2009 in China and Southeast Asian countries. Reports proved that strains of *Vibrio* with plasmid carrying gene *pirA*- and *pirB*- These genes encodes for toxins and harms the shrimp gut. EMS has cost the global shrimp sector an estimated \$1 billion in damages to date. Also, the World Bank advises to carry research in shrimp diseases and invests about 275 million dollars, estimating that there have been around US\$3 billion in losses worldwide as a result of shrimp sickness [7].

Due to a higher antimicrobial resistance rate (prediction: 10 million deaths by 2050 if the current trend continues). These investigations led to the conclusion of developing probiotic species from the intestinal gut of shrimp. Several microbiome studies have been initiated

to analyze the gut microbiome of wild shrimp under biotic factors. Due to their inability to thrive in the marine environment, terrestrial bacterial strains are of no use. Additionally, the majority of research that was published focused on prospective probiotic strains that were simple to grow on the existing medium. However, it has been observed that marine bacterial endosymbionts cannot be grown on the medium currently in use. Since they are well-established antagonistic symbionts, endosymbiotic hostile microorganisms may, in theory, have probiotic efficacy. Consequently, investigating marine bacterial endo-symbionts might be a potential strategy for creating new shrimp probiotics.

The majority of research on the use of probiotics in shrimp farming is limited to bacterial pathogen control and shrimp health enhancement. There are few reports on managing or controlling viral pathogens. Albert explained the immune effects of probiotics against the white spot syndrome virus show that chemicals and biological agents, mainly extracted from algal species can possibly inactivate viruses.

MODE OF ACTION:

The mechanism by which the shrimp gut microbiota protects the viral infections in marine species is discussed below:

Competitive inhibition:

One of the ecological processes used to alter the species makeup of soil, aquatic body, or other microbiological environment is competitive exclusion. Changes in species dominance will be triggered by even the smallest adjustments to variables that influence growth or death rates. By using the competitive exclusion principles, we are still far from understanding the variables which regulate enzyme kinetics

rate, much alone the full species makeup. This might not be sufficient in the case of shrimp probiotics as they possess non-specific antagonistic properties with a wide range of nutritive habitats [8].

Bioaugmentation:

The application of bioremediation is widespread, although the degree of effectiveness varies widely relies on the types of items utilized and the practical knowledge at the back-end user's disposal. To accomplish the intended results, the species introduced has to be chosen with certain functionalities, and receptive to bioremediate the population and increase yield with proper condition of habitat. Efficiency of this, mainly depends on the competitive inhibition between gut microbiota. Studies carried out by Wang et.al claim that the application of probiotics greatly reduces the chemical contents in ponds but there is no effect on the microbial concentration observed [9].

Quorum sensing Blocking:

Tinh et al. have shown a novel mode of action for the shrimp gut microbiome. Quorum sensing blocking has been proven to be a cutting-edge and successful disease control method against *Vibrio* spp. in shrimp. Quorum signaling is mediated by N- acyl homoserine lactone in aquaculture species. Enrichment cultures of microbes that break down N-acyl homoserine lactone in *L. vannamei* were identified to have potential probiotic effects in *Brachionus* cultures [10].

IMPROVING AQUA-SPECIES WITH SHRIMP GUT MICROBIOTA AS PROBIOTICS: FIELD REALITIES

Even though probiotics are developing as a potential option to avoid shrimp deaths at a larger scale. Research is carried out on the

efficacy and reliability of microbiome use in rearing areas is limited. Moriarty et.al successfully experimented with altering probiotics instead of antibiotics to prevent vibriosis in shrimp. The results obtained show that those shrimp supplemented with probiotics grow larger with a higher survival rate.

Another field study explains the competitive inhibition of probiotics which replaces the need for antibiotics in shrimp gut. The necessity for the addition of these microbes has risen higher in gut microbiome production in terms of its survival rate. Albeit explained the use of LAB for improving the shrimp survival rate. The results show a greater performance rate of treated shrimp upon dosage with probiotics than with untreated one and still some investigations are required in terms of its stability should be done. Nevertheless the usage of shrimp survival after ozonation results in a higher survival rate. Wang et.al states that the probiotic bacteria tested in the field condition show the following properties:

- Increased microbial communities
- Higher algal growth
- Attaining a stable environment between probiotics and pathogenic species [11]

ALTERATION IN GUT MICROBIOME IN CORRELATION WITH MARINE DISEASES

This section will summarize on the co-relation between the effect of micro-organisms with their impact in shrimp production.

Diseases in Penaeids:

There were no significant variations in the microbial count of "diseased" shrimp, which were bred in East Asian countries were characterized by lethargic development, loss of appetite, empty bowel, and increased death rate [12].

Acute hepatopancreatic necrosis disease: The illness is due to diminution of the HP which finally leads to cessation of the tubules of HP. The causative agent here is plasmid-borne *Vibrio*, which is carried by numerous species of *Vibrio*. According to its incidence, in *L. vannamei*, there was a considerable decline in bacterial heterogeneity of the HP in relation to healthy persons. This shrimp condition exhibits a decline in diversity of over 53% in just 7 days. The individuals with AHPND-positive had high existence of "*Candidatus Bacilloplasma* along with *Vibrio* clusters. When both interact with each other, results in either encouraging or preventing infection by the community.

White spot syndrome:

The greatest danger to the global health of shrimp is the White Spot Syndrome. Despite primarily affecting shrimp, its severe pathophysiology causes growth to be reduced and finally leads to significant mortality rates in a variety of cultured species. Recently, it was demonstrated that the gut microbiota of *L. vannamei*, was drastically changed concerning WSSV infection. A considerable rise in Proteobacteria and Fusobacteria in the gut, as well as a decrease in Bacteroidetes and Tenericutes, are seen in individuals with WSSV infection. Though the abundance of phyla is observed during this infection, no change in bacterial OTU richness is observed. According to evidence, shrimp reared in a system with variable-sized bacteria had bacterial communities similar to those containing solely medium-sized bacteria, therefore the presence of a bio floc could possibly change the microbiome-mediated resistance to infection [1].

White faeces syndrome:

This syndrome is characterized by white-golden intestinal contents and white faeces

threads, which remains unclear. Initially, it was believed that WFS is closely related to the presence of *Enterocytozoon hepatopenaei*. This syndrome is usually associated with alteration in gut microbiome. Ascomycota and basidiomycota were prevalent in healthy and sick persons, with a rise in pathogenic *Candida* spp. in those presenting clinical indications of WFS, according to Li et al. (2019). In people with WFS infection, Dai et al. (2019) identified a high prevalence of Ascomycota and Basidiomycota [13].

Poor Growth:

The bacterial gut microbiome can affect shrimp development by altering the action of digestive enzymes. Body growth and weight substantially and positively linked with amylase, pepsin, and lipase activity after raising larvae of *L. vannamei* for 70 days. Structural equation modeling highlighted how the composition of the gut's microbial and eukaryotic communities contributed to the considerable favorable impacts on the enzyme. In retarded shrimp, bacterial diversity was greatly decreased but the relative abundance of gamma proteobacteria rose significantly. When compared to healthy individuals, retarded shrimp also harbored less phylogenetically grouped gut communities, showing a decrease in host determination in the composition of bacterial gut communities [14]. The eukaryotic microbiota of WFS-infected shrimp displayed more stochastic assembly as compared to healthy individuals, indicating that this change in ecological processes is not simply restricted to bacterial community assembly [2]. We hypothesize that early stochastic events might lead to differences in microbiotas across shrimp community members, which would later predispose certain individuals to illness. This phenomenon might also assist to explain

differences in disease susceptibilities within populations [15].

Although many reports support the potential of probiotics in shrimp aquaculture, it is unknown how long these products will remain on the market, whether they will last throughout the entire culture cycle, or whether they will change into a native strain (autochthonous) for a longer period. Bacterial communities are in a stable state that is produced by the organisms themselves when they are in homeostasis. The control of populations in certain proportions, one to the other, is influenced by competition for resources and habitat, and inhibition of one group by the metabolic products of another species. The allochthonous microbiota seems to have a very small possibility of becoming an autochthonous microbiome, according to the homeostasis phenomenon [4]. It is highly challenging for allochthonous (produced in another area) microorganisms, whether unintentionally or purposefully brought into an environment, to establish themselves since all of the ecological niches are filled in a controlled bacterial community [3]. As a result, probiotic sustainability in shrimp farms is a complicated ecological phenomenon that calls for a multifaceted approach to identifying the prebiotic elements necessary for the development and growth of probiotic microbe in shrimp farms. The results of the study suggest that the criteria used for the selection of putative probiotic strains, such as predominant growth on primary isolation media, and ability to produce extracellular enzymes and siderophores, did not bring about the desired effect in vivo and improve the immune system in shrimp [16]. Though lyophilized cultures are proven to be successful in human [4] and veterinary applications, their efficacy in aquaculture systems are being an unresolved enigma. There are no serious changes during the initial stages of farming aquatic organisms

when the stocked biomass is small and their metabolism rate and amounts of supplementary feed are low. However, with the progress of culture leading to a rapid increase in biomass, consequently water quality deteriorates, mainly as a result of the accumulation of metabolic waste of cultured organisms, decomposition of unutilized feed, and decay of biotic materials [3]. Thus, the mid-culture outbreaks in shrimp farms are highly devastating. Therefore the application of probiotics needs to be rationalized and optimized for the reduction of toxic metabolites/bacterial load during the late mid-culture period, instead of applying probiotics as feed additive or water enrichments for the entire culture cycle [17] which ultimately increase the cost of production. It was found that production of crab have increased following the addition of bacterial strains to the culture water. If probiotics are to be persisted to make any sustained contribution to the indigenous microbiota, their introduction would need to be regularly and/or at a concentration higher than that of the already established microbial community [5].

CONCLUSION:

Sustainable shrimp farming has been envisioned as requiring a cost-effective holistic/integrated management approach. The current proactive management techniques utilizing immunostimulants and natural products may only to a limited extent guarantee shrimp health; nevertheless, none of the products ensures the management of shrimp and the environment holistically [18,19]. As a result, the only newly developed or prospective preventive measure that might guarantee shrimp health and environmental management comprehensively is probiotics [6]. Probiotics role in shrimp health management is well known, but there are still issues with their

capacity for bioremediation and measures for biocontrol (antagonism). Instead of using terrestrial strains, it is essential to screen the vast marine microorganisms to produce a prolonged probiotic effect under field circumstances. The marine bacterial endosymbionts are emerging as a promising resource in this area. According to the "pathobiome" concept [4] Pathogenesis may not be directly linked to changes in interactions between multiple taxa and the host, but rather to changes in the relative abundance of a particular taxon [20]. However, there is not yet enough data to make generalizations about the gut microbiome of various shrimp species concerning

REFERENCES

- Holt CC, Bass D, Stentiford GD, van der Giezen M. Understanding the role of the shrimp gut microbiome in health and disease. *J Invertebr Pathol.* 2021 Nov;186:107387. doi: 10.1016/j.jip.2020.107387. Epub 2020 Apr 21. PMID: 32330478.
- Zhang S, Sun X. Core Gut Microbiota of Shrimp Function as a Regulator to Maintain Immune Homeostasis in Response to WSSV Infection. *Microbiol Spectr.* 2022 Apr 27;10(2):e0246521. doi: 10.1128/spectrum.02465-21. Epub 2022 Apr 12. PMID: 35412375; PMCID: PMC9045241.
- Tinh NT, AsankaGunasekara RA, Boon N, Dierckens K, Sorgeloos P, Bossier P. N-acyl homoserine lactone-degrading microbial enrichment cultures isolated from *Penaeus vannamei* shrimp gut and their probiotic properties in *Brachionus plicatilis* cultures. *FEMS Microbiol Ecol.* 2007 Oct;62(1):45-53. doi: 10.1111/j.1574-6941.2007.00378.x. Epub 2007 Sep 5. PMID: 17784866.
- Vandenberghe J, Verdonck L, Robles-Arozarena R, Rivera G, Bolland A, Balladares M, Gomez-Gil B, Calderon J, Sorgeloos P, Swings J. Vibrios associated with *Litopenaeus vannamei* larvae, postlarvae, broodstock, and hatchery probionts. *Appl Environ Microbiol.* 1999 Jun;65(6):2592-7. doi: 10.1128/AEM.65.6.2592-2597.1999. PMID: 10347048; PMCID: PMC91383.
- Bass D, Stentiford GD, Wang HC, Koskella B, Tyler CR. The Pathobiome in Animal and Plant Diseases. *Trends Ecol Evol.* 2019 Nov;34(11):996-1008. doi: 10.1016/j.tree.2019.07.012. Epub 2019 Sep 12. PMID: 31522755; PMCID: PMC7479508.
- Felipe do Nascimento Vieira, Celso Carlos Buglione Neto, José Luiz Pedreira Mourinho, Adolfo Jatobá, Cristina Ramirez, Mauricio Martins, Margherita A Barracco, Luis Vinatea. Time-related action of *Lactobacillus plantarum* in the bacterial microbiota of shrimp digestive tract and its action as immunostimulant. Vieira et al. 2008 June
- MdZoqratt MZH, Eng WWH, Thai BT, Austin CM, Gan HM. Microbiome analysis of Pacific white shrimp gut and rearing water from Malaysia and Vietnam: implications for aquaculture research and management. *PeerJ.* 2018 Oct 30;6:e5826. doi: 10.7717/peerj.5826. PMID: 30397546; PMCID: PMC6214229.
- Albert G J Tacon. Global trends in aquaculture and compound aquafeed production. January 2003 4:28–35.
- A.S. Ninawe & Joseph Selvin (2009) Probiotics in shrimp aquaculture: Avenues and challenges, Critical Reviews in

- Microbiology, 35:1, 43-66, DOI: 10.1080/10408410802667202
- Xuexi Wang, Hongjie Luo, Dejuan Wang, Yunzong Zheng, Wenbo Zhu, Weini Zhang, Zhengbang Chen, Xinhua Chen, Jianchun Shao, Partial Substitution of Fish Meal with Soy Protein Concentrate on Growth, Liver Health, Intestinal Morphology, and Microbiota in Juvenile Large Yellow Croaker (Larimichthys crocea), Aquaculture Nutrition, 10.1155/2023/3706709, 2023, (1-15), (2023).
- Xiong J, Zhu J, Dai W, Dong C, Qiu Q, Li C. Integrating gut microbiota immaturity and disease-discriminatory taxa to diagnose the initiation and severity of shrimp disease. Environ Microbiol. 2017 Apr;19(4):1490-1501. doi: 10.1111/1462-2920.13701. Epub 2017 Mar 23. PMID: 28205371..
- Lee CT, Chen IT, Yang YT, Ko TP, Huang YT, Huang JY, Huang MF, Lin SJ, Chen CY, Lin SS, Lightner DV, Wang HC, Wang AH, Wang HC, Hor LI, Lo CF. The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin. Proc Natl AcadSci U S A. 2015 Aug 25;112(34):10798-803. doi: 10.1073/pnas.1503129112. Epub 2015 Aug 10. Erratum in: Proc Natl AcadSci U S A. 2015 Sep 29;112(39):E5445. Lin, Shih-Shuen [corrected to Lin, Shih-Shun]. PMID: 26261348; PMCID: PMC4553777.
- Chou-Min Chong, Mohamed ZahuwaanShakir, Kok-Song Lai, Hon Jung Liew, Jiun-Yan Loh, Microbes and fish diseases, Recent Advances in Aquaculture Microbial Technology, 10.1016/B978-0-323-90261-8.00009-2, (65-102), (2023).
- Li, L., Yan, B., Li, S. et al. A comparison of bacterial community structure in seawater pond with shrimp, crab, and shellfish cultures and in non-cultured pond in Ganyu, Eastern China. Ann Microbiol 66, 317–328 (2016). <https://doi.org/10.1007/s13213-015-1111-4>
- Craig J Plante, Kristina M Hill-Spanik, Rowan Emerson, Inputs don't equal outputs: bacterial microbiomes of the ingesta, gut, and feces of the keystone deposit feeder *Ilyanassa obsoleta*, FEMS Microbiology Ecology, 10.1093/femsec/fiac152, 99, 1, (2022).
- Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009 May;294(1):1-8. doi: 10.1111/j.1574-6968.2009.01514.x. Epub 2009 Feb 13. PMID: 19222573.
- Most J, Penders J, Lucchesi M, Goossens GH, Blaak EE. Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women. Eur J Clin Nutr. 2017 Sep;71(9):1040-1045. doi: 10.1038/ejcn.2017.89. Epub 2017 Jun 7. PMID: 28589947.
- Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol. 2016 Jan;14(1):20-32. doi: 10.1038/nrmicro3552. Epub 2015 Oct 26. PMID: 26499895; PMCID: PMC4837114.
- Li C, Wang S, He J. The Two NF-κB Pathways Regulating Bacterial and WSSV Infection of Shrimp. Front Immunol. 2019 Jul 30;10:1785. doi: 10.3389/fimmu.2019.01785. PMID: 31417561; PMCID: PMC6683665.

Zhang S, Xin F, Zhang X. The compound packaged in virions is the key to trigger host glycolysis machinery for virus life cycle in the cytoplasm. *iScience*. 2020 Dec 9;24(1):101915. doi: 10.1016/j.isci.2020.101915. PMID: 33385116; PMCID: PMC7770649.

Lo, Chu Fang et al. "Specific genomic DNA fragment analysis of different geographical clinical samples of shrimp white spot syndrome virus." *Diseases of Aquatic Organisms* 35 (1999): 175-185.

Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hämäläinen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lähdesmäki H, Huttenhower C, Gevers D, Cullen TW, Knip M; DIABIMMUNE Study Group; Xavier RJ. Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell*. 2016 May 5;165(4):842-53. doi: 10.1016/j.cell.2016.04.007. Epub 2016 Apr 28. Erratum in: *Cell*. 2016 Jun 2;165(6):1551. PMID: 27133167; PMCID: PMC4950857.

Adak A, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci*. 2019 Feb;76(3):473-493. doi: 10.1007/s00018-018-2943-4. Epub 2018 Oct 13. PMID: 30317530.