International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijamr Volume 11, Issue 3 -2024

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2024.11.03.007

Development of effective bio-formulations for the control of viral diseases, such as WSSV and IHNNV for the benefit of aqua culture

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Keywords

Litopenaeus vannamei,
Aquaculture,
Seafood,
Viral Disease,
Bio- formulations.

Abstract

The development of effective bio-formulations for controlling viral diseases in aquaculture is crucial for ensuring the sustainability and profitability of the industry. Traditional control methods like chemical treatments and biosecurity protocols have limitations, necessitating alternative approaches. Bio-formulations, which utilize natural compounds or microorganisms to combat pathogens, offer promising solutions. They are species-specific, environmentally sustainable, and promote ecological balance. However, developing effective bio-formulations faces challenges such as formulation stability, delivery mechanisms, and regulatory hurdles. Overcoming these challenges requires interdisciplinary collaboration, advanced biotechnological tools, and clear regulatory frameworks. Strategies include deepening our understanding of viral pathogenesis, utilizing advanced technologies, and fostering collaborative partnerships. Investing in the development of bio-formulations can safeguard the health and productivity of aquaculture systems, ensuring a secure and sustainable seafood supply for future generations. By addressing the challenges and harnessing the potential of bio-formulations, aquaculture can better withstand the threats posed by viral diseases, contributing to global food security and economic prosperity.

Introduction

Viruses pose significant threats to the aquaculture industry, often causing devastating diseases that result in substantial economic losses. Among these, White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) stand out as major pathogens affecting various aquatic species, including shrimp and fish. Given the limited

options for controlling viral diseases in aquaculture, there is an urgent need to develop effective bio-formulations capable of mitigating viral infections and promoting the health and productivity of aquaculture systems[3].

WSSV is one of the most virulent and widespread pathogens affecting shrimp farming globally. It causes White Spot Disease (WSD), characterized by severe mortality rates and significant reductions in shrimp production. Similarly, IHHNV is a highly contagious virus that primarily affects penaeid shrimp species, leading to necrosis and hematopoietic tissue disorders[23]. The impact of these viral diseases extends beyond economic losses, as they also disrupt the ecological balance of aquatic ecosystems and threaten food security in regions dependent on aquaculture[7].

In response to these challenges, the development of bio-formulations offers a promising approach to disease control in aquaculture. Bio-formulations typically consist of beneficial microorganisms, plant extracts, or other natural compounds that possess antiviral properties or enhance the immune response of aquatic organisms[10]. By leveraging the principles of biological control, these formulations aim to suppress viral replication, strengthen the host's immune defenses, and mitigate the spread of infection within aquaculture systems.

The use of bio-formulations aligns with the growing demand for sustainable aquaculture practices that minimize reliance on chemical treatments and antibiotics. Unlike conventional antiviral drugs, bio-formulations are biodegradable, environmentally friendly, and pose minimal risks of adverse effects on non-target organisms. Moreover, they offer a multifaceted approach to disease management by targeting viral pathogens while simultaneously supporting the overall health and resilience of aquatic species[13].

To develop effective bio-formulations for controlling WSSV, IHHNV, and other viral diseases in aquaculture, multidisciplinary research efforts are essential. This includes screening and selecting candidate microorganisms or natural compounds with antiviral properties, optimizing formulation techniques for maximum efficacy and stability, and conducting rigorous testing under controlled laboratory conditions and field trials in aquaculture facilities. Additionally, research should focus on understanding the mechanisms of action underlying the antiviral activity of bio-formulations and their potential interactions with aquatic host organisms and microbial communities[19].

As the aquaculture industry continues to expand to meet the growing demand for seafood, the prevalence of viral diseases poses a significant challenge to its sustainability and productivity. Viral pathogens such as White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) have emerged as major threats, causing devastating outbreaks and economic losses in shrimp and fish farming operations worldwide. Conventional methods of disease control, such as chemical treatments and antibiotics, are often ineffective against viral infections and can have detrimental effects on aquatic ecosystems and human health. Therefore, there is a pressing need for alternative strategies that are safe, environmentally sustainable, and capable of effectively managing viral diseases in aquaculture settings[24].

Bio-formulations offer a promising approach to disease control in aquaculture by harnessing the natural defense mechanisms of beneficial microorganisms and plant-derived compounds. These formulations can act as biocontrol agents, targeting viral pathogens directly or modulating the host's immune response to enhance resistance to infection. By leveraging the complex interactions between microorganisms, plants, and aquatic organisms, bio-formulations have the potential to provide long-term protection against viral diseases while minimizing the risk of environmental pollution and the development of antibiotic resistance[16].

The development of effective bio-formulations for controlling viral diseases in aquaculture requires a multidisciplinary approach that integrates

expertise from microbiology, immunology, molecular biology, and aquaculture science. Researchers are actively exploring novel sources of antiviral compounds, screening microbial strains for their ability to inhibit viral replication, and optimizing formulation techniques to ensure stability and efficacy in aquatic environments. Field trials are essential for evaluating the performance of bio-formulations under real-world conditions and validating their effectiveness in reducing viral disease outbreaks and improving overall farm productivity[20].

In addition to their direct antiviral effects, bio-formulations can contribute to the sustainability of aquaculture systems by promoting ecological balance, enhancing water quality, and reducing the reliance on chemical inputs. By fostering a more harmonious relationship between aquaculture operations and the surrounding environment, bio-formulations offer a holistic approach to disease management that aligns with the principles of sustainable aquaculture[11]. As research in this field continues to advance, the development and adoption of bio-formulations hold great promise for ensuring the long-term viability and resilience of global aquaculture production.

The emergence of viral diseases poses a significant threat to the aquaculture industry, which plays a crucial role in meeting global demand for seafood. Among these diseases, White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) stand out as major concerns due to their devastating impacts on shrimp and fish populations[21]. Traditional control methods such as chemical treatments and antibiotics have proven insufficient in managing viral outbreaks and often have adverse environmental consequences. Therefore, there is a critical need for innovative approaches that can effectively combat viral diseases while minimizing environmental harm and promoting sustainable aquaculture practices[4]. In this context, the development of bio-formulations offers a promising avenue for controlling viral diseases in aquaculture by leveraging the natural defense mechanisms of beneficial microorganisms and plant-derived compounds. Through strategic research and collaboration across disciplines, bio-formulations have the potential to revolutionize disease management practices in aquaculture, ensuring the long-term viability and resilience of the industry[18].

The development of effective bio-formulations holds great promise for combatting viral diseases in aquaculture and improving the sustainability and resilience of the global aquaculture industry. By harnessing the power of nature's own defenses, these bio-based solutions offer a viable and environmentally friendly alternative to conventional disease control methods, paving the way for a more sustainable future for aquaculture[12].

Materials and Methods

Collection of WSSV infected shrimps

Based on the symptoms severity the WSSV infected *Litopenaeus vannamei* were collected from the hatcheries of Nellore region of AP. The symptoms of the shrimps were in a scale 0, 1,2,3,4 and 5. The collected shrimps were transferred carefully in tight packed water bags to lab of DCLS, Salem along with healthy shrimps were also brought to the lab for further viral transmission studies. The DNA of infected shrimps was isolated based on the kit method and send for the sequencing for the confirmation of WSSV in selected species[2].

Collection of IHHNV infected shrimps

Based on the symptoms severity the IHNNV infected *Litopenaeus vannamei* were collected from the hatcheries of Nellore region of AP. The symptoms of the shrimps were in a scale 0,1,2,3,4 and 5. The collected shrimps were transferred carefully in tight packed water bags to lab of DCLS, Salem along with healthy shrimps were also brought to the lab for further viral transmission studies. The DNA of infected shrimps was isolated based on the kit method and

send for the sequencing for the confirmation of IHHNV in selected species[8].

Rearing of infected aquatic animals in customized scale

The collected both WSSV and IHHNV infected shrimps were brought into the laboratory and reared on the healthy water that contain 30% of salinity as per recommended protocol. Based on the symptoms severity the shrimps were separated in different container and taken for further evaluation

Isolation of Effective bacteria from the healthy shrimps and pond water

The healthy and infected shrimp/fishes were collected from the hatchery of Chidambaram, Tamil Nadu, India. The pond water also collected from the same region for the isolation of beneficial bacterial sps. The dissected shrimp/fish parts were serially diluted and cultured on Tryptic soy agar (TSB) (Table), Thio-sulphate citrate bile salt sucrose agar (Table) and Zobell marine agar. The media were prepared with 0.2% NaCl. The inoculated plates were incubated at 25°C for up to 48h.

Identification of bacteria through 16srDNA sequencing method

The bacteria grown on selective media were further sub-cultured on the selective broth media and the total DNA of bacteria was isolated by kit method. The colonies were grown on TSA medium supplemented with 2% sodium chloride and incubated at 25°C for 24 h. Bacterial colonies were harvested. The extraction of genomic DNA was carried out according to Ferments DNA extraction kit manufacturer's instructions[15]. Two sets of oligonucleotide primers were used for identification general 16s rRNA. The eubacterial universal pair of primers were 63f (5'-CAGGCCTAACACATGCAAGTC) and 1387r (5'- GGGCGGWGTGTACAAGGC) to generate an amplified product of 1.3 kb and send for further sequencing through 16srDNA method (Table of Primer, PCR setup) as outsourcing.

The received DNA of selective strains were further analyzed in NBLAST sequencing through online and identified the *Vibrio sps* based on its homology.

Preparation of Bio-Formulations for the treatment of infected shrimps/fish

PCR amplifications of the toxA and lasB genes were performed in 25 µl reaction mixture containing 0.5 µl of dNTPs (10 mM), 0.5 µl of each primer (10 pmol), 1.5 µl MgCl2 (25 mM), 0.2 µl Taq DNA polymerase (5 U/µl) as outsourcing to check their virulence. Non-pathogenic bacteria were identified and further sub cultured in selective media for mass cultivation of bio-formulation preparation[25]. *Vibrio alginolyticus, Bacillus subtilis, Roseobacter gallaeciensis, and Pseudomonas aestrumarina* were originally isolated from the gastrointestinal tract of adult shrimp *Litopenaeus vannamei*, which were selected because of their antimicrobial activities against shrimp pathogens. These bacteria along with other common beneficial bacterial *consortia (bacillussps, lactobacillus,Nitrobacter, Nitrosomonas, rhodobacter)* were taken and cultured as mas Bio-bac and prepared according to the cfu in mix.

Treatment of Bio-bac with infected species

The combined consortia were prepared in minimal media and directly given to tanks with different ages of infected shrimp. These cultures were used for inoculums (cell density 2 × 108) in WSSV/IHHNV challenge experiments. One gram of WSSV/IHHNV-infected shrimp was homogenized in 9 mL of TNE buffer (Tris 50 mM, NaCl 100 mM and EDTA 1 mM, pH 7.4), the homogenized was centrifuged at 4000 rpm for 10 min and the supernatant was filtered with 0.25 µM syringe filter and the clear filtrate was stored at −70 °C for further study. The 2 mL of bacterial culture broth was added into a glass culture tank containing 10 numbers of *L. vannamei* (0.2 g each), and 0.5 mL of WSSV/IHHNV extract was added into the culture tank. The behavior of the shrimps was observed everyday as well as their survival ratio, level of

white spot formation, overall health status, and the hardness of outer shell.

Validation of the product

The *L. vannamei* was adapted for 2–3 days; after that, 0.1% (cell density 2×10^8) of cultured bacterial isolate was inoculated into the culture tank. After 2 days, 0.01% of WSSV/IHHNV (10^4 copies) was directly added into the culture tank. During shrimp culture, shrimp mortality was monitored twice daily, and the dead shrimps were examined by PCR to confirm WSSV infection. The level of white spot formation, the overall health status, and hardness of outer shell were also observed[1].

Results

The infected shrimps such WSSV and IHHNV shrimps were collected and the sap of the solution were directly inoculated to the healthy shrimps in a artificial tanks and studied for further effects of bio-bac effects on shrimps.

WSSV infected shrimp

IHHNV infected shrimps

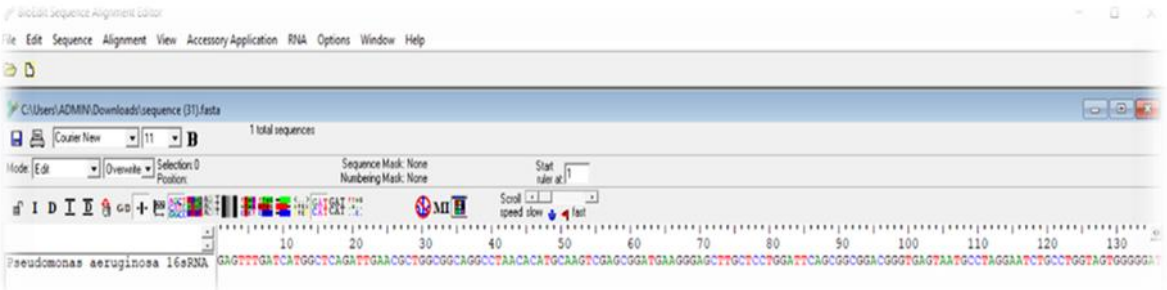
Shrimp seedlings were inoculated with sap of infected shrimp extracts to induce the infection of WSSV and IHHNV. The presence of infection was further confirmed by sending outsourcing through PCR analysis.

Isolation of bacteria from healthy shrimps collected from infected pond

Pseudomonas bacteria were isolated on TCBS

Staining of Pseudomonas bacteria under microscope

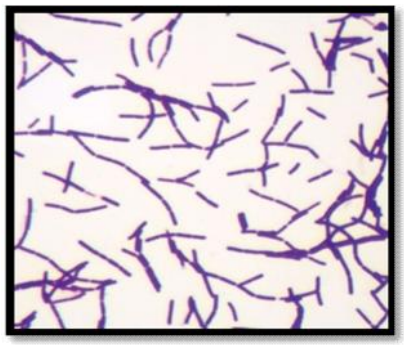
16sRNA sequencing of Pseudomonas bacteria



>*Pseudomonas aeruginosa* 16sRNA

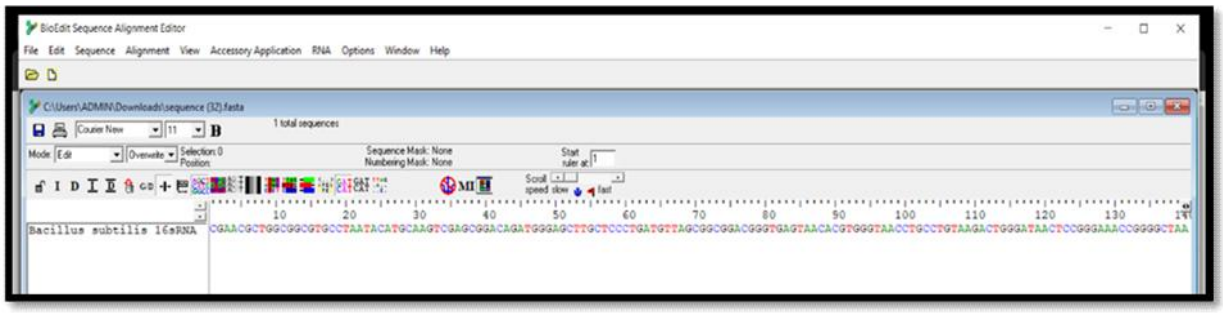
GAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGAT
GAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGT
AGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGG
GGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAG
GCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGAT
CCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAG
GGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGT
GCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGC
GCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAA
ACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA
TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGA
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTA
GCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGT
ACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGA
TGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTG
GGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCA
TGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGC
GAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGT
GAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGG
GGACGGTTACCACGGAGTGATTCATGACTGGGGTGAAGTCGTAACAG.

Bacillus species isolated on Zobell marine agar



Staining of *Bacillus species* bacteria under microscope

16sRNA sequencing of Bacillus species

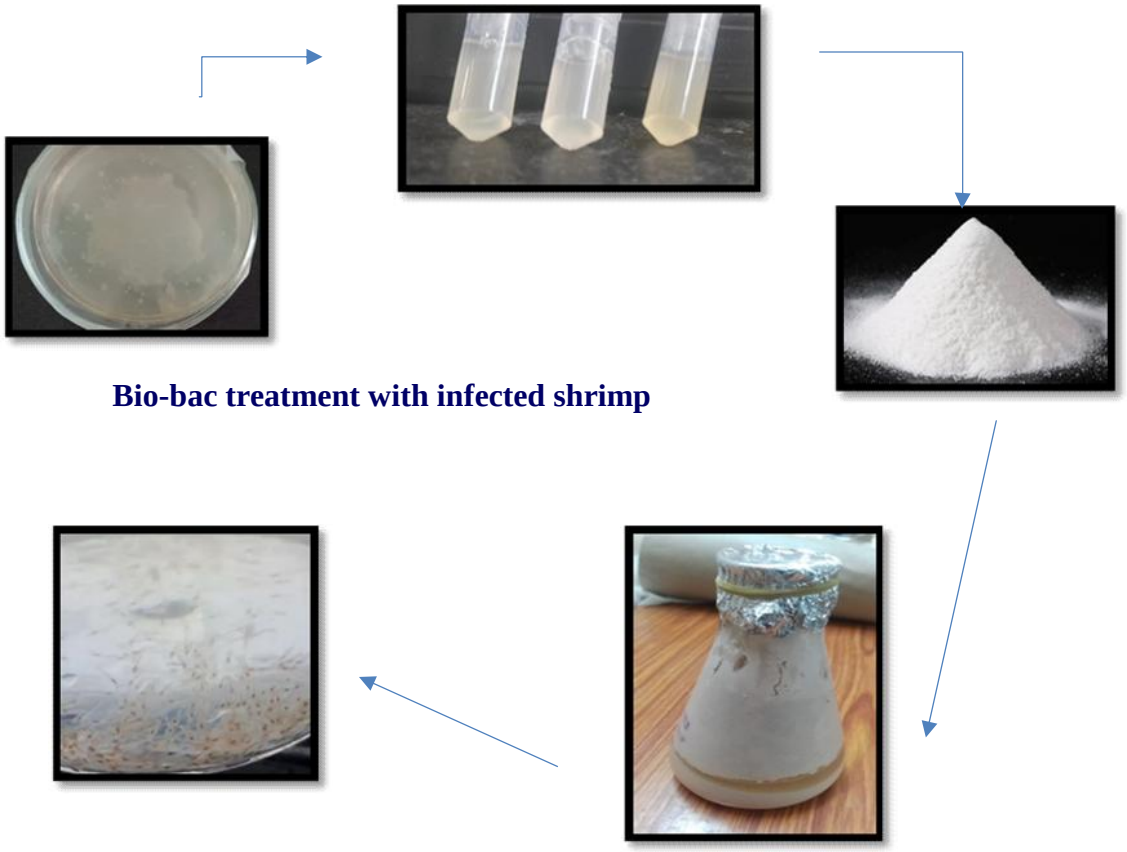


>*Bacillus subtilis* 16sRNA

CGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGG
GAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTC
GGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG
GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGT
TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCG
GTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAA
CTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA
GGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGG
GGAGACGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAG
GGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCG
CAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACG
TCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCT
AAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT
ATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTA
AACCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTG
GAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTAGGAGCCAGC
CGCCGAAGGTGGGACAGATGATTGGGG

Effects of Bio-bac on IHNNV and WSSV infected shrimps

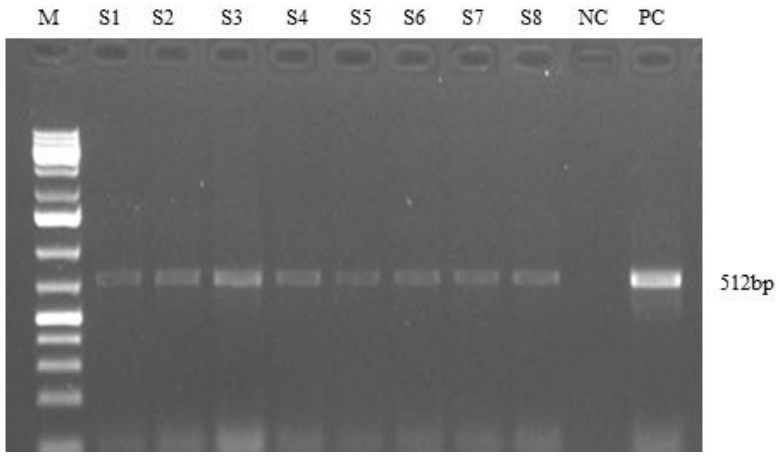
The bio-bac was prepared with combinations of two bacteria *Bacillus* and *Pseudomonas* in feed based formulation. The total combinations of consortia were prepared with talc-powder enriched with calcium carbonate and carboxy methyl cellulose powder to enhance the feeding rate of infected shrimps



Talc+CMC+COns

Bio-bac treatment with infected shrimp

The results were observed for the change in symptoms in infected shrimps also the presence of viral load through PCR.



Random shrimps were collected with 8 samples along with positive control were done on the PCR and analyzed the results. In that all samples were positive but the growth rate somewhat extended when we applied to the shrimp culture compared to control shrimps.

The shrimps infected with WSSV and IHNNV were not cured with treatment but for control it improved the feeding capacity rate (FCR). Thus my finding of bio-bac provided health improvement in control and made the shrimps immunized for further infection of nay pathogen.

Discussion

Aquaculture plays a crucial role in meeting the increasing global demand for seafood. However, viral diseases pose significant threats to the sustainability and profitability of aquaculture operations. Among these, White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) are particularly devastating[14]. Traditional control measures, such as chemical treatments and biosecurity protocols, have limitations. Thus, there's a pressing need for alternative approaches. Bio-formulations, utilizing natural compounds or microorganisms to combat pathogens, offer promising solutions. This discussion explores the potential and challenges of developing bio-formulations for controlling viral diseases in aquaculture.

WSSV and IHHNV are highly contagious viruses affecting various aquaculture species worldwide. WSSV primarily infects shrimp, causing up to 100% mortality within days. Similarly, IHHNV affects shrimp and prawns, leading to significant economic losses[17]. Current control methods, including chemical disinfectants and quarantine protocols, have limitations in terms of efficacy and environmental impact. Moreover, the emergence of antiviral resistance underscores the urgency for alternative strategies.

Bio-formulations harness the power of nature to control pathogens while minimizing environmental impact. They consist of beneficial microorganisms, plant extracts, or naturally occurring compounds with antiviral properties. Unlike chemical treatments, bio-formulations are often species-specific, reducing the risk of harming non-target organisms. Furthermore, they offer sustainable solutions by promoting ecological balance and reducing reliance on synthetic chemicals[22]. The potential of bio-formulations in aquaculture extends beyond viral disease control, encompassing other aspects such as water quality management and probiotic supplementation.

Despite their promise, developing effective bio-formulations for viral disease control in aquaculture presents several challenges. Formulation stability, ensuring the viability and activity of active ingredients under varying environmental conditions, remains a significant concern. Additionally, optimizing delivery mechanisms to ensure proper dispersion and uptake by aquatic organisms is essential[6]. Regulatory hurdles, including approval processes and standardization requirements, further complicate the development and commercialization of bio-formulations. Interdisciplinary collaboration among biologists, chemists, engineers, and regulatory experts is crucial to address these challenges effectively.

To overcome these challenges, interdisciplinary research efforts are needed to deepen our understanding of viral pathogenesis and host-pathogen interactions. Advanced biotechnological tools, such as nanotechnology and genetic engineering, can enhance the efficacy and delivery of bio-formulations[9]. Collaborative partnerships between academia, industry, and regulatory agencies facilitate knowledge exchange and streamline the development process. Establishing clear regulatory frameworks ensures the safety and efficacy of bio-formulations while fostering innovation and market access.

Effective control of viral diseases in aquaculture is essential for ensuring the sustainability and resilience of the industry. Bio-formulations offer a promising avenue for combating pathogens while minimizing environmental impact. Despite challenges, interdisciplinary research, technological innovation, and regulatory support can unlock the full potential of bio-formulations for viral disease control in aquaculture[5]. By investing in these efforts, we can safeguard the health and productivity of aquaculture systems, ensuring a secure and sustainable seafood supply for future generations.

In conclusion, the development of effective bio-formulations for controlling viral diseases in aquaculture represents a promising avenue for ensuring the sustainability and resilience of the industry.

Viral diseases such as White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) pose significant threats to aquaculture, leading to economic losses and environmental degradation.

Bio-formulations offer several advantages over traditional chemical treatments, including species-specificity, environmental sustainability, and promotion of ecological balance. Despite facing challenges such as formulation stability, delivery mechanisms, and regulatory hurdles, interdisciplinary collaboration, technological innovation, and regulatory support can overcome these obstacles.

Investing in the development of bio-formulations is essential for safeguarding the health and productivity of aquaculture systems. By deepening our understanding of viral pathogenesis, leveraging advanced biotechnological tools, and fostering collaborative partnerships, we can unlock the full potential of bio-formulations for viral disease control in aquaculture.

Ultimately, by addressing these challenges and harnessing the potential of bio-formulations, aquaculture can better withstand the threats posed by viral diseases, contributing to global food security and economic prosperity. The continued advancement and adoption of bio-formulations will play a crucial role in ensuring a secure and sustainable seafood supply for future generations.

Acknowledgments

We thank Thiru. K. Rajavinayagam, Secretary, Dr. I. Carmel Mercy Priya, Principal, AVS College of Arts and Science (Autonomous), Salem for their encouragement.

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Quick Response Code

DOI:10.22192/ijamr.2024.11.03.007

How to cite this article:
G. Dheepan, D. Arul Selvan. (2024). Development of effective bio-formulations for the control of viral diseases, such as WSSV and IHNNV for the benefit of aqua culture. Int. J. Adv. Multidiscip. Res. 11(3): 66-77.
DOI: http://dx.doi.org/10.22192/ijamr.2024.11.03.007