CHAPTER - 10

Application of Probiotics in Mariculture

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Introduction

The global demand for the marine cage culture industry is increasing due to its role as an alternative livelihood and its contribution to protein and export value for coastal communities. Over the past two decades, mariculture has expanded significantly worldwide, accounting for 25.5% of global aquaculture production (FAO 2017). In India, the Central Marine Fisheries Research Institute has played a significant role in standardizing breeding and cage culture technologies for various marine finfish species such as cobia, silver pompano, orange-spotted grouper, Indian pompano, seabreams, rabbit fish, and john's snapper and achieved successful production in different maritime states. To ensure the sustainable growth and production of marine finfishes, it is crucial to maintain a health protocol for both the fish and the environment in all types of culture systems. Therefore, the implementation of effective health management practices is essential for the sustainable production of cage culture. Disease outbreaks pose a significant challenge in intensive culture systems, leading to a reduction in profitability in aquaculture industries. Aquatic organisms establish a strong connection with their surrounding environment, over crowing, improper feeding practices etc., thereby increasing their vulnerability to several diseases. The application of antibiotics is a common practice in aquaculture/mariculture industry to control the outbreak of diseases. The application of antibiotics as a precautionary measure has been linked to the emergence of drug-resistant aquatic pathogens when used for longer periods. Several countries have imposed restrictions on the use of specific antibiotics in aquaculture industries. As a result, the implementation of probiotics and dietary supplementation has emerged as a highly effective approach to combat pathogenic agents. This alternative method offers a range of mechanisms to counteract these agents, serving as a viable substitute for antibiotic treatment.

Probiotics in Aquaculture

Probiotics are a type of microbial supplement that contains living microorganisms. These microorganisms have beneficial effects on the host by modifying the microbial community associated with the host or its cultivation environment. They also help improve the utilization of artificial feed and enhance the nutritional value of the feed. Additionally, probiotics enhance the host's response to diseases and improve its overall vigor. The application of probiotics provides effective and sustainable methods for maintaining good water quality and increasing the biomass of natural food organisms in various culture systems. Using water and feed probiotics in hatchery tanks, as well as feed probiotics in cage culture, is considered the best preventive measure against bacterial infections. It is recommended to use probiotics in Mariculture systems, both open and closed, along with other water quality management practices, to control bacterial infections.

Advantages of probiotic application in aquaculture

Probiotic research is gaining momentum in the aquaculture sector as a means to address the potential risks associated with disease enhancement, thereby promoting the development of sustainable practices in the industry. Probiotics application in aqua/mariculture has the following advantageous: 1. enhance growth and survival rates and immunity of fish 2. Enhances nutrient utilization 3. Exhibit bacteriostatic and bactericidal activity against pathogens 4. Prevention of colonization of fish pathogens.

Major probiotic bacteria used in Mariculture

The use of probiotics in mariculture has become increasingly popular, particularly when sourced from the gut of fish. Lactic acid bacteria (LAB), Bacillus, and Streptococcus are among the most favoured bacterial candidates. Although the application of probiotics is a relatively new approach, it has garnered attention for its potential to regulate various physiological activities in aquatic organisms. Currently, several probiotics have been identified as highly effective, including *Bacillus subtilis, Lactobacillus helveticus* and *Enterococcus faecium* which have shown significant benefits. Additionally, there have been reports suggesting that Gram-negative facultative symbiotic anaerobes like *Vibrio, Pseudomonas, Plesiomonas, and Aeromonas* could also be potential probiotic candidates found in the gastrointestinal tract (GIT) of fish and shellfish. Apart from laboratory-based probiotics, various experimentally approved commercial probiotics available in the market are also found effective in aquaculture.

Fig. 1: Probiotics: Commercial probiotics (Fig 1& 2); and laboratory-developed probiotics (Fig.3)

Screening of probiotics

The utilization of probiotics in aquaculture has been widespread due to their diverse range of biological activities. The initial and essential step in this process is the screening of probiotics, which requires a systematic and scientific approach.

The criteria for selecting probiotics consist of the following: (1) It must not cause harm to the host; (2) it should not invade into the tissues and cause tumors; (3) it should effectively reach the intended site within the host; (4) it should contain plasmids without antibiotic and virulence resistance genes; (5) it should colonize the host for a sustained period and be able to replicate; and (6) it should demonstrate efficacy in host model systems rather than just in vitro studies.

In invitro screening for potential probiotics, the inhibitory or antagonistic activity of bacteria need to be checked. In vitro screening for inhibitory substances commonly employs four methods: the double layer method, the well diffusion method, the cross-streak method, and the disc diffusion method. All of these methods are based on the same fundamental principle, where a bacterium generates an extracellular substance that can inhibit either itself or another bacterial strain.

Application of probiotics

Application of probiotics in aquaculture employs several key procedures: (1) acquiring a comprehensive understanding of the probiotic application, (2) conducting both in vivo and in vitro assessments to determine their pathogenicity, and (3) conducting a long-term practical evaluation of the treated probiotics. Additionally, modern techniques such as ERIC-PCR, PCR-DGGE/TGGE, FISH, and 16S rRNA gene sequencing are the best molecular tools for the selection and evaluation of probiotics.

1. The utilization of probiotics extends beyond their role in promoting the growth of various cultivated species in aquaculture. For instance, the addition of Bacillus spp., *Enterococcus* spp. and *Pseudomonas spp*. at concentrations of 10^7 and 10^9 CFU/g in the diet resulted in notable weight gain in fish.
2. Probiotic supplementation enhances feed utilization and weight gain in cultured fish. Probiotics can stimulate feed palatability by breaking down indigestible components, producing vitamins, and detoxifying poisonous compounds in the diet. Probiotics increase fish resistance to stress caused by environmental and technological hazards. The application of beneficial bacteria provides micronutrients such as vitamins, fatty acids, and essential amino acids to support the healthy growth of cultured fish.
3. Microorganisms can establish themselves in the gastrointestinal tract (GIT) because they reproduce at a faster rate than they are expelled after being administered for an extended period. Probiotics are continuously introduced into fish cultures to promote health by boosting the expression of various immunological factors and by occupying physical space in the gut mucus layer, thereby reducing the presence of pathogens. Probiotic candidates also play a vital role in nutrient enhancement in the host. Enhancement of crude lipid, total protein, and body weight in fish fed with probiotics *Lactobacillus* sp. *Bacillus* spp. and *Streptococcus* spp.

Methods of probiotic application

The application of probiotics plays a crucial role in attaining goals like disease prevention and treatment. The administration can be done through feed or water, depending on several factors such as the specific probiotics utilized, the form of supplementation, the mode of administration, the dosage level, and the duration of application. These variables influence the choice of method for probiotic application.

Probiotics can be categorized into two main groups according to their mode of action. The first group is gut probiotics, which are taken orally with food to enhance the beneficial microbial flora in the gut. The second group is water probiotics, which thrive in water environments and effectively eliminate pathogenic bacteria by consuming all the nutrients available in the specific medium, ultimately starving the harmful bacteria and eliminating them.

Oral administration

The most commonly used method involves incorporating probiotics into the feed. Probiotics can also be introduced into the tank or pond water to protect against infections. Parabiotics, on the other hand, are inactive microbial cells derived from probiotics. They contain cell components like peptidoglycans and surface proteins, offering advantages such as being available in a pure form, easy to produce and store, and having a higher likelihood of triggering specific responses through ligand-receptor interactions. Probiotics can be administered continuously or at regular intervals. Many studies have focused on continuous feeding of the host fish for varying durations, ranging from 15 to 94 days.

Application of multi-strain probiotics

The use of multiple-strain products offers the benefit of being effective against a wider range of conditions and species. It is also common to combine probiotics with prebiotics and/or plant products. Research on the application of multistrain probiotics suggests that probiotics containing *Bacillus spp*. ($1x10^9$ CFU/mL) and *Lactobacillus spp*. ($1x10^{11}$ CFU/mL), provided at concentrations of 0, 0.5, and 1.0 ml/l in water for 8 weeks, can enhance the growth of fish by improving the health of their gut, liver, and muscles. Numerous reports have demonstrated the positive impact of probiotics in aquaculture/mariculture, including improved fish growth performance, immune response, and resistance against certain pathogenic bacteria.

Fig.2: Indian pompano, Trachinotus mookalee fed with laboratory-developed probiotics

Probiotics in improving water quality

Bacillus sp. is particularly linked to the use of probiotics in enhancing the quality of culture water. This is because Gram-positive bacteria, such as *Bacillus* sp., have a superior ability to convert organic matter into CO_2 compared to Gram-negative bacteria. The presence of high levels of Gram-positive bacteria, facilitated by the use of *Bacillus* sp., can effectively reduce the accumulation of dissolved and particulate organic carbon. Consequently, the utilization of Bacillus sp. in aquaculture systems leads to improved water quality, enhanced survival and growth rates, as well as better health conditions for cultured shrimp and fish. The utilization of commercially available water and feed probiotics in marine culture systems is highly recommended as the most effective preventive measure against bacterial infections.

Conclusion

Probiotics have garnered attention in the aquaculture/mariculture industry, prompting research efforts to investigate their utilization and potential advantages. This is due to the increasing demand for probiotics in the aquaculture of animals. Investigations should focus on screening host-specific probiotic strains from aquaculture-rearing systems to effectively manage their quality and functional properties. Although probiotic bacteria provide numerous advantages to the host, there exist certain restrictions that need to be addressed. For instance, the antimicrobial compounds or bacteriocins produced by probiotic candidates against pathogenic bacteria are not specific to particular species. To increase the efficiency of probiotic bacteria, it is imperative to prioritize strain improvement. In conclusion, the application of single or multistrain probiotic bacteria plays a great role in the sustainable production of disease-resistant fish in cage culture or any kind of other mariculture systems.

Suggested Readings

Loka, J., Ghosh, S., Ranjan, R., Xavier, B., Megarajan, S., Sadu,N., Shiva,P., Gopalkrishnan, A. 2022. Potential of laboratory-developed marine bacterial consortium as antibacterial and growth promoting agent in mariculture. Marine Fisheries Information Service Technical & Extension Series . 252; 15.

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