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Prospects of using Bacillus species as Probiotic in the Aquaculture sector

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ABSTRACT

The utilization of probiotics in aquaculture has garnered significant attention as a potential microbial strategy for enhancing the health and overall well-being of various aquatic species reared in aquaculture settings. Among the diverse array of microbial options available, Bacillus probiotics have emerged as a particularly advantageous choice. This predilection is due to their special qualities, which include their ability to produce antimicrobial chemicals, the fact that they may be mixed with fish feed without causing any negative effects or toxicity, and the fact that they can create spores, which allows them to survive in harsh environments. Consequently, Bacillus species have gained prominence as the foremost probiotic option for augmenting feed consumption, mitigating stress responses, bolstering immune defenses, enhancing disease resistance, maintaining tissue integrity, and improving water quality. This mini-review retriving mainly about characteristics, mechanisms and importance of Bacillus probiotic in aquaculture industry.

KEYWORDS

Probiotics, aquaculture, antimicrobial compounds, Bacillus sp., disease resistance

Introduction

Aquaculture a multidisciplinary science (Shang, 1986) and a global food manufacturing sector that is expanding quickly (Cai and Leung, 2017). Disease outbreaks during culture cause enormous economic loss, which has a disastrous effect on aquaculture, making them a critical concern (Liu *et al*., 2010). Regular application of various therapeutic/preventative, including growth-promoting agents, has had a number of detrimental effects, including an increase in the frequency and dissemination of antibiotic resistance patterns in pathogens, as well as a disparity in the normal gut microbiota due to the possibility of aquatic habitats being a source of transmission, lasting effects in tissues, suppression and/or elimination of certain beneficial bacteria, worsening of the culture environment, and other factors (Cabello, 2006). On the other hand, there are only a small number of vaccines available against specific diseases, making them ineffective as a general aquaculture disease management plan.

The aquaculture sector investigated and created a number of methods that are, above all else, sustainable, environmentally benign, and just as effective as antibiotics, medicines, and chemotherapeutic agents (Balcazar *et al*., 2006; Lazado *et al*., 2015). In aquaculture, probiotics are a safe supplement that enhance host health by fostering development, supplying nutrients, and managing microbial colonization, improving immune responses, enhancing the use of feed, raising the activity and digestibility of digestive enzymes, enhancing the quality of the water, and reducing disease (Selim & Reda, 2015). Gram-positive probiotic microorganisms that are frequently employed in aquaculture include the following examples: *Bacillus*, *Lactobacillus*, *Micrococcus*, *Streptococcus*, *Enterococcus*, *Carnobacterium*, and *Weissella* species can be found in the environment. (Irianto & Austin, 2002; Nayak, 2010). The advantages of probiotics in aquaculture farming encompass a number of outcomes, including (Nayak, 2010; Mohapatra *et al.*, 2013): (i) Immune system stimulation in both in vivo and in vitro conditions (ii) modulation of the immune system; (iii) microbial colonisation of the digestive tract improves feed conversion on the organism; and (iv) disease prevention.

In addition to controlling and outcompeting pathogenic microorganisms, probiotics can be added to the culture medium to promote the growth of organisms like Bacillus spp., which are Gram-positive endospore-forming bacteria. Additionally, when administered to aquatic species, microbes such as *Bacillus* spp. are not harmful or pathogenic and do not cause any adverse consequences. (Farzanfar, 2006). Enhancing aquatic species' growth, nutrition, resistance to illness, and immunity are just a few of the many advantages. This bacteria uses a range of dissimilar and overlapping methods, including competitive exclusion, antagonistic synergism, and immune-stimulating effects. This article provides a summary of this bacterium's uses in aquaculture as a probiotic, its mode of administration and several mechanisms.

Bacillus- An effective probiont

According to Seong et al. (2018), *Bacillus* is included in the phylum Firmicutes, class

Bacilli, and order Bacillales. They have a rod-shaped morphology (between 2.5 and 10 µm), are endosporing, aerobic or facultatively anaerobic, and are positive for catalase. (Amoa-Awua & Jakobsen, 1995). These species are ubiquitious in nature illustation shown in Fig. 1.

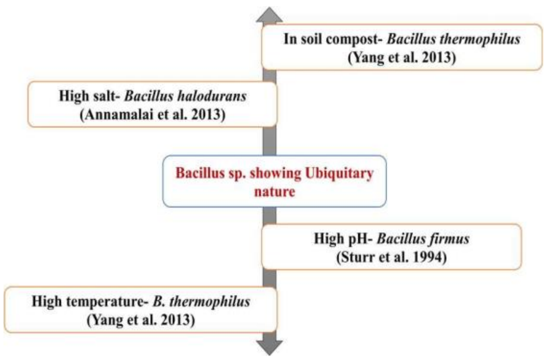

Fig. 1: Figure representing ubiquitins nature of Bacillus sp.

Bacillus spp., which include *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans*, and *Bacillus licheniformis*, are some of the probiotic bacteria that produce spores. The spores can withstand heat, cold, radiation, desiccation, and disinfectants. Differential sterilisation and fumigation techniques are often validated using *B. subtilis* subsp. globigii, a heat, chemical, and radiation resistant bacteria. The spores of the obligate thermophile *B. stearothermophilus* are used to test heat sterilisation protocols. The breakdown of waste materials, whether it occurs naturally or artificially, depends on specific *Bacillus* species.

Structural descriptions: The two primary divisions of the family Bacillaceae, which comprises rod-shaped bacteria that create endospores, are the anaerobic spore-producing bacteria of the genus Clostridium and the aerobic or facultatively anaerobic spore-producing bacteria of the genus *Bacillus*. The majority of Bacillus species are saprophytes. Because of their unique physiological traits, Bacillus endospores may endure or even flourish in hostile conditions, such as desert sands, hot springs, Arctic soils, fresh waters, and marine sediments, in addition to being resistant to unfavourable physical and chemical conditions. The representatives of genus Bacillus may thrive at extreme temperatures, pH levels, and salt concentrations level where few other creatures could survive. These representatives include thermophilic, psychrophilic, acidophilic, alkaliphilic, and halophilic species.

Sporulation: Spores are usually produced when the vegetative organism endures stress by limiting the supply of nutrients. Pathogens allow the vegetative organism to remain in a latent state outside of the aerobic or anaerobic environment (Atrih and Foster, 2002). Similar to this, when nutrients are few, the Gram-positive bacterium *B. subtilis* initiates the sporulation process. Spores can endure environmental stresses like heat, radiation, toxic chemicals, and high pH levels for extended periods of time while dormant. Spores of the *Bacillus* species can survive for years in their resistant, dormant forms, but they can swiftly reactivate and start to multiply anew when they come into contact with specific nutrients. The process of sporulation begins with the asymmetric division of the rod-shaped B. subtilis, which results in the development of a "polar septum" that separates into two genetically similar but physically different compartments: a bigger "mother cell" and an inferior "forespore." Each of these compartments will ultimately experience different cell fates (Tan and Ramamurthi, 2014). A "simple" form of cell differentiation was studied using sporulation as a model system, and it is still used to research basic cell biological processes today.

Several advantages of Spore producing bacteria over that do not produce spores, such as *Lactobacillus* spp. That is mentioned by Cutting, (2011): (i) The viability of desiccated spores is unaffected by storage at room temperature.; and (ii) Spores can tolerate the low pH of the gastrointestinal barrier.

Important features of Bacillus sp.

1. Produces phytase, chitinase, xylanase, cellulase, tannase, protease, and lipase (Ghosh *et al*., 2018)
2. Produces bacteriocins, peptide and lipopeptide antibiotics, and other antimicrobial compounds (Abriouel *et al*.,2011)
3. Sporulation potential (Elisashvili *et al*., 2019)
4. Bacillus may colonise various settings and improve the host's nutrition.

Mode of administrations of Bacillus species

Aquaculture systems employ a variety of probiotic dosing methods. Probiotics can be added straight to water or taken as dietary supplements (as live foods as Artemia, rotifers, or pellet food) (Skjermo and Vadstein, 1999). In addition, probiotic injectable administration has also been documented (LaPatra *et al*.,2014). Probiotics operate better when they are administered properly, and understanding how they work in combination with effective administration techniques can be crucial for their use in aquaculture (Dawood and Koshio, 2016). A small number of studies have looked at the possible health advantages of probiotics given directly into water, and most studies that have looked at probiotic qualities in aquaculture leaned on dietary supplements. The aquaculture system, fish size and age, as well as all other contributing aspects, should be taken into consideration while choosing the optimal administration method.

Probiotics are often given to aquaculture via a limited number of methods, including injection, direct inclusion into the water, and dietary additives to pelleted or live feed (Jahangiri and Esteban, 2018).

Bacillus sp.'s mechanisms for reducing infections in aquaculture

1. **Production of bacteriocins:** Many bacteria create bacteriocins, which are extracellularly released bioactive antimicrobial peptides. Bacteriocins can be used to eradicate or halt the growth of prokaryotes, dangerous microorganisms, and microbes that are antibiotic-resistant (Zou et al., 2018). It is known that certain Bacillus species produce the effective pathogen inhibitors bacteriocins and bacteriocin-like inhibitory substances (BLISs).
2. **Modulating antioxidant enzymes:** To efficiently combat free radicals, Bacillus species produces antioxidant enzymes SOD and glutathione (Li et al., 2012). Fish typically contain the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase, and catalase (Giulio et al., 1993). Antioxidant enzymes provide information about an aquatic organism's antioxidant state and oxidative stress (Hindu et al., 2018).
3. **Quorum quenching:** In the bacterial regulatory mechanism known as quorum sensing (QS), bacteria create, release, and detect tiny signal

molecules known as autoinducers in order to coordinate gene expressions in a way that is dependent on cell density (Chu et al., 2014). Bacteria use N-acyl homoserine lactone (AHL) signals to synchronise target gene expression and keep track of their population density (Dong et al., 2004). Disrupting QS has been proposed and demonstrated to be an approach to manage pathogenic bacteria in the fields of animal husbandry and aquaculture. QS controls how pathogenicity traits in bacteria are regulated (Piewngam et al., 2018). The QQ (Quorum Quenching) ability of Bacillus species is one of its mechanisms for reducing the pathogenicity of harmful bacteria (Wee et al., 2018).

4. **Improving feed utilization and growth:** To enhance the growth and advance the welfare of farmed fish, many chemicals and dietary supplements have been utilised. One of the most important of these supplements is probiotics, which are employed as a straightforward and secure additive to increase the activity of digestive enzymes and provide nutrients to the host in order to promote growth, which improves feed utilisation and digestibility (Reda & Selim, 2015). The presence of digestive enzymes and the level of their activity in fish are indicators of the relative acceptance of food, digestive ability in relation to the type of feed provided, trophic niche in natural conditions, and fish feeding ecology (Solovyev et al., 2014). One of the reasons Bacillus is chosen as a probiotic to enhance digestive enzyme activity is because its enzymes are effective at metabolising a wide variety of lipids, proteins, and carbohydrates (Han et al., 2015).
5. **Competition for adhesion sites-** Another commonly hypothesised method by which probiotics reduce the multiplication of infections is competition for adhesion sites (Ige, 2013). One of the ways probiotics combat dangerous infections is by colonisation of the gut and other tissue surfaces and competition for adhesion space (Ringø et al., 2007). Probiotic microorganisms develop more quickly than pathogenic germs, which contributes to competition for adhesion sites and the exclusion of pathogenic microbes. The ability of microorganisms to adhere to surfaces is greatly influenced by a variety of elements, including adhesins, lipoteichoic acids, passive forces, hydrophobic, steric forces, and electrostatic interactions (Mohapatra et al., 2013).
6. **Enhancing immune system of the host:** Supplementing with probiotics has several major advantages, one of which is improved host immunity. Probiotics have the ability to alter innate immunity via altering humoral immune reactions and the proliferation of immunity-related genes (Verschuere et al., 2000). Fish's humoral and cell-mediated immune responses are triggered by Bacillus. Fish with dietary Bacillus species treatment showed improved cellular and humoral immune responses, which translated to higher disease resistance (Yi et al., 2018).

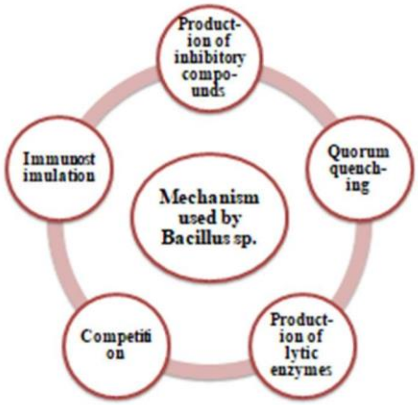

Fig. 2: Bacillus sp. mechanisms in combating the infections

Application of Bacillus sp. In Aquaculture

1. As an additive- It is best used as an additive in functional feed.
2. As a part of fermented aquafeeds to enhance nutritional status.
3. As a bioremediating supplement.
4. As a live feed supplement.

Conclusions and Future perspectives

The use of probiotics in aquaculture is now increasingly being viewed as an environmentally friendly method of reducing health-related difficulties. Probiotics' ability to prevent infections is achieved by increasing resistance and discouraging germs. Aquaculture makes extensive use of probiotic Bacillus strains, which are gaining importance. Several studies have shown that specific probiotics usage can be an optional method for protecting aquatic organisms against diseases. However, it is impossible for aqua culturalists to foresee the disease onset in order to supply probiotic nourishing in the weeks prior to disease. Subsequently, encourage work on the impacts of treatment is required if the onset has as of now happened. It has been established that the probiotic Bacillus is effective in reducing the negative impacts of pathogens in aquaculture, including bacterial and viral infections. Furthermore, Bacillus species are commonly employed as bioremediating agents, eliminating organic wastes, heavy metals, and other contaminants from the environment. They can also be introduced into the growth medium to suppress and outcompete pathogenic bacteria.

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