Aquaculture

Review

***Bacillus firmus*: Applications and Potentialities as a Probiotic for Aquaculture**

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ABSTRACT

Background: Nowadays, numerous bacterial strains, genus Bacillus, are used as probiotics to promote the growth of cultivated aquatic organisms, particularly in their larval stages. **Aim:** To conduct a review of the different applications of *Bacillus firmus* in the industry, with emphasis on their use as a probiotic for aquaculture. **Development:** *B. firmus* is a widely used beneficial bacterium as a nematicide to protect crops, the bioremediation of contaminated environments, enzyme production, and as a probiotic for aquaculture. Its potential for shrimp culture is outstanding for controlling diseases and water quality management in the culture pond. **Conclusions:** The specific mechanisms of *B. firmus* for shrimp culture have not been studied broadly, since it is particularly relevant in providing all the benefits that the bacterium offers to this economic sector.

Keywords: bacterium, shrimp, culture, probiotics (*Source*: *MeSH*)

INTRODUCTION

Aquaculture is one of the fastest-growing economic activities, contributing with approximately 50% of the world's fish supply (Ramírez-Fernández, 2018). Shrimp culture is one of the most profitable sectors due to the growing demand in the international market. Overall, the size of the shrimp market was $ 39 billion by late 2017, and the estimations reveal that it will reach the $ 67 billion by 2027 (FAO, 2020). The white shrimp is the most frequently aquacultured marine crustacean, accounting for 75.7% of the world production of all the marine shrimp species cultivated (Toledo *et al*., 2018). However, the intensification of that sector is creating stress conditions that lead to high-mortality causing diseases. In view of that situation, probiotics

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appear as a viable alternative for sustainable and environmentally-friendly aquaculture (Toledo *et al*., 2018).

Several studies have focused on the applications and beneficial effects of probiotics on aquaculture (Peñalosa-Martinell *et al*. 2020; Valdes Vaillant *et al*., 2020), particularly on bacteria of genus Bacillus (Kuebutornye and Abarike, 2019). Today, numerous bacterial strains of this genus are used as probiotics to promote growth in cultivated aquatic organisms, especially during the larval stages. Nevertheless, researchers are becoming particularly interested in the isolation and characterization of new strains, in keeping with the need to isolate more probiotic strains of *P. vannamei* in enhanced culture media. Besides, further research must be done to sequence the genomes of probiotics to identify functional genes and develop novel enzymatic products through *in vitro* assays for the modulation of the physiological state of *P. vannamei* in the diet.

In this context, bacterium *Bacillus firmus* is widely used to control diseases in crop farming and aquaculture, from which hundreds of strains have been isolated in different environments. The aim of this paper is to conduct a review of the different applications of *B. firmus* in the industry, specifically as a probiotic for aquaculture.

DEVELOPMENT

Bacillus firmus was identified by Bredemann and Werner in 1933, a Gram-positive bacterium that belongs to risk group I reported as GRAS (Generally Recognized as Safe), with aerobic growth and ellipsoid endospore formation. The colonies are 2-4 mm diameter, with irregular contours and gummy in agar. Some strains of this species are alkaline tolerant, and can grow in environments with a pH=11. Due to the similarity of the morphological characteristics of the bacterium to *B. pumilus* and *B. subtilis,* its identification relies on physiological differences and genetic traits like the 16S rDNA (Geng *et al*., 2014).

It is used as a probiotic in different systems of livestock production and aquaculture; several studies also report its beneficial effects in agriculture, bioremediation, extracellular enzyme and nuclease production, and biohydrogen production (Sinha and Pandey, 2014; d'Errico *et al*., 2019; Baramee *et al*., 2020; Barathi *et al*., 2020; Bhatt *et al*., 2021; Huang *et al*., 2021).

Bacillus firmus as a probiotic

Today, numerous bacterial strains of the genus Bacillus are used as probiotics to promote growth of cultivated aquatic organisms, especially in the larval stages. Several research studies recognize the probiotic activity of *B. firmus* in organisms, such as the Nile Tilapia, the Gibel Carp, rotifers, and the white shrimp (Aly *et al*., 2008; Li *et al*., 2018; Ruiz-Toquica *et al*., 2020). The inclusion of this bacterium in the diet of these organisms has demonstrated a capacity to enhance survival and several production indicators, as well as the potentialities for aquaculture use; However, the number of studies related to this topic is low.

Aly *et al*. (2008) demonstrated that the probiotic activity of *B. firmus in O. niloticus* (the Nile Tilapia), showed inhibitor effects against *in vitro A. hydrophila*, which caused no signs of the disease or mortality when it was injected in the fish. Sun *et al*. (2013) found *B. firmus* strains in the digestive tract of healthy shrimps, and demonstrated that they significantly increased *P. vannamei* survival and immunity in the presence of the white spot virus when supplied in the diet (Sun *et al*., 2013). Jamali *et al*. (2015), in his study of the beneficial effect as a probiotic that promotes growth and survival in *P. vannamei* larval culture (Jamali *et al*., 2015)

Other authors, such as Li *et al*. (2018) evaluated the effects of supplementing the diet with *B. firmus* (10^8 colony forming units (cfu)/g) on the activity of digestive enzymes present in the intestine, and on the bacterial composition, in *L. vannamei*. In their study, shrimps that received a diet containing *B. firmus* showed the highest amylase, pepsin, and lipase activities. These findings suggest that probiotics might enable the utilization of nutrients to enhance the activity of *Penaeus vannamei*'s digestive enzymes, thus shaping the composition of the intestinal microbiota (Li *et al*., 2018).

Ruiz-Toquica *et al*. (2020) stressed that *B. firmus* is tolerant to biliary salts, a strong phosphatase activity and microbial activity against pathogens like *Vibrio alginolyticus* and *Aeromonas hydrophila.* These authors claim that following three weeks of administration to shrimp post-larvae (10^6 CFU $mL^{-1} \cdot day^{-1}$), there was a significant increase of the specific growth rate (TEC = 3.8 ± 0.7 % day^{-1}), daily weight gain (ADG = 1.5 ± 0.1 mg day^{-1}), and feed conversion rate compared to the controls that did not receive the bacterium (sterile PBS). Likewise, the administration of *B. firmus* to rotifers after 48h of culture led to an increase of the specific growth rate (TEC = 20.2 ± 1.5 % day^{-1}), fertility (F = 0.4 ± 0.03 eggs $individuals^{-1}$), and productivity (R = 16.0 ± 0.7 individuals mL^{-1}) (Ruiz-Toquica *et al*., 2020).

Li *et al*. (2019) suggested a novel approach on the probiotic mechanism of the bacterium based on its anti-*Quorum sensing* (QS) activity. QS interference, also known as *quorum quenching*, is an alternative method to antibiotic use for the control of diseases in aquaculture. The principal signaling or self-inducing molecules in the QS system are the acyl-homoserine-lactone (El-Esawi *et al*.), which are produced by Gram-negative pathogens, such as *A. hydrophila, A. salmonicida* and *Vibrio harveyi,* which regulate various biological functions, including the release of virulence factors and the formation of pathogen biofilms. In this study, the authors demonstrated *B. firmus*'s capacity to interfere with the detection of the pathogen's QS, and lessen the production of *A. hydrophila*'s virulence factors significantly, including the formation of protease, hemolysin, and biofilm in Gibel Carp culture (Li *et al*., 2019).

Recent research describes and characterizes *B. firmus* as a nematicide, a promising biocontrol agent for integrated management in sustainable agriculture (Huang *et al*., 2021).

Several studies have demonstrated its effectiveness against different plant nematodes, and it inhibits egg hatching and lethal activity, causing paralysis in the parasitic nematodes (Xiong *et al*., 2015). Its activity has a broad spectrum, as it reduces the symptoms and damage caused by different types of nematodes to economically important crops (d'Errico *et al*., 2019). It is very effective on *Meloidogyne* gall-forming nematodes. Its activity has also been described in relation to cyst-forming nematodes (*Heterodera* sp., *Globodera* sp.), and endoparasitic migratory nematodes, namely root nematodes (*Pratylenchus* sp. , *Tylenchulus* sp), and the spiral nematode (*Helicotylenchus* sp.) (Ghahremani *et al*., 2020).

B. firmus is also used as a biological control of *Phytophthora capsici*, in tomato (Lagunas-Lagunas, 2001), and as a biofertilizer (Cuervo Lozada, 2010). It has a great potential to promote plant growth, which was demonstrated in crops such as tomato and cotton (Huang *et al*., 2021). Its utilization as an active ingredient for new generations of biofertilizers and biopesticides make this bacterium an excellent biological control agent to protect crops, whose properties might even be used in aquaculture, particularly in shrimp culture.

Shrimp culture intensification has been affected by the occurrence of disease-causing viruses, bacteria, and parasites, namely metazoan. Penaeid shrimps are intermediate hosts of several metazoan larvae, such as nematodes, cestodes, and trematodes; thus there is a need to use a biological control to eliminate them.

Biological nitrogen-fixing

Genus Bacillus has a great metabolic versatility, and its capacity has demonstrated to fix nitrogen biologically. The biological fixation of nitrogen is a microbial process in which the atmospheric nitrogen is reduced to ammonium and joins the biomass, being the main source of nitrogen for plants (Zlotnikov *et al*., 2001). This strategy is appealing to the scientific community, as a need to implement environmentally-friendly and sustainable strategies that enhance soil productivity and create the best conditions for crops. The literature is not abundant in terms of describing the action mechanism of genus Bacillus nitrogenases.

In 1998, Xie *et al.* isolated strain-generating endospores with ARA (acetylene reduction activity) from soil samples, which corroborated nitrogen fixation activity in strains of *Bacillus licheniformis, B. subtilis, B. cereus, B. pumilus, B. brevis and B. firmus* (Corrales-Ramírez *et al*., 2017)

B. firmus has demonstrated to have the capacity of enhancing nitrogenase activity of isolated microorganisms from other plants, such as *Dactylus glomerata*, as well as improving nitrogenase activity of diazotroph *Klebsiella terrígena*. *B. firmus might protect K. terrígena* nitrogenase from dioxygen, since this enzyme inactivates with the dioxygen tensions that occur in the atmosphere normally, which can be explained through a rise in the amount of nitrogen fixed by the plant, reducing the application of chemical nitrogen fertilizers considerably (Zlotnikov *et al*., 2001).

Cuervo Lozada, (2010) described the *B. firmus* potential as a biological fixator of nitrogen and phosphate solubilizer, with nitrogenase activity in selective media (Cuervo Lozada, 2010).

These features broaden the prospects of this bacterium as a probiotic in shrimp culture, where, in addition to diseases, water quality management difficulties arise due to the accumulation of organic matter and toxic metabolites like nitrogenated compounds. This aspect could help reduce water changes in the culture because of the capacity to transform ammonium into free nitrogen, eliminating toxicity, in the same way as it occurs through a biological filter in a water recirculation system.

The culture tank's water quality is a critical point in the culture process, and it must be controlled according to the physical, chemical, and biological parameters. The parameters must be within the acceptable ranges for proper shrimp development. Otherwise, the population in the culture might grow slowly, with the proliferation of pathogens and disease outbreaks, ensued mortality, and poor quality of the end product.

Bioremediation

The utilization of *B. firmus* to remove heavy metals from industrial residual waters demonstrate the adjustment to toxic environments, and the capacity of the bacterium to use arsenic, cobalt, zinc, cadmium, and mercury, becoming an attractive alternative in bioremediation. Microorganisms, especially plant beneficial bacteria, are effective in azoic dye bleaching, tolerating metals and saline stress (Mahmood *et al*., 2020).

Salehizadeh and Shojaosadati, (2003) demonstrated the bio absorption potential of Pb, Cu, and Zn through a novel polysaccharide produced by *B. firmus*, whereas Keung *et al*. (2008) mentioned the possibility of *B. firmus* to solubilize Cd and Zn. Moreover, Bachate *et al*. (2013) considered it a potential candidate for bioremediation of As and Cr-contaminated environments, they reported the capacity of the bacterium to reduce Cr (VI) and oxidize As (III) into less toxic forms (Bachate *et al*., 2013).

Dino *et al*. (2019) reported it as being able of biodegrading aniline from the residual waters of the textile industry, while Barathi *et al*. (2020) reported the capacity of this microorganism to degrade high concentrations of reactive industrial textile dyes RB160, and its capacity to turn them into nontoxic products for the environment.

Besides the inadequate levels of physical, chemical, and biological parameters in the culture ponds, the water contains contaminants that might compromise shrimp production. Among them are hydrocarbons, pesticides, toxic industrial wastes, waste water from nearby settlements, and heavy metals. The exposure of aquatic animals to toxic environments not only can cause damage to the intestinal structure and immune system, but also affect the structure of the intestinal microbiota.

The previous aspects evidence the potentialities of this bacterium and its action mechanism to improve water quality in the culturing tanks where it is added.

Enzyme production

One of the most commonly cited applications in the literature related to *B. firmus* is the production of several extracellular enzymes, such as proteases (Moon and Parulekar, 1993).

Proteolytic enzymes account for 65% of the global market of industrial enzymes thanks to its multiple applications: different industrial processes like food production, pharmaceuticals, and the formulation of detergents (Annamalai *et al*., 2014). These are preferrable to the enzymes from plants and animals, since they have the largest part of the characteristics required for the industrial biotechnological processes.

Some authors referred to the capacity of this bacterium to produce xylanolytic enzymes such as xylanase and β-xyloxidase (Fatmawati *et al*., 2021), cell endonucleases like cyclodextrin glycosyltransferase (CGTase) (Gawande *et al*., 1998; Gawande *et al*., 1999; Mahat *et al*., 2004; Moriwaki *et al*., 2007; Mazzer *et al*., 2008; Pazzetto *et al*., 2011; Bueno *et al*., 2014), fibrinolytic enzymes (Seo and Lee, 2004), and heat-stable xylanases (Tseng *et al*., 2002; Baramee *et al*., 2020).

The utilization of probiotics in the animal diet as growth promoters results mainly from improvements observed in production associated with an increase of nutrient digestion and uptake. Some of the antimicrobial compounds synthesized and secreted by probiotic bacteria are antibiotics, short-chain fatty acids (formic, acetic, propionic, butyric, and lactic), hydrogen peroxide, iron siderophores (chelating compounds), and bacteriolytic enzymes (lysozyme) amylases and proteases (Pérez-Chabela *et al*., 2020).

CONCLUSIONS

The characteristics that distinguish *Bacillus firmus* and its utilization with several industrial applications widen its prospects, and confer this bacterium a probiotic potential for aquaculture. Although numerous studies demonstrate its capacity to improve water quality and *quorum sensing* interference as a probiotic action mechanism, the specific action mechanism for shrimp culture has not been thoroughly studied. Accordingly, this aspect is relevant for the application of all the benefits offered by this bacterium in this field of the economy.

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AUTHOR CONTRIBUTION

Research conception and design: LMGL, MRP, AAC; redaction of the manuscript: LMGL, MRP, AAC.

CONFLICT OF INTERESTS

The authors of this paper confirm the absence of conflicts of interests.