Investigación e Innovación en Nutrición Acuícola

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Investigación e Innovación en Nutrición Acuícola
2022, Monterrey, Nuevo León, México

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Programa Maricultura
Facultad de Ciencias Biológicas
Universidad Autónoma de Nuevo León 2022

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- Autores del escrito. 2022. Nombre del artículo. Editores: Lucía Elizabeth Cruz Suárez, Mireya Tapia Salazar, Martha Guadalupe Nieto López, David Alonso Villarreal Cavazos, Julián Gamboa Delgado y Carlos A. Martínez Palacios. Investigación e innovación en nutrición acuícola, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp.473 ISBN: 978-607-27-1732-9. El cuidado de la presente edición, así como su realización estuvo a cargo de los editores.

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Dirección de edición: Programa Maricultura, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Dra. Lucía Elizabeth Cruz Suárez, Av. Universidad S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, C.P. 66455
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Application of Poly-β-hydroxybutyrate in Shrimp Health Management

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Abstract

Shrimp is one of the most cultured crustacean worldwide due to its high protein and nutrient content. At the same time shrimp aquaculture faces a huge challenge in controlling disease outbreaks. The extensive use of antibiotics to combat the infectious organisms has led to the emergence of antibiotic resistance which is a huge threat to humans and other living forms. Therefore, a more environment friendly treatment strategy should be devised. One such treatment option is the use of biopolymers such as polyhydroxyalkanoates (PHAs). The most widely used PHA is the anti-infective poly-β-hydroxybutyrate which can be fermented into immune stimulating short chain fatty acids by host bacteria or digestive enzymes. PHB produced by marine microorganisms are widely studied and considered to be a good source of PHB for industrial use. PHB has been found to possess multiple benefits to shrimp health which includes immune stimulation, antibacterial properties, increased secretion of digestive enzymes and growth promotion. Moreover, PHB-diet positively influences the gut microbiome of shrimp, therefore, promoting the overall health and growth.

Keywords: *Gut microbiome; PHB-diet; Poly-β-hydroxybutyrate; polyhydroxyalkanoates; shrimp aquaculture*

Introduction

Industrial shrimp monocultures are more prone to infectious diseases that have resulted in complete loss of farm shrimp populations (Selvin, 2010). Disease outbreaks in shrimp aquaculture has become a big hurdle which has stunted the growth and have resulted in the collapse of many shrimp aquaculture industries, irrecoverably (Asche *et al*., 2021). *Litopenaeus vannamei* (Boone, 1931) is a chief commercial shrimp variety that has been farmed worldwide (Wang *et al.*, 2015) because of its disease tolerance, adaptability to different environments and rapid growth (Xu and Pan 2012; Cui *et al.,* 2016). Intensive culture practices have resulted in the outbreak of several infectious diseases that have culminated into mass mortality of farmed shrimp in aquaculture settings (Kumar *et al*., 2015; Joseph *et al*., 2015).

It has been documented by many studies that extensive use of antibiotics in shrimp farms can result in the development of antimicrobial resistance and these microorganisms can infect humans and animals (Wegener *et al*., 1999; Willis 2000). Therefore, antibiotics are no longer a solution for sustainable shrimp farming and other alternatives needs to be developed. Short chain fatty acids (SCFA) produced by bacteria following fermentation have been identified to possess many health benefits including resistance to infectious diseases and being biological and eco-friendly makes them ideal solution for sustainable aquaculture (Weitkunat *et al*., 2015; Dobrowolska *et al*., 2016). However, they are water-soluble and cannot be used directly in rearing systems due to poor intake of the compound by aquatic animals (De Schryver *et al*., 2010). Poly-β-hydroxybutyrate (PHB) is a water insoluble biodegradable polymer which can be digested by intestinal pH into monomeric forms like β-hydroxybutyric acid, a SCFA (De Schryver *et al*., 2010). This makes PHB a suitable chemical that can act as a precursor for SCFA and can be up taken by aquatic animals effectively.

PHB has been identified as one of the promising alternatives for antibiotics in shrimp aquaculture by several researchers (Laranja *et al*., 2014; Laranja *et al*., 2017; Situmorang *et al*., 2020). PHB has been found to possess multiple benefits to shrimp health and this includes immune stimulation, antibacterial properties, increased secretion of digestive enzymes and growth promotion (Duan *et al.,* 2017). This review discusses the sources of PHB and their degradation inside the host, with special emphasis on the beneficial role of PHB in shrimp disease management.

Biopolymers and PHB

Several microorganisms store their carbon and energy in the form of simple macromolecules in the presence of excess carbon and absence of sufficient amounts of nitrogen. Such structurally simple polymers as known as polyhydroxyalkanoates (PHAs). PHAs can be present up to 90% of the dry weight in the form of discrete granules (Anderson and Dawes, 1990; Defoirdt *et al*., 2009). The source of carbon and the bacterial strain determines the chemical composition of PHAs (Simon-Colin *et al*., 2008). Classification of PHAs are based on the number of carbons in the monomers. Monomers containing three to five carbons such as in polyhydroxybutyrate (PHB) and hydroxyvalerate (PHV) are called short-side-chain PHA (scl-PHA), while those containing six to sixteen hydroxyl fatty acids or aliphatic carbon sources are called medium-side-chain PHA (mcl-PHA) (Matsusaki *et al*., 1998; Wu *et al*., 2003; Tian *et al*., 2005; Chien *et al*., 2007).

PHAs have a multitude of applications such as drug delivery agents, nutritional supplements, bioplastics, photographic materials, drugs, medical implants and fine chemicals (Orts *et al*., 2008; Tokiwa & Calabia, 2008; Sudesh & Iwata, 2008; Chen & Wu, 2005a; Chen & Wu, 2005b; Chen, 2009). Due to the biocompatible and biodegradable nature, PHAs are widely used as bioplastics (non-petroleum based plastics) (Chien *et al*., 2007). Bacterial PHAs are studied by polymer experts, microbiologists, chemists, biochemists as well as medical researchers (Chen, 2009) due to their immense applications. Microbial PHA producers have been isolated from the waste outlet of various treatment facilities. Microorganisms like Agrobacterium, Actinobacillus, Sphaerotilius, Azotobacter, Rhodobacter etc. are known for their ability to utilize organic waste for the production of PHA (Madison & Huisman, 1999; López-Cortés *et al*., 2008).

One of the most studied PHAs, is the poly-β-hydroxybutyrate (PHB) (Lee, 1996; Defoirdt *et al*., 2009). PHB is a simple linear polymer of D(-)-3-hydroxybutyric acid, first discovered in 1923 by Maurice Lemoigne, a French scientist. PHB was first identified in an aerobic spore-forming *Bacillus* “M” strain (Laranja & Bossier 2020). Apart from bacterial synthesis, which is the most commonly used production strategy (Lenz & Marchessault, 2005), PHB can also be obtained chemically by synthetic polymerization via ring opening (Vroman & Tighzert, 2009), naturally from natural/transgenic plants (Mousavioun, George & Doherty, 2012). Bacteria such as Bacillus, Pseudomonas, Alcaligenes, Rhizobium etc. store energy and carbon in the form of PHB when phosphorus, nitrogen or oxygen are present in insufficient quantities while, carbon is in excess amount (Anderson and Dawes 1990; Laranja & Bossier 2020). PHB in its native form is amorphous and after cell lysis, a partially crystallized form is released (Gowda & Shivakumar, 2019). Application of PHB in the polymer industry is limited due to its lo thermal stability and brittle nature. Instead, its copolymers like 3-Hydroxyvalerate and 3-Hydroxybutyrate are used to make films, disposable food service ware and compost bags, due to greater toughness and flexibility. The monomers of PHB, i.e., 3-hydroxybuyrate acts as an efficient biocontrol agent, therefore, PHB have various biological applications (Gowda & Shivakumar, 2019). Research suggests the PHB aids in protecting host from various infections (Gowda & Shivakumar, 2019).

The anti-infective nature of PHB could be due to its hydrolysis into 3-hydroxy butyric acid (Ray *et al*., 2017) in the host gut. The resulting SCFAs exhibits anti- pathogenic action based on the physiological condition of the organisms and physicochemical conditions of the external environment. However, the exact mechanism is still unclear (Ricke, 2003). Hence, SCFAs can be useful in treating microbial infections in aquaculture (Defoirdt *et al.,* 2007).

Marine PHB-producers

The increased production of non-biodegradable products has caused immense harm to the environment, therefore, there is huge interest in the production of biodegradable polymers through biological methods (Patnaik, 2006; Arun *et al*., 2009). Biodegradable PHBs produced by marine microbes (Mohanrasu *et al*., 2021), especially bacteria and archaea (Kavitha, Rengasamy & Inbakandan, 2018) find a wide variety of industrial as well as medical applications (Mohanrasu *et al*., 2021). The endosymbionts of a marine sponge *Callyspongia diffusa* was studied for the production of PHB and it was found that *Bacillus subtilis* MSBN17 produced high amounts of PHB when pulp industry waste and tamarind kernel powder were provided as major substrate and co-substrate respectively (Sathiyanarayanan *et al*., 2013). *Vibrio natriegens* isolated from marine sediments was capable of producing PHB and had a short generation time of 9.8 min, which makes it a best candidate for industrial PHB production (Chien *et al*., 2007).

Bacterium *Streptomyces lividans* and marine *Bacillus subtilis* produced low molecular weight PHB (14 000 Da) along with polyphosphate and calcium ions (Reusch, 1999 Kavitha, Rengasamy & Inbakandan, 2018). The bacterium *Ochrobactrum intermedium* isolated from oil waste contaminated sea water at Gulf of Mannar, Tamil Nadu, India, was capable of producing PHB by utilizing hydrocarbon wastes (Mahendhran *et al.,* 2018). A study from Tamil Nadu, India reported eleven naturally PHB producing cyanobacterial species which includes *Phormidium* sp.

(VIT-BMN3) (Gopi, Balaji & Muthuvelan, 2014). The marine sediment isolate *Vibrio azureus* BTKB33 produced 0.21 g/L PHB under submerged fermentation, which was highest among a total of 828 isolates (Sasidharan, Bhat & Chandrasekaran, 2015). Another PHB producer *Vibrio proteolyticus* strain was isolated from a marine environment in Korea (Hong *et al*., 2019). PHB-producer *Pseudodonghicola xiamenensis,* which isolated from the Red Sea in Saudi Arabia could produce higher amount of PHB in a cost effective manner when supplemented with 4% (w/v) date syrup (Mostafa *et al*., 2020a). A moderately halophilic *Vibrio harveyi* MCCB 284, isolated from tunicate *Phallusia nigra,* efficiently utilized glycerol for PHB accumulation up to 72% cell dry weight (Mohandas *et al*., 2017). According to a study, it was found that PHB-producing bacteria such as *Erythrobacter aquimaris* could be isolated from marine mangrove rhizosphere (Mostafa *et al*., 2020b) and hence, such rhizospheres should be further explored for commercially useful PHB-producers.

PHB degradation and anti-pathogenic effect

PHAs can undergo chemical decomposition as well as enzymatic degradation (Defoirdt *et al*., 2009). PHBs being polymers of repeating SCFA monomers, its degradation in host gut results in the production of SCFAs which provide beneficial effects to the host (Gowda & Shivakumar, 2019) (**Fig 1**). PHB provided to the host through feed or bioencapsulation technique is hydrolyzed by gastric digestive enzymes of the host and/ or PHB depolymerase activity of gut microbiome (Kiran *et al.,* 2016; Liu *et al*., 2010; Laranja & Bossier 2020; Defoirdt *et al*., 2007). Low pH of the host gut may also be responsible for the release of β-hydroxybutyrate SCFAs, as revealed by a study on conducted on juvenile seabass fed with PHB-diet (De Schryver *et al*., 2010; Laranja & Bossier 2020). Exposure to β-hydroxy-SCFAs could impact the cellular status of the such as lowering of cytoplasmic pH which makes the pathogen to utilize its energy on maintaining homeostasis, resulting in reduced cell growth, impaired virulence factors or cell death (Defoirdt *et al*., 2009; Laranja & Bossier 2020). A study on *Artemia franciscana* revealed that the release of β-hydroxybutyrate via PHB hydrolysis provided energy to the host and inhibited the growth of pathogenic *Vibrio campbellii* (Defoirdt *et al*., 2007).

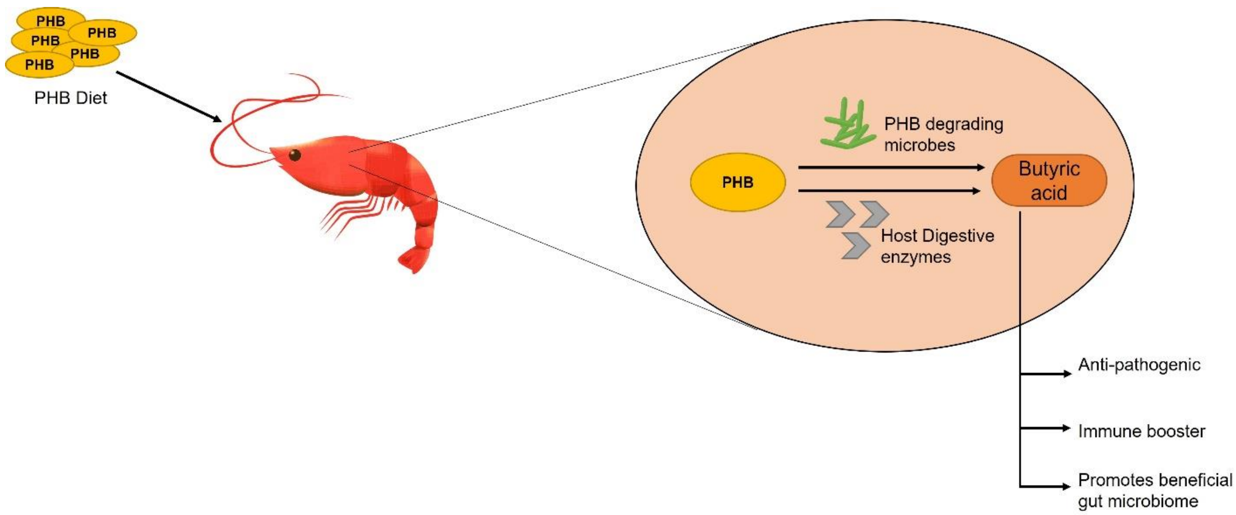

Figure 1: Effect of PHB on shrimp disease control. PHB supplemented in shrimp feed undergoes microbial or enzymatic degradation resulting in the production of butyric acid which shows anti-pathogenic and immune boosting activity along with promoting beneficial gut microorganisms.

The supplementation of PHB feed along with PHB degrading microbes improves the digestion and efficiency of the polymer feed (Gowda & Shivakumar, 2019). PHB-degrading microbes isolated from European sea bass, Siberian sturgeon, and giant river prawn when fed to brine shrimp larvae along with PHB diet increased the survival rate during *Vibrio campbellii* LMG 21363 infection (Liu *et al.,* 2010). Research shows that the PHB also alters the gut microbiome of the host and enhances the growth of beneficial microbes and inhibits the potential pathogenic growth (Laranja & Bossier 2020).

PHB as anti-infectives and immune stimulator

Infectious diseases are a huge burden in shrimp aquaculture industries worldwide and the alarming increase of antimicrobial resistance in them limits the option of antibiotic therapy (Seethalakshmi *et al*., 2021). Polyhydroxy butyrates can be used in aquaculture practices as an effective alternative to antibiotics as they act as immune stimulating agents (Asiri and Chu 2020). Several studies have reported the anti-infective (Defoirdt *et al*., 2007; Laranja *et al.,* 2014) and immune stimulating properties of PHB (Van Cam *et al*., 2009; Laranja *et al*., 2017; Baruah *et al*., 2015). PHB exerts immune stimulatory activity by inducing Hsp70 biosynthesis, which further activates immune regulated genes such as *tgase*, *proPO* and *ftn* (Baruah *et al.,* 2015). Hemocytes are important components of prime immune system and they have significant role in improving cellular and humoral immune responses in shrimp (Robohm 1984; Jiravanichpaisal *et al*., 2006). Shrimp fed with PHB also exhibits enhanced levels of hemocyte, which could contribute to their enhanced immune response (Kiran *et al*., 2020). Moreover, PHB diets are also reported to activate specific immune response in invertebrates. A study by Suguna *et al*. (2014) concluded that PHB diets enhances total peroxidases activity, serum lysozyme activity and antiprotease activity. Another mechanism that could contribute to the immune stimulating property of PHBs could be their ability to enhance the expression of mTOR signaling-related genes like *TOR*, *4E-BP*, *eIF4E2* and *eIF4E1α*, by inhibiting autophagy (Duan *et al*., 2017; Rojas-Morales *et al*., 2016).

Vibriosis is a serious concern in aquaculture settings and accounts to huge economic losses (Lai *et al*., 2015). In addition to its immune stimulatory activities, PHB have also been reported to possess antibacterial activity. Monomeric components of PHB are capable of inhibiting Vibrio pathogens under *in vitro* conditions (Halet *et al*., 2007). The PHB polymer upon reaching shrimp intestine gets hydrolyzed to monomeric forms like short-chain β-hydroxy butyric acid, and can provide resistance to *Vibrio* infections (Defoirdt *et al*., 2007). *In vitro* studies also shows that PHB can also suppress swimming motility of *Vibrio* pathogens which is an important virulence factor to invade and colonize host cells efficiently (Van Hung *et al*., 2018). Elevated concentrations of PHB are also found to inhibit virulence factors in Vibrio spp. such as phospholipase expression and haemolysis, but did not suppress biofilm formation (Van Hung *et al*., 2018). PHB molecules synthesized from *Brevibacterium casei* MSI04 attenuated the expression of virulence factors like haemolysin, bioluminescence, motility and colonization capacity (Kiran *et al*., 2016). Also, these PHB molecules degrades the N-acyl-homoserine lactone and quorum-sensing signaling cascade, which contributes to impairment of biofilm formation in bacterial pathogens (Kiran *et al*., 2016). It can be understood from these studies that PHB molecules from different sources exhibit varying anti-virulent properties. Therefore, it will be worthy to investigate the factors that are responsible for such divergent biological activities.

Influence of PHB on shrimp microbiome

Aquatic species are continually exposed to their surrounding water and any changes in their surrounding microbiome or intestinal microbiome can upset the health and functioning of the host (Rajeev *et al*., 2020). Several studies have pointed out the influence of diet on the shrimp microbiome (Daniel *et al*., 2014; Prathiviraj *et al*., 2021) and therefore a healthy microbiome of shrimp can be modulated by providing formulated dietary regimens. This not only reduces the use of antibiotics for shrimp disease management, but also makes shrimp produces more organic and safer to consume.

The normal microbiome of *Penaeus monodon* comprises of phyla Gammaproteobacteria, Firmicutes, Bacteroidetes, Fusobacteria and Actinobacteria (Rungrassamee *et al*., 2014). Alterations in the microbial community of shrimp intestine can facilitate the colonization of pathogenic bacteria (Holt *et al*., 2021). It has become an established fact that the gut microbiome is closely interconnected to the immune system of host (Sekirov *et al*., 2010) and a depletion in microbial diversity is often predicted to be the chief reason for pathogenesis (Holt *et al*., 2021).

PHB supplementation has been reported to increase the abundance of beneficial microflora in the intestine (Qiao *et al*., 2020). *Mesorhizobium* is a crucial bacterium for sustaining the normal growth of shrimp and its absence has been found to cause imbalance in the intestinal flora of starved shrimp (Dai *et al*., 2018). PHB diets has been proven to increase the abundance of this bacterium in gibel carp, (Qiao *et al.,* 2020) and hence can be expected to show similar results in shrimp as well. The microbiome modulation activity of PHB diet could be because of the fact that PHB gets monomerized to SCFA at intestine and SCFA can further promote the growth of probiotic bacteria in the intestine (Forchielli and Walker 2007). SCFA in the intestine also modulates immune response and metabolic output of the host (Rajeev *et al*., 2021). Dietary supplementation of PHB was found to enrich the beneficial bacteria such as *Bacillus*, *Lactococcus*, *Lactobacillus*, *Clostridium* and *Bdellovibrio* in *L. vannamei* (Duan *et al*., 2017). Another study proved that amorphous PHB extracted from *Halomonas* sp. enriched the symbiotic microbial population of shrimp intestine and decreased the abundance of *Vibrio* sp. much lower than crystalline PHB (Gao *et al*., 2019).

Conclusion

The extensive use of antibiotics in shrimp aquaculture has led to the emergence of antibiotics resistance, and this calls for an alternative disease treatment strategy. Biopolymers due to their biodegradable nature is a suitable eco-friendly option. The use of PHB in shrimp disease management is of huge interest these days due to its anti-pathogenic and immune stimulating abilities. PHB exerts its activity by microbial or enzymatic degradation into β-hydroxybutyric acid which is known for its anti-infective and immune stimulating activities. Marine PHB producing microbes are found to be a great source of PHB when compared to chemically synthesized PHB. The use of PHB also positively modulates gut microbiome composition of the shrimp. However, the exact mechanism of PHB degradation and its antimicrobial effect is still unclear. Therefore, further research is required to understand the exact mechanism of PHB degradation. The use other biopolymers for disease treatment in aquaculture could also be studied, which would provide better environmental friendly treatment options. Therefore, the use of PHB could be better alternative to harmful antibiotics in shrimp disease management.

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