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A Strategic Review on Use of Polyhydroxyalkanoates as an Immunostimulant in Aquaculture

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Abstract

Background and Objective: Increasing concerns over the use of antibiotics in aquaculture have emerged researchers to focus on short chain fatty acids and other biocompatible molecules as alternatives for disease prophylaxis and treatment. Polyhydroxyalkanoates well studied as biopolymeric materials for using in packaging and biomedicine were not focused much for their abilities to act as antimicrobial agents in aquaculture until recent years. Application studies of polyhydroxyalkanoates as aquafeed additives have highlighted their promising roles as eco-friendly alternatives for commercial antibiotics with strong immunomodulatory effects in fish-es and shrimps. The major aim of this review was to explore up-to-date scientific research studies on use of polyhydroxyalkanoates as aquafeed additives and their immunomodulatory effects.

Results and Conclusion: Up-to-date, limited scientific literatures have been published on the use of polyhydroxyalkanoates and their copolymers as alternatives to antibiotics in aquaculture. This research field includes a great scope of development due to the promising immu-nomodulatory and antimicrobial activity of polyhydroxyalkanoates against common pathogens in aquaculture, as reported in literatures. Although several hypothesis and research data for explaining the mechanisms behind their immunostimulatory effects were suggested by various researchers, genetic and molecular bases underlying these phenomena are yet to be explored. Further research and development in this area can introduce these biopolymers as the most promising eco-friendly alternatives for antibiotics in aquaculture.

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1. Introduction

Global aquaculture sector with an output of 179 million tons in 2018 has truly served as the backbone of global food sector to reply growing demands of the exponentially increasing human populations over the last few decades [1]. The world population size is expected to reach 10 billion by 2050 and aquaculture is expected to include promising roles in providing food needs of the increasing populations [2]. Aquaculture is still the fastest growing sector in food production industries with an average yearly growth rate of nearly 7.5% since 1970 [3]. The global fish exports increased from 7.8 to 164 billion USD from 1976 to 2018 [4]. Sudden diseased outbreaks in aquaculture sector slower increases in production rates, jeopardizing overall integrity of the aquatic ecosystems [5]. The traditional practice of using disinfections of rearing water and administration of antibiotics directly or in feeds are becoming further undesirable as they lead to emergence of resistant microorganisms [6,7]. Thus, further research focuses were emphasized on development of sustainable alternatives to these chemotherapeutic agents in aquaculture. Some promising alternatives include development of specific pathogen free (SPF) animals and use of probiotics and prebiotics as feed additives [8,9]. Recently, use of immunostimulants for the prophylaxis and infection control in aquaculture has become further popular due to their eco-friendly biocompatible nature. Immunostimulants are basically compounds capable of modulating immune system of the hosts to increase resistance against pathogens [10]. Diverse groups of biologicals, chemical agents, plant secondary metabolites and microbial derivatives have been reported to include

immunomodulatory effects against common pathogens in aquaculture [11].

Use of biofloc technology, using a wide variety of heterotrophic microorganisms forming bioflocs, has been suggested to include significant immunomodulatory effects on cultured organisms by means of their cellular components, metabolites and derivatives [12]. The high costs and labor-intensive nature of this technology associated with immunomodulatory methods such as vaccination and phage therapy have made rendered these methods to be no longer sustainable for aquaculture. This urged novel studies on the use of short chain fatty acids (SCFAs) as immunostimulants in aquaculture [13]. The SCFAs are basically organic fatty acids containing 1-6 carbon atoms. They are usually derived from oligosaccharides, polysaccharides, proteins, peptides and glycoproteins through microbial fermentation [14,15]. Polyhydroxyalkanoates (PHAs) are from the most widely studied SCFAs for their roles in immunostimulation in aquaculture in several experimental studies carried out majorly on fishes and shrimps [16,17]. The PHAs are a class of microbial polyesters accumulated during nutrient imbalance conditions (carbon in excess with nitrogen or any other essential nutrients in limited quantities) in many microorganisms to serve as intracellular energy and carbon sources [18]**.** The poly (3-hydroxybutyrate) (PHB) is the most widely studied type of PHAs. Chemically, PHAs are polymers of hydroxyalkanoates, accumulated inside many microorganisms as energy reserves and enhance the organism ability to fight with several stress factors [19]. In fact, PHAs are truly biodegradable and biocompatible making them ideal candidates for use in food packaging [20,21], drug delivery and other biomedical uses [22-24].

Commercialization of PHAs can seriously be difficult due to high costs associated with substrates, production strains, fermentation strategies and downstream processing. With the evolution of agricultural/industrial waste based production strategies [25-35] and eco-friendly methods for the extraction and purification of chemicals, focuses on PHAs are still increasing. Statistical modelling of bioprocess variables and fermenters to enhance production of PHAs have recently been addressed to strengthen their commercialization and competence in global markets [36-39]. Use of PHAs in aquaculture is a novel research field with multiple reports, suggesting their strong immune modulatory and growth promoting effects on organisms fed with PHA incorporated aquafeed [40,41]. Therefore, the aim of the current review study was to present up-to-date scientific literatures of PHA use as immunostimulants in aquaculture. The study further provides commercialization aspects of PHAs and future challenges for effective uses of PHAs as sustainable immunostimulants in aquaculture.

2. Biosynthesis and enzymology of polyhydroxyalkanoates

In microorganisms, energy generation, as well as synthesis of carbon-rich molecules, is derived from carbon sources during the metabolism. The most common carbon sources for microbes are sugar molecules. Microbes produce pyruvates from sugar molecules through glycolysis, which are converted to acetyl-CoA and enter the Krebs cycle. Under nutrient-rich conditions, high quantities of coenzyme A from Krebs cycle inhibit 3-ketothiolase and thereby blocks PHA synthesis. Under such conditions, acetyl-CoA enters Krebs cycle for cell growth and energy production [42]. Therefore, microbes are stressed with unbalanced levels of nutrients (essential nutrients such as nitrogen or phosphorus are limited with excess carbon supplementations) for production of PHAs. Under unbalanced nutrient conditions, coenzyme A is non-inhibitory to 3-ketothiolase and thus the acetyl-CoA is directed into the PHA biosynthesis pathway [43,42]. In-depth research on the PHA biosynthesis pathway have shown that multiple biosynthetic pathways exist for PHA production, each including significant differences based on the classes of PHAs and microbial strains [44]. The most widely accepted pathway has been demonstrated in *Cupriavidus necator* [45]. In *Cupriavidus necator*, *pha*A, *pha*B and *pha*C genes encode major enzymes involved in PHA production. The β-Ketoacyl-CoA thiolase encoded by *pha*A gene is responsible for the conversion of acetyl-CoA molecules to acetoacetyl-CoA, which is condensed to (R)-3-hydroxybutyrate monomer units by the catalyzing action of NADPH-dependent acetoacetyl-CoA dehydrogenase encoded by *pha*B gene (Figure 1). Then, (R)-3-hydroxybutyrate monomers are polymerized into PHA synthase enzyme encoded by *pha*C gene [18,46]. Another enzyme, which is greatly important in PHA metabolism, is PHA deploymerase that helps breakdown of PHAs into carbon and energy sources for supporting growth of bacteria under stress conditions [47]. The PHA depolymerases are encoded by *pha*Z gene and can act intracellularly and extracellularly [19].The *pha*Z and oligomer hydrolases studies are widely carried out in *Cupriavius necator*. This microbe is reported to be capable of producing seven PHA depolymerase types (phaZ1–phaZ7) and two types of oligomer hydrolyses (phaY1 and phaY2) [48]. The PHA biosynthesis and degradative pathways are interconnected in microbes, described as PHA cycle. This PHA cycle not only describes the size and number of PHA granules in the bacterial cells, but also provides carbon and energy sources for the bacterial metabolism [49].

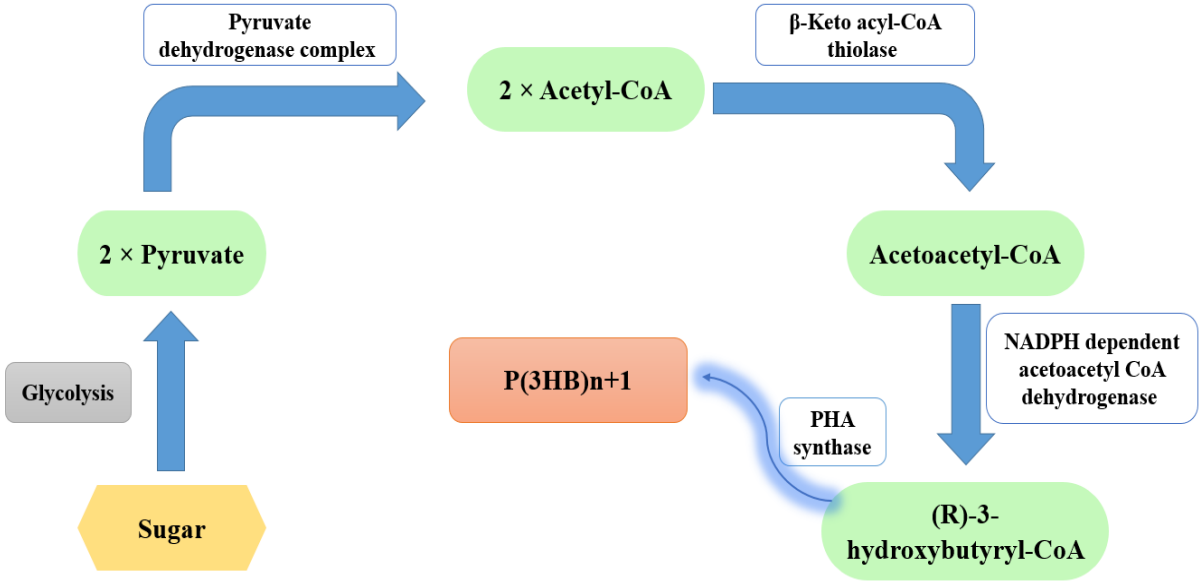

Figure 1. PHA biosynthesis in *Cupriavidus necator*

3. Biodegradation of polyhydroxyalkanoates

In biological systems, degradation of PHB occurs intracellularly or extracellularly [50]. Intracellular degradation or PHA mobilization occurs when bacteria are stressed due to carbon limitation [51]. The accumulated PHAs are then degraded into carbon and energy sources [47]. First, PHAs are broken down into PHA oligomers by PHA depolymerase enzyme. This is followed by conversion of PHA oligomers into respective PHA monomers by oligomer hydrolase enzyme [52]. Then, PHA monomers undergo dehydrogenation using nicotinamide adenine dinucleotides to form acetoacetic acid. Acetoacetyl-CoA synthase enzyme acts on acetoacetic acid by utilizing adenosine triphosphate to produce acetoacetyl-CoA. The acetoacetyl-CoA is converted into acetyl-CoA by 3-ketothiolase, which further enters Krebs cycle for energy generation [53]. In extracellular degradation, exogenous PHAs are broken down by non-PHA accumulating organisms. Extracellular PHA depolymerase affects PHA molecules, resulting in production of dimers and monomers that are water soluble with a low molecular size. These molecules can easily be taken up by the cells and proceed with the energy production [54].

4. Polyhydroxyalkanoates in aquaculture

Use of PHB in aquaculture is increasing, compared to other widespread uses of PHB in food processing and medical industries. The major use of PHB in aquaculture includes its incorporation as a feed additive and in denitrification systems as a part of biofloc technology [55]. Although use of biopolymers such as PHB in biofloc technology for cleaning aquaculture systems has increased in the last few decades, use of PHB as a feed additive is still growing. Effects of PHA incorporation, as an aqua feed additive, on growth profiles and immunological responses in fishes and shrimps were studied by research groups in the last few decades. The molecular mechanisms, underlying immunomodulatory and growth enhancing properties of PHAs, are not fully described. Thus, the aim of the present review was to summarize and discuss the major experimental studies that report use of PHAs as growth enhancers and immunostimulants in aquaculture.

4.1. Polyhydroxyalkanoates as growth enhancers in aquaculture

Effects of PHB supplementation in enhancing growth profile and development of Chinese mitten crab zoea larvae were studied by Sui et al. [56]. Feeding trials included two batches, one batch of larvae fed with PHB molecules and the other batch with live feeds (*Rotifers* and *Artemia*) enriched with PHB. Study revealed increased survival rates and development qualities in larvae fed with PHB molecules. These findings can be correlated with the fact that although PHB acted as carbon reserves in the larvae, it failed to supplement other essential nutrients for the growth and survival of the organisms. In the second batch fed with PHB enriched live feed, superior growth qualities and survival rates were seen. This could be connected to the contribution of energy from PHB and other vital nutrients that accelerated the growth of the larvae. Another interesting observation regarding effects of PHB to provide osmotic shock tolerance for the larvae was reported in this study. The PHB enrichment in feeds provides additional energy to

colonic cells, which enhances absorption ability and bioavailability of trace elements. The PHB molecule acts as an energy source to intestinal microbial ecosystem and hence limits infections by the opportunistic pathogens [56]. Moreover, PHB in the feed affected metabolism of larvae and physical fitness of the larvae enhanced. The bioencapsulation of PHB in *Rotifers* and *Artemia* decreased feed quantity provided to the larvae for optimal growth by a quarter. In another study by Yaqoob et al. [57], *Oncorynchus mykiss* fingerlings were fed with feed containing various concentrations of PHB (1, 2 and 3%) thrice a day for studying effects of PHB on their growth, gut enzyme activity and body composition. No significant effects were observed on these parameters during the first two weeks, possibly due to the absence of PHB hydrolase that breaks down the PHB in developing fingerlings. Dietary supplementation of PHB was reported to increase digestive enzyme activity and hematological parameters of the shrimps [57].

Indirect supplementation of PHA to hosts using live feeds such as *Artemia* grown in PHA containing media was reported in the literature. Nhan et al. [58] fed *Macrobrachium rosenbergii* larvae with Instar II *Artemia*, which was pre cultured in three various media of one with PHB, another one with highly unsaturated fatty acids (HUFA) and the last one with a combination of PHB and HUFA. Although it was reported that the larvae fed with PHB enriched *Artemia* showed increased developmental and survival rates even after exposure to *Macrobachium rosenbergii*, the highest growth and survival rates were seen in larvae fed with *Artemia* enriched with PHB and HUFA. The highest survival proportion was reported as 80% after ten days and 58% after 28 days, respectively. This can be correlated to the fact that when PHB alone was supplemented, the feed lacked other essential nutrients needed for growth. When such a modified feed was incorporated to HUFA lipid emulsions, it could provide the essential nutrients needed for the growth and development of larvae. The total count of pathogenic bacteria such as *Vibrio* spp. reported normal in natural larvae decreased significantly when PHB was present in the feed. Similar results were reported in brine shrimp larvae (*Artemia franciscana*) fed with PHB and thereby provided supporting evidence to the pathogen inhibition capability of PHB. Generally, PHB entering guts is partially degraded to β-hydroxybutyrate. In absence of molecular studies, it was hypothesized that this fatty acid provides additional energies to gut epithelia, making them further resistant against the pathogens. The β-hydroxybutyrate can inhibit growth of pathogens as it is toxic to them [59]. In experiments with *Macrobachium rosenbergii* larvae, larvae were not able to digest PHB supplements and the hydrolysis was carried out by the gut microbial flora [58]. For brine shrimp larvae, the larval gut showed ability to produce PHB hydrolysing enzymes [59].

De Schryver et al. [60] studied juvenile European sea bass (*Dicentrarchus labrax)* and various concentrations of PHB supplementation (2, 5, 10 and 100%) on the overall growth performance of the sea basses as well as their effects on intestinal microflora. The researchers reported that the average weight increase for the sea bass juveniles with no PHB supplies was 216%. The juveniles fed with 2 and 5% of PHB included average weight increases of 243 and 271%, respectively. This additionally verified the hypothesis that the aquatic organisms fed with PHB included increased growth rates, compared to that those with normal fed did. They also reported that the survival rate of PHA fed juveniles was much higher than that of others, clearly seen at Week 6 of the study. The intestinal pH values of PHB fed juveniles showed significant decreases, possibly due to the degradation of PHB into SCFAs (3-hyroxybutyrate), which resulted in decreases in pH. Significant changes were reported in the microbial population after Week 2. The reason for this phenomenon was that the fish gastrointestinal enzymes partially degraded PHB into monomers and oligomers of β-hyroxybutyrate, which could be used by the gut microbes as carbon and energy sources. The possibility of nonspecific lipases and esterases in hydrolysis of PHA could be correlated with these findings. The other reason was linked to PHB degrading microbes, which were not reported in the gut microbiota of sea basses, either the microbes reached the gut from the outside and colonized the gut or the existing microbiota evolved (switched on the genes) to degrade the available PHB. The researchers stated that changes in microbial population could be due to a combination of the highlighted factors. Effects of PHB on growth and development of large yellow croaker (*Larimichthys crocea*) fishes and piglets were assessed by incorporating various quantities of PHB into the normal feed [61]. It was seen that 0.5 to 2% of PHB supplementation increased the survival rate, specific growth rate, final body weight and weight gain rate of the yellow croaker drastically. Moreover, the highest survival rate was 84.88% ±3.49 in 2% PHB treatments and the specific growth rate was 1.27% ±0.06, the final body weight was 66.33% ±3.03 and the weight gain rate was 122.95% ±8.42 in 1% PHB treatments. Contradictory results were seen in Nile tilapia (*Oreochromis niloticus*) fed with PHB supplements [62]. This outlined that PHB effects could be species specific. The antioxidant activity of yellow croaker fishes nourished with PHB increased significantly, compared to control. This could be one of the reasons for the increased survival rate, as the antioxidant enzymes eliminated reactive oxygen (ROX) and nitrogen (NOX) species and hence promoted immunity and fitness of the fishes. The study further reported that when PHB was fed in excess to the yellow

croakers, increases were seen in the bacterial growth within the gut epithelia, leading to the host inflammatory responses and activation of the immune system. This resulted in decreases in final body weight of the fishes. When PHB was administrated to weaned piglets, no negative effects on the animal growth were reported and, in fact, the piglet growth performance increased. This result was correlated with the effects of PHB degradation by the intestinal microbes as the microbes released β-hydroxybutyrate and their oligomers, which enhanced production of digestive enzymes in the weaned piglets.

Effects of PHB on the immunity and growth of organisms are usually studied only in their larval stages because of the fact that the larvae are more vulnerable than adult organisms physically and physiologically. This is due to the absence of adaptive immunity in larvae, which makes them further susceptible to infections by pathogens [63]. The larval body can show instant reactions to any compounds that they are fed with; thus, studying larval stage is widely preferred. Franke et al. [64] fed European sea bass yolk-sac larvae (*D. labrax)* with PHB to study the compound effects on the larval immune system and growth as well as gut microbes. The larvae were fed with PHB at various concentrations during various stages of their life cycle. High and low concentrations of PHB were fed for mouths opening and first feeding stages. Death rates of the larvae decreased significantly and the maximum survival rates were reported in the mouth-opening stage larvae fed with low concentrations of PHB (approximately 35%). Decreases in death rates could be correlated to the increased immunity. Indeed, PHB was degraded in the larval gut to SCFAs of β-hydroxybutyrate, which decreased the intestinal pH and served as an additional energy source for the intestinal cells. This helped them in differentiation and increased mucin production. Decreased pH limited infection of the pathogens and helped proliferation of the selected microbes. Studying roles of ferritin, dicentracin and other genes in inducing secretion of immune complexes, it was found that PHB boosted immune responses. It has been reported that SCFAs of β-hydroxybutyrate act on the larval immune system as in mammals by binding to G-protein coupled receptors. The pH of gut plays a major role in early immune responses of the larvae. It has previously been shown that the low pH created by PHB in the larval intestine favors growth of certain microbes. This can stimulate the microbe-associated molecular patterns, which can activate early cellular and humoral immune responses [65]. The prebiotic effects of PHB supplemented feed have been reported in experimental studies on fishes and prawns [60,66,67].

The Siberian sturgeon (*Acipenser baerii*) fingerlings were fed with 2 and 5% of PHB to understand the effects of PHB on growth performance and gut microbial community in the fingerlings [66]. The fingerlings were fed three times a day with feeds containing PHB. Increases in weight gain, specific growth rate and survival of the fingerlings fed with 2% of PHB were reported. The weight gain and specific growth rate for the 2%-PHB fed fingerlings were 58.1% ±3 and 2.2% ±0.2, respectively. These values for control fed with no PHB were 51.5% ±8 and 2.3% ±0.1, respectively. The survival rate was as highly as 96.6% ±3.4 for the fingerlings fed with 2% of PHB and 89.1% ±9.3 for controls. Diversity of the microbial community in the gut was analyzed using 16S rRNA fingerprinting techniques. The major microbes in the gut were characterized as *Bacillus* and *Ruminococcaceae* genera. Lorenz curves and Shannon index of Biolog Ecoplate data were analyzed and results indicated bacteria in the guts of PHB treated fingerlings. However, the bacterial aerobic metabolic potentials varied compared to controls [66].

4.2. Immunostimulatory effects of polyhydroxyalkanoates in aquaculture

In addition to in-depth research studies on the effects of PHA and other SCFAs on growth promotion and feed utilization, effects of these additives on pathogen defense and immunostimulation of aquatic organisms have received further attentions recently [13]. A study on the immunostimulatory effects of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (P (HB-co-HV) copolymers was carried out on *O. mossambicus* [41]. In this study, *O. mossambicus* was fed with aquafeed enriched with various concentrations (0, 1, 2 and 5%) of P (HB-co-HV) and the specific immune response was studied by measuring antibody responses to sheep RBC in 5-d intervals. Nonspecific immune responses were recorded by studying lysozyme, total peroxidase and antiprotease activities. Total immune responses were studied by assessing infections of virulent *Aeromonas hydrophila*. The overall immunity was enhanced in fishes fed with P (HB-co-HV). The lysozyme activity increased significantly. Similar patternd were recorded when *Labeo rohita* was fed with 0.5 or 2.0% of n-3 HUFAs [68]. Lysozyme enzymes were suggested to provide protection from Gram-positive and Gram-negative pathogens [69]. Lysozymes in fishes are mostly associated with phagocytic neutrophils; therefore, increased levels of lysozymes in P (HB-co-HV) fed fishes suggested that P (HB-co-HV) might increase the number of active neutrophils or enhanced production rate of lysozymes or both [41]. The level of total serum protease is a measure of antiprotease activity. Pathogens normally secrete enzymes, which lyse tissue cells to facilitate their invasion [70]. These enzymes need chelation to activate. Antiproteases naturally prevent this chelation; thereby, blocking the pathogens. Increased antiprotease activity was reported in the study [41]. Clements et al. [71] reported that β-hydroxy SCFAs produced by partial

or full degradation of PHB could directly enter pathways of lipid biosynthesis.

Another study reported immuno-stimulatory effects of PHB on giant tiger shrimp (*Penaeus monodon*) larvae fed with *A. nauplii*, enriched with PHB producing *Bacillus* JL47 [72]. The bacteria produced 55% of PHB of their dry cell weight. The larvae were fed with PHB for 15 d (twice a day) and exposed to pathogenic *Vibrio* (*V.*) *campbellii* LMG 21363 by immersing in media with 10^6 cells ml^{-1}. Immunostimulatory effects were studied by measuring expression of three genes, including prophenoloxidase (ProPO), transglutaminase (TGase) and heat shock protein 70 (Hsp70) genes. Expression of the three genes were upregulated by the PHB and were significant even before exposure to pathogens [72,73]. Naturally, transglutaminase enzymes help coagulation processes in shrimps; thereby, decreasing pathogen invasion and hemolymph loss [74]. Heat shock proteins serve as molecular markers, which refold and repair proteins that are denaturized due to heat shock stresses [75]. These effects include innate immunity of the larvae; hence, PHB was concluded to have priming effects on the immune system in their studies.

Effects of PHB enriched diet on growth, intestinal microbial community and immune responses in soiny mullet *(Liza haematocheila*) were studied by feeding mullets with feeds containing various quantities of PHB (0, 0.5, 1, 2, 4 and 8%) [76]. Specific growth rate and weight gain increased in fishes, which were fed with diets containing PHB. The highest rates were reported in groups fed with 2% of PHB. Specific growth rate and weight gain of the mullets respectively increased to 74.32 and 90.74%, compared to groups fed with no PHB. Ighodaro and Akinloye [77] reported major antioxidant enzymes, which were important in the first defense line of immune systems, including catalase and superoxide dismutase as well as total anti-oxidant capacity. Bagnyukova et al. [78] showed that catalase played critical roles of converting peroxides to water and oxygen, preventing hydroxyl radical toxicity. The superoxide dismutase was also described by Meng et al. [79] as the enzyme, preventing adverse reactions by removing the excess reactive oxygen species. The study showed that catalase and superoxide dismutase levels included increased activities in groups fed with 0.5, 1 and 2% of PHB after 30 d. However, total antioxidant capacity was not affected from the increased levels of catalase and superoxide dismutase and was almost similar to groups fed with no PHB. Expression of immune linked genes was studied to investigate immune responses. Immune-linked genes encoding aldehyde oxidase (AOX), penicillin-binding protein A (PbpA), interleukin-8 (IL-8) and major histo-compatibility complex class II (MHC II) in soiny mullets were studied using quantitative real-time PCR (qRT-PCR). The PbpA and IL-8 encoding genes were reported to play major roles in immune responses against the pathogens [76]. The PbpA protein helps crosslinking peptidoglycans of the intestinal microbiota in fishes. Cross-linked peptidoglycans are known for their immunopotentiation effects on the host immune system [80, 81]. As stated by Baggiolini et al. [82], IL-8 is a chemotactic factor, which is synthesized by the cells expressing receptors for pathogen recognition to communicate with white blood cells (WBC). Wright et al. [83] described that aldehyde oxidase, belonging to the family of xanthine oxidases, played critical roles in pathophysiological processes. Ryhanen et al. [84] demonstrated that MHC II were responsible for the activation of innate immunity by recognizing pathogens and presenting the pathogen specific antigens to immune cells. The study reported that PbpA and IL-8 gene expressions increased in groups fed with 2 and 4% of PHB, compared to other groups. Furthermore, the MHC II gene expression was significantly upregulated in groups fed with 2% of PHB. It was reported that the aldehyde oxidase transcriptional level was downregulated in PHB fed groups, compared to groups fed with no PHB. Semova et al. [85] reported that the intestinal microbial communities of aquatic organisms played major roles in epithelial proliferation, physiological development, harvest, health status and immune responses. The most commonly reported bacterial genera in soiny mullets were *Bacillus*, *Delftia*, *Lactococcus*, *Carnobacterium* and *Achromobacter*. It was reported that the intestinal *Bacillus* spp. in groups fed with 2% of PHB were significantly higher than those in groups fed with no PHB. Using analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, it was found that genes involved in major metabolism pathways, secondary metabolites biosynthesis, immune system, signaling molecules and interacttions as well as other downregulated genes involved in disease pathways were significantly upregulated in PHB fed organisms. The major literature discussing immunomodulatory effects of PHA supplementation in aquafeeds is listed in Table 1.

Table 1. Effects of polyhydroxyalkanoate supplementation on organisms

Common name of the host	Scientific name	Effect of PHA supplementation on the organism	Reference
Nile tilapia	*Oreochromis niloticus*	5 % PHB increased immune response, serum lysozyme activity and serum peroxidase activity significantly, showed resistance towards virulent *Aeromonas hydrophila* strain	[41]
		Increase in the lipase activity. Increase in survival by 20% when the larvae fed with PHB was challenged with *Edwardsiella ictaluri* gly09R	[62]
White leg shrimp	*Litopenaeus vannamei*	Increase in survival rate and final weight when fed with PHB and glucose	[86]
		Enhanced survival, growth and robustness of the larvae when exposed and not exposed to pathogenic *Vibrio anguillarum.*	[40]
Chinese mitten crab	*Eriocheir sinensis*	Increase in the Developmental rate, survival and osmotic stress tolerance	[56]
Chinese mitten crab	*Eriocheir sinensis*	Enhanced growth and survival when challenged and not challenged with *Vibrio anguillarum*	[87]
Brine Shrimp	*Artemia franciscana*	Increased the survival when the starved nauplii were challenged with *Vibrio campbellii*	[59]
		Significant survival observed when fed with 100 mg l^{-1} when challenged with *Vibrio campbellii*	[88]
		Better Survival when challenged with V*ibrio campbellii*	[89]
Blue mussel	*Mytilus edulis*	Improvement in the growth and development and increase in the survival	[90]
Giant freshwater prawn	*Macrobrachium rosenbergii*	Increase in the development of larvae and survival	[58]
		Increased survival and larval development	[91]
Rainbow trout	*Oncorhynchus mykiss*	Higer weight gain, specific growth rate and significantly higher specific activity of the amylase and total protease when fed with 1 % PHB. Increased survival and immunostimulation when challenged with *Yersinia ruckeri.*	[92]
European sea bass	*Dicentrarchus labrax*	Increased survival when fede with 2 %, 5 % and 10 %, maximum weight gain at 5 %PHB feed and overall decrease in the pH	[60]
Siberian sturgeon	*Acipenser baerii*	Increase in the content of whole-body lipid, and activity of pepsin when fed with PHB and PHB-HUFA	[67]
		Increased the weight gain, Specific Growth Rate and survival when fed with 2 % PHB	[66]
Giant tiger prawn	*Penaeus monodon*	Increased survival and growth of the larvae which were exposed and not exposed to pathogens, and highest survival when exposed with ammonium chloride	[93]

4.3. Antiadhesive activity of polyhydroxyalkanoates on biofilm forming fish pathogens

Biofilms are formed when microorganisms adhere to a surface, embedding in their extracellular matrix [94]. Biofilms are produced majorly to counteract various stresses. Biofilms can protect against antibiotics and help survival of the microbes. Pathogens that are capable of biofilm production are very difficult to eradicate. Anti-biofilm activity or antiadhesive activity on biofilm formers is greatly important in aquaculture as it provides a sustainable method for fighting against outbreaks associated with biofilm forming bacteria. Kiran et al. [95] studied the antiadhesive activity of PHB against *Vibrio* spp. The PHB for the study was produced using *Brevibacterium casei* MSI04 isolated from *Dendrilla nigra,* a marine sponge. It was reported that *B. casei* produced 25 g l^{-1} PHB in the optimized media when incubated for 96 h. The antiadhesive activity was studied by growing *Vibrio* spp. on glass slides coated with microbial PHB and analyzing them using microplate assay. When 0.6 mg 200 μl^{-1} PHB was used, biofilm activity of the *Vibrio* spp. significantly decreased. Totally, 96% of the antiadhesive activity were reported

against *V. fischeri* and *V. vulnificus*, 92% were reported against *V. alginolyticus* and *V. parahaemolyticus* and 88% against *V. harveyi.* Lee et al. [96] showed that PHB included a low cell adhesion due to the accumulation of methyl groups on the surfaces. Other properties such as immune stimulants and growth inhibitory effects of the PHB were discussed as well. Amorphous PHB (PHB-A) produced by lyophilizing *Ralstonia eutropha* was used to study its effects on *Vibrio* spp. In blue mussel (*Mytilus edulis*) larvae [97]. Larvae were fed with various concentrations of PHB-A (1-10 mg l^{-1}) for 6 or 24 h and infected with *V. splendidus* and *V. coralliilyticus* (10^5 CFU ml^{-1}). When PHB-A was provided 6 h before the infection, the survival rate of larvae increased after 96 h of exposure, compared to that of larvae fed with no PHB-A. The increased survival rates respectively were 55 and 25% against *V. splendidus* and *V. coralliilyticus*, compared to larvae fed with no PHB-A. When concentration of PHB-A in feeds increased from 1 to 10 mg l^{-1}, it showed no effects on the larval survival rate. Researchers carried out studies on how the β-HB affected growth and biofilm formation of *Vibrio* spp. A concentration of 125 mM of β-HB showed maximum inhibition of virulence factors such as hemolysis by *vibrio* spp.; however, this concentration caused caseinase production.

4.4 Effects of polyhydroxyalkanoate supplementation on transcriptional factors

Baruah et al. [88] challenged the brine shrimps of *A. franciscana* with *V. campbellii* after feeding with PHB to study effects of PHB against vibriosis. The shrimp larvae were fed with various concentrations of PHB (10, 100, 250, 500 and 1000 mg l^{-1}) added into water with autoclaved *Aeromonas hydrophila* (10^7 cells). Feeding was carried out for 2 d and then the larvae were exposed to *V. campbellii* (10^7 cells). It was reported that 100 mg l^{-1} of PHB fed larvae resisted vibriosis completely. For resisting complete vibriosis, the necessary concentration was 1000 g l^{-1}. This concentration controversy was solved when sizes of the PHB molecules were compared. In this study, sizes of the PHB molecules were 25-30 µm and size of the PHB in the earlier study included 30 µm averagely. The *Artemia* larvae are non-selective particle-filtration feeders as reported by Fernandez [98]. These larvae only feed on smaller molecules. Effects of vibriosis were studied by analyzing various immune genes, producing Hsp70, prophenoloxidase (proPO), transglutaminase (TGase) and ferritin (ftn). The PHB at 100 mg l^{-1} was reported to induce production of Hsp70; however, the mRNA level for Hsp70 was less. This was possibly linked to half-life of the molecules; previously described by Vogel and Marcotte [99]. The Hsp70 molecule includes more half-life than that its mRNA does. Induced production of Hsp70 was associated to gut pH. Generally, partial digestion of PHB in the gut of larvae form monomers, dimers or oligomers of fatty acids, which decrease pH of the gut. Furthermore, these fatty acids by nonionic diffusion cause cellular acidification, which induces stress as well as production of Hsp70 in epithelial cells. The pro PO is converted into active PO, which induces melanin production, wound healing and encapsulation of pathogens; as discussed earlier. In this study, a 2.5-fold increase in transcription level of *pro*PO gene after 6 h of infection was reported. The TGase is needed for clotting of the hemolymph at the site of pathogen entry, killing pathogens and preventing their further transportation. In this study, a 2-fold increase was seen in mRNA transcript of the TGase after 6 h. Iron plays a major role in survival of all organisms. As described by Weinberg [100], pathogens and hosts compete for iron. Ferritin, a blood protein, prevents iron removal from the host cells. However, no increases were seen in *ftn* transcription levels after feeding with PHB in this study. The study reported that higher concentrations over 100 mg l^{-1} PHB could slightly create toxic effects on the immune system when challenged with pathogens. Moreover, decreases in Hsp70 levels were observed in such cases. This could be due to more acidic pH in gut of the larvae due to partial digestion of a greater number of PHB molecules.

Van Hung et al. [101] studied effects of PHB-A on infections by *V. coralliilyticus* in blue mussels *(M. edulis*) larvae. For the study, larvae were directly exposed to rifampicin resistant *V. coralliilyticus* (10^5 CFU ml^{-1}), another group was administered with PHB-A alone and the rest of groups were fed with *Ralstonia eutropha* with a PHA yield of 75% of cell dry weight for 6 h and were then exposed to *Vibrio* spp. The immune mechanisms were studied by analyzing transcriptional titers of genes producing AMPs mytimycin, mytilinB, defensin and lysozymes. Significant downregulation of mytimycin and lysozyme was observed in larval groups fed with PHB-A and infected with the bacteria. Significant increases were seen in phenoloxidase activity ($p < 0.05$) and defensin gene was upregulated after 48 h of the infection.

5. Sustainable strategies for polyhydroxyalkanoate production: the way forward

Currently, PHAs are visualized as the most ecofriendly replacements for antibiotic use in aquaculture. The major limitations for its widespread use in aquaculture is contributed by high production costs associated with use of synthetic media components for the bacterial fermentation. Agricultural waste residues with appreciable quantities of carbohydrates are described as cost effective substrates for the commercial PHA production. The basic strategy of producing PHA from agricultural wastes is outlined in

Figure 2. The primary step in PHA production using agricultural wastes as substrates is pretreatment of the substrates to make them further appropriate for the fermentation processes. This may involve size decreasing and powdering of the substrates as well as substrate washing to remove undesirable compounds affecting bacterial fermentation [102]. The second step involves hydrolysis to convert complex polysaccharides in the substrates to easily fermentable sugars. Acid hydrolysis, thermal hydrolysis or enzymatic hydrolysis can be used separately or in combination depending on the target substrates [103-105]. Then, PHA production can generally be carried out using two groups of bacteria, first group capable of producing PHA during normal phases of growth and the second group producing PHA when carbon is in excess with limitation of any other essential nutrients such as nitrogen or phosphorus [106]. Either continuous cultivation method or fed batch method is used for PHA fermentation. Fed batch system involves growing the bacterial strain to a desired high cell density followed by creating nutrient limitation for PHA accumulation. In continuous or semi continuous fermentation method, the famous feast and famine strategy is used [107].

The major agricultural wastes reported for PHA production are listed in Table 2. Downstream processing in PHA production is one of the critical steps that describes the efficiency and purity of PHA recovered from the bacterial cells [108]. Extraction of PHA is generally carried out following three steps of pretreatment for cell lysis, separation of PHA and recovery [109]. The commonly used pretreatment methods include heat treatment, alkali/ alkali salt treatment and freeze-thaw methods. The pretreatment lyse or weaken cell walls and membranes of the microorganisms [110]. For PHA separation and extraction, various methods of solvent extraction and enzymatic digestion are used. In solvent extraction methods, various solvents are used to dissolve PHA from the pretreated cells followed by precipitation [111]. The enzymatic lysis methods are complex processes as they need optimal conditions for the enzymes to function actively [112]. Other methods of PHA extraction include mechanical disruption, supercritical fluid extraction, aqueous two phase system based extraction, floatation techniques and irradiation methods [109].

6. Challenges and future prospects

Commercial uses of PHA in aquaculture include major challenges that need further studies to make them sustainable replacements for antibiotic use in aquaculture. Two major challenges include 1) lack of molecular studies on immunomodulatory effects of PHAs in aquaculture and 2) problems associated with decreasing costs of PHA production. Of these, the major challenge that limits commercialization of PHA in aquaculture and other areas is associated with high costs of PHA production and purification. Most of the available studies only have provi-ded basic information on the significance of PHAs and other SCFA as growth promoters and anti-inflammatory agents in aquaculture [13]. For a better understanding of the molecular and genetic bases of immunostimulatory effects of PHA in aquaculture, experimental studies on the nature of receptors, upregulation or downregulation of the genes and expression should be carried out [13]. Stability of the PHA incorporated feeds during various stages of growth in various trophic levels and living ecosystems must be investigated [148]. Although butyrate producing organisms are indicators of healthy microbiota, their relationships with immunomodulation, especially how these microbiota intern control the host immune system, can be included in future studies [149]. Using computational tools, comprehensive studies on characterization of metabolites produced from gut microbiota in response to PHA feeding may provide new insights on how these metabolites help control immune responses in hosts [150].

The second major challenge in commercialization of PHA in aquaculture includes high costs associated with PHA production and downstream processing. Although synthetic media composed of well-known carbon and nitrogen sources are reported highly efficient for PHA production, its use accelerates the overall costs of fermentation processes making it non-commercially viable. This problem can be solved by integrating use of agroindustrial wastes as cost-effective substrates for PHA production. A majority of agricultural wastes are raw polysaccharides, which can serve as appropriate substrates for PHA production, decreasing production costs and associated problems to disposal of agricultural wastes. Use of organic solvents for the extraction of PHA granules poses another challenge as the method is costly and non-ecofriendly.

Figure 2. Basic strategy of producing PHA from agricultural wastes

Table 2. Production of polyhydroxyalkanoates from agricultural wastes

Bacterial culture	Agricultural waste	PHA yield	Reference
Bacillus sp. strain COL1/A6	Hydrolyzed citrus pulp	54.6% CDW	[113]
	Pigeon pea waste	41% CDW	[114]
Psuedomonas aeruginosa	Sugarcane bagasse	60% CDW	[114]
	Rice bran	48% CDW	[114]
Halomonas boliviensis LC1	Wheat bran hydrolysate	4% CDW	[115]
Halomonas campisalis MCM B-1027	Bagasse extract	47% CDW	[116]
Pseudomonas species	Corn oil	35.63% CDW	[117]
Proteobacteria	Hardwood spent sulfite liquor	67.6% CDW	[118]
Recombinant *Escherichia coli*	Molasses	75.5% CDW	[119]
Enterococcus sp. NAP11 *Brevundimonas sp*. NAC1	Cardboard industry waste water	79.27 and 77.63% CDW	[120]
Burkholderia cepacia	Spent coffee grounds (SCG)	56.01% CDW	[121]
Plasticicumulans acidivorans	Paper mill wastewater	77% CDW	[122]
Haloferax mediterranei DSM1411	Whey	73% CDW	[123]
Pseudomonas strains	Grass biomass	34% CDW	[105]
Halomonas i4786	Leguminous processing Water	1.6 g l^{-1}	[124]
Bacillus subtilis	Sugarcane molasses	2.5 mg ml^{-1}	[125]
Ralsthonia eutropha ATCC 17697	Pineapple peel waste	44.8% CDW	[126]
Pseudomonas aeruginosa 42A2	Industrial oil byproduct	4.63 g l^{-1}	[127]
Pseudomonas aeruginosa	Cassava wastewater	39% CDW	[128]
Bacillus subtilis RS1	Sugarcane molasses	70.5% CDW	[129]
Bacillus subtilis (KP172548)	Fish solid waste (FSW)	1.62 g l^{-1}	[130]
Pseudomonas strains	Grass biomass	17% CDW	[105]
Sinorhizobium meliloti MTCC 100	Rice bran hydrolysate	2.71 g l^{-1}	[131]
Bacillus mycoides DFC1	Rice husk hydrolysate	34.5% CDW	[132]
Bacillus subtilis NG220	Sugar industry waste water	5.297 g l^{-1}	[133]
Ralstonia eutropha NCIMB 11599	Saccharified waste potato starch	94 g l^{-1}	[134]
Cupriavidus necator	Waste frying oil	0.62 g l^{-1}	[135]
Azotobacter beijerinickii	Coir hydrolysate	48.19 % CDW	[136]
Recombinant *Escherichia coli*	Soy waste	27.83% CDW	[137]
Recombinant *Escherichia coli*	Algae biomass	51% CDW	[138]
Burkholderia sacchari	Waste paper	44.2% CDW	[139]
Alcaligenes latus (ATCC 29714)	Sugar beet juice	38.66% CDW	[140]
Bacillus subtilis NCDC0671	Papaya peel	4.2 g l^{-1}	[141]
Bacillus subtilis NCDC0671	Orange peel hydrolysate	5.09 g l^{-1}	[142]
Bacillus cereus EGU43	Pea-shell slurry	12.4% CDW	[143]
Pseudomonas citronellolis NRRL B-2504	Apple pulp waste	30% CDW	[144]
Pseudomonas putida KT2440	Waste chicken feather	61% CDW	[145]
Pseudomonas resinovorans DSM 21078	Camelina oil	40% CDW	[146]
Paracoccus sp. LL1	Waste cooking oil	1 g l^{-1}	[147]

A sustainable solution for this process can be supplementation of PHA harboring whole bacterial cells instead of extracted polymers in feeds [72]. Feeding larvae with PHA alone can limit availability of other important nutrients, but feeding with enriched live feeds or directly with PHA containing bacterial cells can solve this problem. Further studies on implementation of the proposed feeding regiments can be favorable solutions. Another important concern is linked to possible toxic effects of high PHB concentrations on larvae. Najdegerami [151] reported that concentrations of PHB higher than 100 mg l^{-1} can include toxic effects on the larval immune system. Only a few reports are available on this issue that need further attentions. More data on PHB toxicity is hence essential for the efficient use of PHA in aqua-culture. In summarize, large gaps exist that need careful monitoring use PHA as a sustainable alternative to anti-biotics in aquaculture. In depth molecular studies and stability and toxicity analyses of the breakdown products in aquaculture systems need further research before the products are available. Sustainable production and purify-cation strategies in combination with proper assessments of biodegradability and toxicity play significant roles in successful use of PHA in aquaculture.

7. Conclusion

In general, use of PHAs as immunostimulants and growth enhancers in aquaculture seems to include potential advantages over the classical antibiotic administration to fight against aquatic pathogens. Despite strong efforts for the commercial use of PHAs in aquaculture within the last decade, problems associated to cost effectiveness of the productions and purification processes are yet to be resolved. To ensure immunomodulatory and growth pro-moting activities of PHAs, further studies and field trials are needed globally. Ensuring non-toxicity of PHAs and their

driven products in hosts must be further studied. Designing improved methods for the PHA production using agricultural wastes may include high effects on decreasing overall costs of fermentation. The appropriate use of PHAs, as alternatives to antibiotics in aquaculture, may create new hopes in battling against emergence and persistence of antibiotic resistant pathogens in aquaculture.

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9. Conflict of interest

The authors declare no conflict of interest.

References

1. Dadar M, Dhama K, Vakharia VN, Hoseinifar SH, Karthik K, Tiwari R, Khandia R, Munjal A, Salgado-Miranda C, Joshi SK. Advances in aquaculture vaccines against fish pathogens: Global status and current trends. Rev Fish Sci Aquac. 2017; 25(3): 184-217. doi: 10.1080/23308249.2016.1261277

2. Pachapur PK, Pachapur VL, Brar SK, Le Bihan Y, Surampalli RY. Food Security and Sustainability. In: Surampalli R, Zhang T, Goyal MK, Brar S, Tyagi R, Editors. Sustainability: Fundamentals and Applications, 1st Edition. John Wiley and Sons, Ltd, 2020; 357-374. doi: 10.1002/9781119434016.ch17

3. Little DC, Newton RW, Beveridge MCM. Aquaculture: A rapidly growing and significant source of sustainable food? Status, transitions and potential. Proc Nutr Soc. 2016; 75(3):274-286. doi: 10.1017/S0029665116000665

4. FAO, editor. The State of World Fisheries and Aquaculture. Vol. 2020. Food and Agriculture Organization of the United Nations. doi: 10.4060/ca9229en

5. Martinez-Porchas M, Martinez-Cordova LR. World aquaculture: Environmental impacts and troubleshooting alternatives. Sci World J. 2012; 1(1):1-9. doi:10.1100/2012/389623

6. Biao X, Kaijin Y. Shrimp farming in China: Operating characteristics, environmental impact and perspectives. Ocean Coast Manag. 2007; 50(7):538-550. doi: 10.1016/j.ocecoaman.2007.02.006

7. Ma D, Hu Y, Wang J, Ye S, Li A. Effects of antibacterials use in aquaculture on biogeochemical processes in marine sediment. Sci Total Environ. 2006; 367(1):273-277. doi:10.1016/j.scitotenv.2005.10.014

8. Ringo E, Olsen RE, Jensen I, Romero J, Lauzon HL. Application of vaccines and dietary supplements in aquaculture: Possibilities and challenges. Rev Fish Biol Fish. 2014; 24(4): 1005-1032. doi:10.1007/s11160-014-9361-y

9. Newman SG. Specific pathogen-free status advances shrimp culture. Global aquaculture advocate. 2009; 12(3):1-4.

10. Bricknell I, Dalmo RA. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol. 2005; 19(5): 457-472. doi:10.1016/j.fsi.2005.03.008

11. Sakai M. Current research status of fish immunostimulants. Aquaculture 1999; 172(1):63-92. doi: 10.1016/S0044-8486(98)00436-0

12. Crab R, Defoirdt T, Bossier P, Verstraete W. Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 2012; 356-357:351-356. doi: 10.1016/j.aquaculture.2012.04.046

13. Hoseinifar SH, Sun Y-Z, Caipang CM. Short-chain fatty acids as feed supplements for sustainable aquaculture: An updated view. Aquac Res. 2017; 48(4):1380-1391. doi: 10.1111/are.13239

14. Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P. Alternatives to antibiotics to control bacterial infections: Luminescent vibriosis in aquaculture as an example. Trends Biotechnol. 2007; 25(10): 472-479. doi:10.1016/j.tibtech.2007.08.001

15. Rajeswari V, Priyadarshini SK, Saranya V, Suguna P, Shenbagarathai R. Immunostimulation by phospholipopeptide biosurfactant from *Staphylococcus hominis* in *Oreochromis mossambicus*. Fish Shellfish Immun. 2016; 48: 244-253. doi:10.1016/j.fsi.2015.11.006

16. Giri SS, Chi C, Jun JW, Park SC. Use of bacterial subcellular components as immunostimulants in fish aquaculture. Rev Aquacult. 2018; 10(2):474-492. doi:10.1111/raq.12182

17. Duan Y, Zhang Y, Dong H, Zheng X, Wang Y, Li H, Liu Q, Zhang J. Effect of dietary poly-β-hydroxybutyrate (PHB) on growth performance, intestinal health status and body composition of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Fish Shellfish Immunol. 2017; 60:520-528. doi:0.1016/j.fsi.2016.11.020

18. Anderson AJ, Dawes EA. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol Rev. 1990; 54(4): 450-472. doi: 10.1128/MMBR.54.4.450-472.1990

19. Obruca S, Sedlacek P, Slaninova E, Fritz I, Daffert C, Meixner K, Sedrlova Z, Koller M. Novel unexpected functions of PHA granules. Appl Microbiol Biotechnol. 2020; 104(11): 4795-4810. doi:10.1007/s00253-020-10568-1

20. Khosravi-Darani K, Bucci DZ. Application of poly (hydroxyalkanoate) in food packaging: Improvements by nanotechnology. Chem Biochem Eng Q. 2015; 29(2):275-285. doi: 10.15255/CABEQ.2014.2260

21. Koller M. Poly (hydroxyalkanoates) for food packaging: Application and attempts towards implementation.Apple food Biotechnol. 2014; 1(1):3-15. doi: 10.22037/afb.v1i1.7127

22. Chee JY, Lakshmanan M, Jeepery IF, Hairudin NHM, Sudesh K. The potential application of *Cupriavidus necator* as polyhydroxyalkanoates producer and single cell protein: A review on scientific, cultural and religious perspectives. Appl Food Biotechnol. 2019; 6(1): 19-34. doi: 10.22037/afb.v6i1.22234

23. Umesh M, Priyanka K, Thazeem B, Preethi K. Biogenic PHA nanoparticle synthesis and characterization from *Bacillus subtilis* NCDC0671 using orange peel medium. 2018; 67(17): 996-1004. doi:10.1080/00914037.2017.1417284

24. Umesh M, Thazeem B. Biodegradation studies of polyhydroxyalkanoates extracted from *Bacillus subtilis* NCDC 0671. Res J Chem Environ. 2019; 23(6):107-114.

25. Koller M, Marsalek L, de Sousa Dias MM, Braunegg G. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N Biotechnol. 2017; 37(1):24-38. doi: 10.1016/j.nbt.2016.05.001

26. Koller M, Braunegg G. Potential and prospects of continuous polyhydroxyalkanoate (pha) production. Bioengineering (basel). 2015; 2(2):94-121. doi: 10.3390/bioengineering2020094

27. Koller M, Marsalek L. Potential of diverse prokaryotic organisms for glycerol-based Polyhydroxyalkanoate production. Appl Food Biotechnol. 2015; 2(3):3-15. doi:10.22037/afb.v2i3.8271

28. Koller M, Dias MM de S, Rodriguez-Contreras A, Kunaver M, Zagar E, Krzan A, Braunegg G. Liquefied wood as inexpensive precursor-feedstock for bio-mediated incorporation of (R)-3-hydroxyvalerate into polyhydroxyalkanoates. Mater. 2015; 8(9):6543-6557. doi:10.3390/ma8095321

29. Priyanka K, Umesh M, Thazeem B, Preethi K. Polyhydroxyalkanoate biosynthesis and characterization from optimized medium utilizing distillery effluent using *Bacillus endophyticus* MTCC 9021: A statistical approach. Biocatal Biotransfor. 2020;1(1):1-13.

30. Khosravi DK, Vasheghani FE, Tanaka K. Hydrogen oxidizing bacteria as poly (hydroxybutyrate) produ-cers. Iranian J Biotechnol. 2006; 4(3):193-196.

31. Khosravi-Darani K, Mokhtari Z-B, Amai T, Tanaka K. Microbial production of poly(hydroxybutyrate) from C1 carbon sources. Appl Microbiol Biotechnol. 2013; 97(4): 1407-1424. doi:10.1007/s00253-012-4649-0

32. Khosravi-Darani K, Yazdian F, Babapour F, Amirsadeghi AR. Poly (3-hydroxybutyrate) production from natural gas by a methanotroph native bacterium in a bubble column bioreactor. Chem Biochem Eng Q. 2019; 33(1):69-77. doi: 10.15255/CABEQ.2017.1263

33. Ghoddosi F, Golzar H, Yazdian F, Khosravi-Darani K, Vasheghani-Farahani E. Effect of carbon sources for PHB production in bubble column bioreactor: Emphasis on improvement of methane uptake. J Environ Chem Eng. 2019; 7(2):1-6. doi: 10.1016/j.jece.2019.102978

34. Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Heidarzadeh-Vazifekhoran A, Shojaosadati SA, Karimzadeh R, Khosravi Darani K. Statistical media optimization for growth and PHB production from methanol by a methylotrophic bacterium. Bioresour Technol. 2009; 100(8):2436-2443. doi:10.1016/j.biortech.2008.11.024

35. Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Shojaosadati SA, Karimzadeh R, Heidarzadeh-Vazifekhoran A. Effect of feed composition on PHB production from methanol by HCDC of *Methylobacterium extorquens* (DSMZ 1340). J Chem Technol Biotechnol . 2009; 84(8):1136-1139.

36. Shahhosseini S, Khosravi DK. Simulation and model validation of batch PHB production process using *Ralstonia eutropha*. Iran J Chem Chem Eng. 2003; 22(2): 35-42.

37. Darani K, Farahani KVE, Shojaosadati SA. Application of the plackett-burman design for the optimization of poly (β-Hydroxybutyrate) production by *Ralstonia eutropha*. Iran J Biotechnol. 2003; 1: 155.

38. Khosravi DK, Vasheghani FE, Shoja ASA. Application of the Taguchi design for production of poly (β-hydroxybutyrate) by *Ralstonia eutropha*. Iran J Chem Chem Eng. 2004; 23(1):131-136.

39. Khosravi-Darani K, Vasheghani-Farahani E. Application of supercritical fluid extraction in biotechnology. Crit Rev Biotechnol. 2005; 25(4): 231-242. doi:10.1080/07388550500354841

40. Gao M, Du D, Bo Z, Sui L. Poly-β-hydroxybutyrate (PHB)-accumulating Halomonas improves the survival, growth, robustness and modifies the gut microbial composition of *Litopenaeus vannamei* postlarvae. Aquaculture 2019; 500(1):607-612. doi: 10.1016/j.aquaculture.2018.10.032

41. Suguna P, Binuramesh C, Abirami P, Saranya V, Poornima K, Rajeswari V, Shenbagarathai R. Immunostimulation by poly-β hydroxybutyrate–hydroxyvalerate (PHB–HV) from *Bacillus thuringiensis* in *Oreochromis mossambicus*. Fish Shellfish Immun. 2014; 36(1): 90-97. doi: 10.1016/j.fsi.2013.10.012

42. Tan G-Y, Chen C-L, Li L, Ge L, Wang L, Razaad I, Li Y, Zhao L, Mo Y, Wang J-Y. Start a research on biopolymer polyhydroxyalkanoate (PHA): A review. Polyme. 2014; 6(3): 706-754. do:10.3390/polym6030706

43. Jung YM, Lee YH. Utilization of oxidative pressure for enhanced production of poly-beta-hydroxybutyrate and poly (3-hydroxybutyrate-3-hydroxyvalerate) in *Ralstonia eutropha*. J Biosci Bioeng. 2000; 90(3):266-270. doi: 10.1016/S1389-1723(00)80080-8

44. Naik S, Venu Gopal SK, Somal P. Bioproduction of polyhydroxyalkanoates from bacteria: a metabolic approach. World J Microbiol Biotechnol. 2008; 24(10): 2307-2314. doi:10.1007/s11274-008-9745-z

45. Liebergesell M, Mayer F, Steinbuchel A. Anaylsis of polyhydroxyalkanoic acid-biosynthesis genes of anoxygenic phototrophic bacteria reveals synthesis of a polyester exhibiting an unusal composition. Appl Microbiol Biotechnol. 1993; 40(1): 292-300. doi:10.1007/bf00170383

46. Tsuge T, Yano K, Imazu S-I, Numata K, Kikkawa Y, Abe H, Taguchi S, Doi Y. Biosynthesis of polyhydroxyalkanoate (PHA) copolymer from fructose using wild-type and laboratory-evolved PHA synthases. Macromol Biosci. 2005; 5(2):112-117. doi:10.1002/mabi.200400152

47. Luengo JM, Garcia B, Sandoval A, Naharro G, Olivera ER. Bioplastics from microorganisms. Curr Opin Microbiol. 2003; 6(3): 251-260. doi: 10.1016/S1369-5274(03)00040-7

48. Martinez-Tobon DI, Gul M, Elias AL, Sauvageau D.

Polyhydroxybutyrate (PHB) biodegradation using bacterial strains with demonstrated and predicted PHB depolymerase activity. Appl Microbiol Biotechnol. 2018; 102(18):8049-8067.
doi: 10.1007/s00253-018-9153-8

49. Prieto A, Escapa IF, Martinez V, Dinjaski N, Herencias C, de la Pena F, Tarazona N, Revelles O. A holistic view of polyhydroxyalkanoate metabolism in *Pseudomonas putida*. Environ Microbiol. 2016; 18(2):341-357.
doi:10.1111/1462-2920.12760

50. Hiraishi T, Taguchi S. Protein engineering of enzymes involved in bioplastic metabolism. In: Ogawa t, protein engineering-technology and application. 1st edition. InTech, London, 2013:133-165.
doi: doi.org/10.5772/55552

51. Ong SY, Chee JY, Sudesh K. Degradation of poly-hydroxyalkanoate (PHA): A review. J Sib Fed Univ, Biol. 2017; 10(2):211-225.
doi: 10.17516/1997-1389-0024

52. Kobayashi T, Uchino K, Abe T, Yamazaki Y, Saito T. Novel intracellular 3-hydroxybutyrate-oligomer hydrolase in *Wautersia eutropha* H16. J Bacteriol. 2005; 187(15): 5129-5135.
doi:10.1128/JB.187.15.5129-5135.2005

53. Lemes AP, Montanheiro TLA, Passador FR, Duran N. Nanocomposites of Polyhydroxyalkanoates Reinforced with Carbon Nanotubes: Chemical and Biological Properties. In: Thakur VK, Thakur MK, Eco-Friendly Polymer Nanocomposites: Processing and Properties. 1st Edition. Springer, New Delhi, India; 2015:79-108.
doi:. 10.1007/978-81-322-2470-9_3

54. Blevins HM, Blue MKD, Cobbs BD, Ricotilli TA. Characterization of an extracellular polyhydroxyalkanoate depolymerase from *Streptomyces* sp. SFB5A. J Bioremediat Biodegrad. 2018; 9(5):1-11
doi: 10.4172/2155-6199.1000452

55. Kheti B, Kamilya D, Choudhury J, Parhi J, Debbarma M, Singh ST. Dietary microbial floc potentiates immune response, immune relevant gene expression and disease resistance in rohu, *Labeo rohita* (Hamilton, 1822) fingerlings. Aquaculture 2017; 468: 501-507.
doi: 10.1016/j.aquaculture.2016.11.018

56. Sui L, Liu Y, Sun H, Wille M, Bossier P, De Schryver P. The effect of poly-β-hydroxybutyrate on the performance of Chinese mitten crab (*Eriocheir sinensis* Milne-Edwards) zoea larvae. Aquac Res. 2014; 45(3):558-565.
doi: 10.1111/are.12077

57. Yaqoob T, Khan N, Arslan M, Korkmaz F, Tacer A, Suzer C, Dogar S. Dietary Supplementation of Poly-AA^2-Hydroxybutyrate on the Growth, Digestive Enzymes Activity and Body Composition of Rainbow Trout (*Onchorynchus mykiss*). Res Rev J Zool Sci. 2018;6(1):38–42.

58. Nhan DT, Dinh The Nhan, Wille M, De Schryver P, Defoirdt T, Bossier P, Sorgeloos P. The effect of poly β-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 2010; 302(1-2):76-81.
doi:10.1016/j.aquaculture.2010.02.011

59. Defoirdt T, Halet D, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P, Bossier P, Verstraete W. The bacterial storage compound poly-beta-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. Environ Microbiol. 2007; 9(2): 445-452.
doi:10.1111/j.1462-2920.2006.01161.x

60. De Schryver P, Sinha AK, Kunwar PS, Baruah K, Verstraete W, Boon N, De Boeck G, Bossier P. Poly-beta-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass, *Dicentrarchus labrax*. Appl Microbiol Biotechnol. 2010; 86(5): 1535-1541.
doi: 10.1007/s00253-009-2414-9

61. Wang X, Jiang X, Wu F, Ma Y, Che X, Chen X, Liu P, Zhang W, Ma X, Chen G. Microbial poly-3-hydroxybutyrate (PHB) as a feed additive for fishes and piglets. Biotechnol J. 2019; 14(12): 1-8.
doi: 10.1002/biot.201900132

62. Situmorang ML, De Schryver P, Dierckens K, Bossier P. Effect of poly-β-hydroxybutyrate on growth and disease resistance of Nile tilapia *Oreochromis niloticus* juveniles. Vet Microbiol. 2016; 182:44-49.
doi: 10.1016/j.vetmic.2015.10.024

63. Castro R, Jouneau L, Tacchi L, Macqueen DJ, Alzaid A, Secombes CJ, Martin SAM, Boudinot P. Disparate developmental patterns of immune responses to bacterial and viral infections in fish. Sci Rep. 2015; 5:1-12.
doi: 10.1038/srep15458

64. Franke A, Roth O, Schryver PD, Bayer T, Garcia-Gonzalez L, Künzel S, Bossier P, Miest JJ, Clemmesen C. Poly-β-hydroxybutyrate administration during early life: Effects on performance, immunity and microbial community of European sea bass yolk-sac larvae. Sci Rep. 2017; 7(1): 1-11.
doi: 10.1038/s41598-017-14785-z

65. Franke A, Clemmesen C, De Schryver P, Garcia-Gonzalez L, Miest JJ, Roth O. Immunostimulatory effects of dietary poly-β-hydroxybutyrate in European sea bass post larvae. Aquac Res. 2017; 48(12): 5707-5717.
doi: 10.1111/are.13393

66. Najdegerami EH, Tran TN, Defoirdt T, Marzorati M, Sorgeloos P, Boon N, Bossier P. Effects of poly-β-hydroxybutyrate (PHB) on Siberian sturgeon (*Acipenser baerii*) fingerlings performance and its gastrointestinal tract microbial community. FEMS Microbiol Ecol. 2017; 79(1): 25-33.
doi: 10.1111/j.1574-6941.2011.01194.x

67. Najdegerami EH, Baruah K, Shiri A, Rekecki A, Van den Broeck W, Sorgeloos P, Boon N, Bossier P, De Schryver P. Siberian sturgeon (*Acipenser baerii*) larvae fed *Artemia nauplii* enriched with poly-β-hydroxybutyrate (PHB): Effect on growth performance, body composition, digestive enzymes, gut microbial community, gut histology and stress tests. Aquac Res. 2015; 46(4): 801-812.
doi: 10.1111/are.12231

68. Misra S, Sahu NP, Pal AK, Xavier B, Kumar S, Mukherjee SC. Pre and post-challenge immuno-haematological changes in *Labeo rohita* juveniles fed gelatinised or non-gelatinised carbohydrate with n-3 PUFA. Fish Shellfish Immunol. 2006; 21(4): 346-356.
doi: 10.1016/j.fsi.2005.12.010

69. Alexander JB, Ingram GA. Noncellular nonspecific defence mechanisms of fish. Annu Rev Fish Dis. 1992; 2:249-279.
doi: 10.1016/0959-8030(92)90066-7

70. Fu GH, Bai ZY, Xia JH, Liu F, Liu P, Yue GH. Analysis of two lysozyme genes and antimicrobial functions of their

recombinant proteins in Asian seabass. Plos One. 2013; 8:1-12.
doi: 10.1371/journal.pone.0079743

71. Clements KD, Gleeson VP, Slaytor M. Short-chain fatty acid metabolism in temperate marine herbivorous fish. J Comp Physiol B. 1994; 164: 372-327.
doi: 10.1007/bf00302552

72. Laranja JLQ, Amar EC, Ludevese-Pascual GL, Niu Y, Geaga MJ, De Schryver P, Bossier P. A probiotic *Bacillus* strain containing amorphous poly-beta-hydroxybutyrate (PHB) stimulates the innate immune response of *Penaeus monodon* postlarvae. Fish Shellfish Immunol. 2017; 68:202-210.
doi: 10.1016/j.fsi.2017.07.023

73. Amparyup P, Charoensapsri W, Tassanakajon A. Prophenoloxidase system and its role in shrimp immune responses against major pathogens. Fish Shellfish Immunol. 2013; 34(4): 990-1001.
doi:10.1016/j.fsi.2012.08.019

74. Maningas MBB, Kondo H, Hirono I, Saito-Taki T, Aoki T. Essential function of transglutaminase and clotting protein in shrimp immunity. Mol Immunol. 2008; 45(5): 1269-1275.
doi: 10.1016/j.molimm.2007.09.016

75. Morimoto RI. Regulation of the heat shock transcriptional response: Cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. 1998; 12(24): 3788-3796.
doi: 10.1101/gad.12.24.3788

76. Qiao G, Xu C, Sun Q, Xu D-H, Zhang M, Chen P, Li Q. Effects of dietary poly-β-hydroxybutyrate supplementation on the growth, immune response and intestinal microbiota of soiny mullet (*Liza haematocheila*). Fish Shellfish Immunol. 2019; 91:251-263.
doi:10.1016/j.fsi.2019.05.038

77. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018; 54(4):287-293.
doi: 10.1016/j.ajme.2017.09.001

78. Bagnyukova TV, Vasylkiv OY, Storey KB, Lushchak VI. Catalase inhibition by amino triazole induces oxidative stress in goldfish brain. Brain Res. 2005; 1052(2):180-186.
doi: 10.1016/j.brainres.2005.06.002

79. Meng Q, Chen J, Xu C, Huang Y, Wang Y, Wang T, Zhai X, Gu W, Wang W. The characterization, expression and activity analysis of superoxide dismutases (SODs) from *Procambarus clarkii*. Aquaculture. 2013; 406-407:131-140.
doi: 10.1016/j.aquaculture.2013.05.008

80. Ishida K, Hung TV, Liou K, Lee HC, Shin C-H, Sohng JK. Characterization of *pbpA* and *pbp2* encoding penicillin-binding proteins located on the downstream of clavulanic acid gene cluster in *Streptomyces clavuligerus*. Biotechnol Lett. 2006; 28(6): 409-417.
doi: 10.1007/s10529-005-6071-5

81. Fujimoto Y, Konishi Y, Kubo O, Hasegawa M, Inohara N, Fukase K. Synthesis of crosslinked peptidoglycan fragments for investigation of their immunobiological functions. Tetrahedron Lett. 2009; 50(26): 3631-3634.
doi: 10.1016/j.tetlet.2009.03.081

82. Baggiolini M, Dewald B, Moser B. lnterleukin-8 and related chemotactic cytokines-CXC and CC chemokines. Adv Immunol. 1993; 55:97-179.
doi: 10.1016/s0065-2776(08)60509-x

83. Wright RM, Weigel LK, Varella-Garcia M, Vaitaitis G, Repine JE. Molecular cloning, refined chromosomal mapping and structural analysis of the human gene encoding aldehyde oxidase (*AOX1*), a candidate for the *ALS2* gene. Redox Rep. 1997;3 (3):135-144.
doi:10.1080/13510002.1997.11747101

84. Ryhanen P, Surcel HM, Ilonen J. Decreased expression of class II major histocompatibility complex (MHC) molecules on monocytes is found in open-heart surgery related immunosuppression. Acta Anaesthesiol Scand. 1991; 35(5):453-456.
doi: 10.1111/j.1399-6576. 1991.tb03327.x

85. Semova I, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, Rawls JF. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe. 2012; 12(3):277-288.
doi: 10.1016/j.chom.2012.08.003

86. Luo G, Liu Z, Shao L, Tan H. Using poly-β-hydroxybutyric as an additional carbohydrate for biofloc in a shrimp *Litopenaeus vannamei* bioflocs nursery system with brackish water. Aquaculture. 2019; 506: 181-187.
doi: 10.1016/j.aquaculture.2019.03.021

87. Sui L, Cai J, Sun H, Wille M, Bossier P. Effect of poly-β-hydroxybutyrate on Chinese mitten crab, *Eriocheir sinensis*, larvae challenged with pathogenic *Vibrio anguillarum*. J Fish Dis. 2012; 35(5): 359-364.
doi: 10.1111/j.1365-2761.2012. 01351.x

88. Baruah K, Huy TT, Norouzitallab P, Niu Y, Gupta SK, De Schryver P, Bossier P. Probing the protective mechanism of poly-ß-hydroxybutyrate against vibriosis by using gnotobiotic *Artemia franciscana* and *Vibrio campbellii* as host-pathogen model. Sci Rep. 2015; 5:1-8.
doi: 10.1038/srep09427

89. Halet D, Defoirdt T, Van Damme P, Vervaeren H, Forrez I, Van de Wiele T, Boon N, Sorgeloos P, Bossier P, Verstraete W. Poly-beta-hydroxybutyrate-accumulating bacteria protect gnotobiotic *Artemia franciscana* from pathogenic *Vibrio campbellii*. FEMS Microbiol Ecol. 2007; 60(3): 363-369.
doi: 10.1111/j.1574-6941.2007. 00305.x

90. Van Hung N, De Schryver P, Tam TT, Garcia-Gonzalez L, Bossier P, Nevejan N. Application of poly-β-hydroxy-butyrate (PHB) in mussel larviculture. Aquaculture 2015; 446:318-324.
doi: 10.1016/j.aquaculture.2015.04.036

91. Thai TQ, Wille M, Garcia-Gonzalez L, Sorgeloos P, Bossier P, De Schryver P. Poly-ß-hydroxybutyrate content and dose of the bacterial carrier for Artemia enrichment determine the performance of giant freshwater prawn larvae. Appl Microbiol Biotechnol. 2014; 98(11):5205-5215.
doi: 10.1007/s00253-014-5536-7

92. Najdegerami H E. Immunostimulatory and growth-promoting potential of poly-β-hydroxybutyrate in rainbow trout (*Oncorhynchus mykiss*) fingerlings culture. Iran J Fish Sci. 2020; 19(2): 847-865.

93. Laranja JLQ, Ludevese-Pascual GL, Amar EC, Sorgeloos P, Bossier P, De Schryver P. Poly-β-hydroxybutyrate (PHB) accumulating *Bacillus spp*. improve the survival, growth and robustness of *Penaeus monodon* (Fabricius, 1798)

postlarvae. Vet Microbiol. 2014; 173(3-4):310-317. doi: 10.1016/j.vetmic.2014.08.011

94. Costerton JW, William Costerton J, Lappin-Scott H. Introduction to Microbial Biofilms. Microbial Biofilms. 1995:1-12. doi: 10.1017/cbo9780511525353.002

95. Kiran GS, Lipton AN, Priyadharshini S, Anitha K, Suarez LEC, Arasu MV, Choi KC, Selvin J, Al-Dhabi NA. Antiadhesive activity of poly-hydroxy butyrate biopolymer from a marine *Brevibacterium casei* MSI04 against shrimp pathogenic vibrios. Microb Cell Fact. 2014; 13:114. doi: 10.1186/s12934-014-0114-3

96. Lee CW, Song BK, Jegal J, Kimura Y. Cell adhesion and surface chemistry of biodegradable aliphatic polyesters: Discovery of particularly low cell adhesion behavior on poly(3-[RS]-hydroxybutyrate). Macromolecular Research. 2013; 21:1305-1313. doi: 10.1007/s13233-013-1181-8

97. Van Hung N, Bossier P, Hong NTX, Ludeseve C, Garcia-Gonzalez L, Nevejan N, De Schryver P. Does *Ralstonia eutropha*, rich in poly-β hydroxybutyrate (PHB), protect blue mussel larvae against pathogenic vibrios ?. J Fish Dis. 2019; 42(6):777-787. doi: 10.1111/jfd.12981

98. Fernandez RG. Artemia bioencapsulation 1. Effect of particle sizes on the filtering behavior of *Artemia franciscana*. J Crustacean Biol. 2001; 21(2): 435-442. doi: 10.1163/20021975-99990144

99. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012; 13(4):227-232. doi: 10.1038/nrg3185

100. Weinberg ED. Iron availability and infection. Bba-Gen Subjects. 2009; 1790(7):600-605 doi: 10.1016/j.bbagen.2008.07.002

101. Van Hung N, De Schryver P, Dung NV, Nevejan N, Bossier P. *Ralstonia eutropha*, containing high poly-β-hydroxy-butyrate levels, regulates the immune response in mussel larvae challenged with *Vibrio coralliilyticus*. Fish Shellfish Immunol. 2019; 84:196-203. doi: 10.1016/j.fsi.2018.09.066

102. Preethi K, Vineetha UM. Water hyacinth: A potential substrate for bioplastic (PHA) production using *Pseudomonas aeruginosa*. Int J Appl Res Vet Med. 2015; 1(11):349-354.

103. Yin F, Li D, Ma X, Li J, Qiu Y. Poly (3-hydroxybutyrate-3-hydroxyvalerate) production from pretreated waste lignocellulosic hydrolysates and acetate cosubstrate. Bioresour Technol. 2020; 316: 123911. doi: 10.1016/j.biortech.2020.123911

104. Gowda V, Shivakumar S. Agrowaste-based Polyhydroxy-alkanoate (PHA) production using hydrolytic potential of *Bacillus thuringiensis* IAM 12077. Braz Arch Biol Technol. 2014; 57(1):55-61. doi: 10.1590/S1516-89132014000100009

105. Davis R, Kataria R, Cerrone F, Woods T, Kenny S, O'Donovan A, Guzik M, Shaikh H, Duane G, Gupta VK, Tuohy MG, Padamatti RB, Casey E, O'Connor KE. Conversion of grass biomass into fermentable sugars and its utilization for medium chain length polyhydroxyalkanoate (mcl-PHA) production by *Pseudomonas* strains. Bioresour Technol. 2013; 150:202-209. doi: 10.1016/j.biortech.2013.10.001

106. Lee SY. Plastic bacteria? Progress and prospects for polyhydroxyalkanoate production in bacteria. Trends Biotechnol. 1996; 14(11):431-438.

107. Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P. Short-chain fatty acids and poly-β-hydroxyalkanoates: (New) Biocontrol agents for a sustainable animal production. Biotechnol Adv. 2009; 27(6):680-685. doi: 10.1016/j.biotechadv.2009.04.026

108. Madkour MH, Heinrich D, Alghamdi MA, Shabbaj II, Steinbüchel A. PHA recovery from biomass. Biomacromolecules. 2013; 14(9): 2963-2972. doi: 10.1021/bm4010244

109. Kunasundari B, Sudesh K. Isolation and recovery of microbial polyhydroxyalkanoates. Express Polym Lett. 2011; 5(7): 620-634. doi: 10.3144/expresspolymlett.2011.60

110. Kourmentza C, Placido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN, Reis MAM. Recent advances and challenges towards sustainable Polyhydroxyalkanoate (PHA) production. Bioengin. 2017; 4(2)1-43. doi: 10.3390/bioengineering4020055

111. Anis SNS, Iqbal NM, Kumar S, Al-Ashraf A. Increased recovery and improved purity of PHA from recombinant *Cupriavidus necator*. Bioengineered 2013; 4(2): 115-118. doi: 10.4161/bioe.22350

112. Neves A, Muller J. Use of enzymes in extraction of polyhydroxyalkanoates produced by *Cupriavidus necator*. Biotechnol Progr. 2012; 28(6):1575-1580. doi: 10.1002/btpr.1624

113. Santimano MC, Prabhu NN, Garg S. PHA Production Using Low-Cost Agro-Industrial Wastes by *Bacillus* sp. Strain COL1/A6. Res J Microbiol. 2009; 4(3): 89-96. doi: 10.3923/jm.2009.89.96

114. Khandpur P, Jabeen ET, Rohini KVL, Varaprasad Y. Study on production, extraction and analysis of polyhydroxy-alkanoate (PHA) from bacterial isolates. IOSR J Pharm Biol Sci. 2012; 1:31-38. doi: 10.9790/3008-0113138

115. Van-Thuoc D, Quillaguaman J, Mamo G, Mattiasson B. Utilization of agricultural residues for poly(3-hydroxy-butyrate) production by *Halomonas boliviensis* LC1. J Appl Microbiol. 2008; 104(2): 420-428. do:10.1111/j.1365-2672.2007. 03553.x

116. Kulkarni SO, Kanekar PP, Jog JP, Sarnaik SS, Nilegaonkar SS. Production of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) by *Halomonas campisalis* MCM B-1027 using agro-wastes. Int J Biol Macromol. 2015; 72:784-789. doi: 10.1016/j.ijbiomac.2014.09.028

117. Chaudhry WN, Jamil N, Ali I, Ayaz MH, Hasnain S. Screening for polyhydroxyalkanoate (PHA)- producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. Ann Microbiol. 2011; 61(3): 623-629. doi: 10.1007/s13213-010-0181-6

118. Queiros D, Rossetti S, Serafim LS. PHA production by mixed cultures: a way to valorize wastes from pulp industry. Bioresour Technol. 2014; 157: 197-205. doi: 10.1016/j.biortech.2014.01.099

119. Saranya V, Shenbagarathai R. Production and characterization of PHA from recombinant *E. coli* harbouring

phaC1 gene of indigenous *Pseudomonas sp*. LDC-5 using molasses. Braz J Microbiol. 2011; 42(3):1109-1118. doi: 10.1590/S1517-83822011000300032

120. Bhuwal AK, Singh G, Aggarwal NK, Goyal V, Yadav A. Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper, and cardboard industry wastes. Int J Biomater. 2013; 2013:1-10. doi:10.1155/2013/752821

121. Obruca S, Benesova P, Petrik S, Oborna J, Prikryl R, Marova I. Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. Process Biochem. 2014; 49(9): 1409-1414. doi: 10.1016/j.procbio.2014.05.013

122. Jiang Y, Marang L, Tamis J, van Loosdrecht MCM, Dijkman H, Kleerebezem R. Waste to resource: Converting paper mill wastewater to bioplastic. Water Res. 2012; 46(17): 5517-5530. doi: 10.1016/j.watres.2012.07.028

123. Koller M. Recycling of waste streams of the biotechnological poly (hydroxyalkanoate) production by *Haloferax mediterranei* on Whey. Int J Polym Sci. 2015; 2015:1-9. doi: 10.1155/2015/370164

124. Elain A, Le Grand A, Corre Y-M, Le Fellic M, Hachet N, Le Tilly V, Loulergue P, Audic J-L, Bruzaud S. Valorisation of local agro-industrial processing waters as growth media for polyhydroxyalkanoates (PHA) production. Ind Crops Prod. 2016; 80:1-5. doi: 10.1016/j.indcrop.2015.10.052

125. Anjali M, Sukumar C, Kanakalakshmi A, Shanthi K. Enhancement of growth and production of polyhydroxyalkanoates by *Bacillus subtilis* from agro-industrial waste as carbon substrates. Compos Interfaces. 2014; 21(2):111-119. doi:10.1080/15685543.2013.834200

126. Vega-Castro O, Contreras-Calderon J, Leon E, Segura A, Arias M, Perez L, Sobral PJA. Characterization of a polyhydroxyalkanoate obtained from pineapple peel waste using *Ralsthonia eutropha*. J Biotechnol. 2016; 231:232-238. doi:10.1016/j.jbiotec.2016.06.018

127. Rodriguez-Carmona E, Bastida J, Manresa A. Utilization of agro-industrial residues for poly(3-hydroxyalkanoate) production by *Pseudomonas aeruginosa* 42A2 (NCIMB 40045): Optimization of culture medium. J Am Oil Chem Soc. 2012; 89(1):111-122. doi:10.1007/s11746-011-1897-6

128. Costa SGVAO, Lepine F, Milot S, Deziel E, Nitschke M, Contiero J. Cassava wastewater as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by *Pseudomonas aeruginosa*. J Ind Microbiol Biotechnol. 2009; 36(8): 1063-1072. doi:10.1007/s10295-009-0590-3

129. Rathika R, Janaki V, Shanthi K, Kamala-Kannan S. Bioconversion of agro-industrial effluents for polyhydroxyalkanoates production using *Bacillus subtilis* RS1. Int J Environ Sci Technol. 2019; 16(10): 5725-5734 doi:10.1007/s13762-018-2155-3

130. Mohapatra S, Sarkar B, Samantaray DP, Daware A, Maity S, Pattnaik S, Bhattacharjee S. Bioconversion of fish solid waste into PHB using *Bacillus subtilis* based submerged fermentation process. Environ Technol. 2017; 38(24): 3201-3208. doi:10.1080/09593330.2017.1291759

131. Saranya Devi E, Vijayendra SVN, Shamala TR. Exploration of rice bran, an agro-industry residue, for the production of intra- and extra-cellular polymers by *Sinorhizobium meliloti* MTCC 100. Biocatal Agric Biotechnol. 2012; 1(1):80-84. doi: 10.1016/j.bcab.2011.08.014

132. Narayanan A, Sajeev Kumar VA, Ramana KV. Production and characterization of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) from *Bacillus mycoides* DFC1 using rice husk hydrolyzate. Waste Biomass Valori. 2014; 5(1):109-118. doi:10.1007/s12649-013-9213-3

133. Singh G, Kumari A, Mittal A, Yadav A, Aggarwal NK. Poly β-hydroxybutyrate production by *Bacillus subtilis* NG220 using sugar industry waste water. Biomed Res Int. 2013; 2013:1-10. doi:10.1155/2013/952641

134. Haas R, Jin B, Zepf FT. Production of poly (3-hydroxybutyrate) from waste potato starch. Biosci Biotechnol Biochem. 2008; 72 (1): 253-256. doi:10.1271/bbb.70503

135. Verlinden RA, Hill DJ, Kenward MA, Williams CD, Piotrowska-Seget Z, Radecka IK. Production of polyhydroxyalkanoates from waste frying oil by *Cupriavidus necator*. AMB Express. 2011; 1(1): 11. doi:10.1186/2191-0855-1-11

136. Prabu CS, Murugesan AG. Effective utilization and management of coir industrial waste for the production of poly- β- hydroxybutyrate (PHB) using the bacterium *Azotobacter beijerinickii*. Int J Environ Res 2010; 4(3): 519-524.

137. Hong K, Leung YC, Kwok SY, Law KH, Lo WH, Chua H, Yu PH. Construction of recombinant *Escherichia coli* strains for polyhydroxybutyrate production using soy waste as nutrient. Appl Biochem Biotechnol. 2000; 84-86:381-390. doi:10.1385/abab:84-86:1-9:381

138. Sathish A, Glaittli K, Sims RC, Miller CD. Algae biomass based media for poly (3-hydroxybutyrate) (PHB) production by *Escherichia coli*. J Polym Environ. 2014; 22(2): 272-277. doi:10.1007/s10924-014-0647-x

139. Al-Battashi H, Annamalai N, Al-Kindi S, Nair AS, Al-Bahry S, Verma JP, Sivakumar N. Production of bioplastic (poly-3-hydroxybutyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing *Burkholderia sacchari*. J Clean Prod. 2019; 214: 236-247. doi: 10.1016/j.jclepro.2018.12.239

140. Wang B, Sharma-Shivappa RR, Olson JW, Khan SA. Production of polyhydroxybutyrate (PHB) by *Alcaligenes latus* using sugarbeet juice. Ind Crops Prod. 2013; 43:802-811. doi: 10.1016/j.indcrop.2012.08.011

141. Umesh M, Priyanka K, Thazeem B, Preethi K. Production of single cell protein and polyhydroxyalkanoate from *Carica papaya* waste. Arab J Sci Eng. 2017; 42(6):2361-2369. doi:10.1007/s13369-017-2519-x

142. Umesh M, Mani VM, Thazeem B, Preethi K. Statistical optimization of process parameters for bioplastic (PHA) production by *Bacillus subtilis* NCDC0671 using orange peel-based medium.Iran J Sci Technol Trans A Sci. 2018; 42(4): 1947-1955.

doi:10.1007/s40995-017-0457-9

143. Patel SKS, Kumar P, Singh M, Lee J-K, Kalia VC. Integrative approach to produce hydrogen and polyhydroxybutyrate from biowaste using defined bacterial cultures. Bioresour Technol. 2015; 176:136-141. doi:10.1016/j.biortech.2014.11.029

144. Rebocho AT, Pereira JR, Freitas F, Neves LA, Alves VD, Sevrin C, Grandfils C, Reis MAM. Production of medium-chain length polyhydroxyalkanoates by *Pseudomonas citronellolis* grown in apple pulp waste. Appl Food Biotechnol. 2019; 6(1):71-82. doi:10.22037/afb.v6i1.21793

145. Pernicova I, Enev V, Marova I, Obruca S. Interconnection of waste chicken feather biodegradation and keratinase and mcl-PHA production employing *Pseudomonas putida* KT2440. Appl Food Biotechnol. 2019; 6(1):83-90. doi:10.22037/afb.v6i1.21429

146. Bustamante D, Tortajada M, Ramon D, Rojas A. Camelina oil as a promising substrate for mcl-PHA production in *Pseudomonas sp*. Cultures. Appl Food Biotechnol. 2019; 6(1): 61-70. doi:10.22037/afb.v6i1.21635

147. Kumar P, Kim BS. *Paracoccus* sp. Strain LL1 as a single cell factory for the conversion of waste cooking oil to polyhydroxyalkanoates and carotenoids. Appl Food Biotechnol. 2019; 6(1): 53-60. doi:10.22037/afb.v6i1.21628

148. Tran NT, Li Z, Wang S, Zheng H, Aweya JJ, Wen X, Li S. Progress and perspectives of short-chain fatty acids in aquaculture. Rev Aquacult. 2020; 12(1):283-298. doi: 10.1111/raq.12317

149. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 2014; 12(10): 661-672. doi:10.1038/nrmicro3344

150. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. Nature 2016; 535(7610):75-84. doi:10.1038/nature18848

151. Najdegerami EH. Immunostimulatory and growth-promoting potential of poly-β-hydroxybutyrate in rainbow trout (Oncorhynchus mykiss) fingerlings culture. Iran J Fish Sci. 2020;19(2): 847-65

Review Article

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بررسی استراتژیک کاربرد پلی‌هیدروکسی‌آلکانوآت‌ها به عنوان تعدیل کننده سیستم ایمنی در آبزی‌پروری

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- تعدیل‌کننده سیستم ایمنی
- پلی‌هیدروکسی‌آلکانوآت‌ها

***نویسنده مسئول**

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چکیده

سابقه و هدف: افزایش نگرانی درباره استفاده از آنتی‌بیوتیک‌ها در آبزی‌پروری موجب شده است که محققان بر اسیدهای چرب کوتاه زنجیر و سایر مولکول‌های زیست‌سازگار[۱] به عنوان جایگزین‌هایی برای پیشگیری و درمان متمرکز شوند. پلی‌هیدروکسی‌آلکانوآت‌ها به عنوان مواد زیست‌بسپار[۲] برای کاربرد در بسته‌بندی و زیست‌پزشکی[۳] به خوبی مورد مطالعه قرار گرفتند، اما تا چند سال اخیر، تحقیقات بر سایر توانایی‌های اینها به عنوان ترکیبات ضدمیکروبی در آبزی‌پروری متمرکز نشده است. مطالعات کاربردی پلی‌هیدروکسی‌آلکانوآت‌ها به عنوان افزودنی در آبزی‌پروری، نقش‌های امیدبخش آنها را به عنوان جایگزینی سازگار با محیط زیست[۴] برای آنتی‌بیوتیک‌های تجارتی بر اثرات قوی تعدیل‌کننده سیستم ایمنی در ماهیان و میگوها برجسته کرده است. هدف اصلی این مقاله مروری نشان دادن مطالعات تحقیقی علمی به‌روز درباره پلی‌هیدروکسی‌آلکانوآت‌ها به عنوان افزودنی‌های آبزی‌پروری و اثرات تعدیل‌کننده سیستم ایمنی می‌باشد.

یافته‌ها و نتیجه‌گیری: درباره کاربرد پلی‌هیدروکسی‌آلکانوآت‌ها و هم‌بسپارهای[۵] آنها به عنوان جایگزین آنتی‌بیوتیک‌ها در آبزی‌پروری مطالعات علمی به‌روز و محدودی منتشر شده‌اند. چنانچه در این مطالعات گزارش شده‌است، این زمینه تحقیقاتی پیشرفت‌های گسترده‌ای به لحاظ فعالیت ضدمیکروبی و تعدیل‌کننده سیستم ایمنی امیدبخش پلی‌هیدروکسی‌آلکانوآت‌ها در برابر میکروب‌های بیماریزای متداول در آبزی‌پروری را دربرمی‌گیرد. اگرچه، برخی فرضیات و داده‌های تحقیقی برای تشریح سازوکار مربوط به اثرات تعدیل‌کننده سیستم ایمنی توسط محققان گوناگون ارائه شده است، پایه و اساس مولکولی و ژنتیکی زمین‌ساز این پدیده‌ها هنوز کشف نشده‌است. تحقیق و توسعه بعدی در این زمینه می‌تواند این زیست‌بسپارها را به عنوان امیدبخش‌ترین جایگزین‌ها سازگار با محیط زیست برای آنتی‌بیوتیک‌ها در آبزی‌پروری را معرفی کند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] Biocompatible
[۲] Biopolymeric
[۳] Biomedicine
[۴] Eco-friendly
[۵] Copolymers