Potential role of prebiotics and probiotics in conferring health benefits in economically important crabs

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ABSTRACT

Crab species are economically important crustaceans with high demand in markets. Development of the culture system resulted in an increase in disease outbreaks. Disquisition and application of methods that can prevent and control diseases during the crab culture period are crucially essential. The application of prebiotics and probiotics to replace the overuse of antibiotics has practical significance in sustainability of the crab farming. Previously, both prebiotics and probiotics have shown to confer benefits in growth promotion, immunostimulation, and disease control in crab culture. These components can directly stimulate humoral immunity by increasing activities of lysozyme, phagocytosis, respiratory burst, and phenoloxidase. They also activate the cell-mediated immunity by binding to pattern recognition receptors expressed on immune cells and modulating cell signal transduction pathways. Furthermore, the mechanism of action and effectiveness in use of prebiotics and probiotics are summarized in this study, indicating the potential application of prebiotics and probiotics as feed additives in crab farming.

1. Introduction

Crabs are members of the order Decapoda, including a large number of species with about 4,500 species all over the world [1]. Crabs inhabit different milieu such as oceans, freshwater, and on land. Several crab species, such as the members of mud crab genus (Scylla), Chinese mitten crab (Eriocheir sinensis), and blue swimming crab (Portunus pelagicus), have been known to be commercially important cultivated crustaceans. Among them, members of the mud crab genus (including four species Scylla olivacea, S. paramamosain, S. serrata, and S. tranquebarica) are more popular in marine culture worldwide [2, 3]. Interestingly, crabs become seafood products with high demand in both local and international markets because of their nature in good flavor [3, 4]. In fact, the increased consumption of crab products needs an increase in aquaculture production. The crab aquaculture industry has emerged rapidly in countries and areas, such as Australia, China, Malaysia, Indonesia, Thailand, Philippines, and Taiwan [5, 6]. Crabs are cultured singly in the mangrove area, earthen ponds, concrete ponds, or in combination with shrimps and other species in a poly-culture system [3, 4]. The increase of industrial-scale aquaculture currently makes the cultured animals easy to face many challenges, including disease outbreaks. The disease outbreaks cause major losses in crab farming and indirectly impact sustainable aquaculture [7–13]. In fact, antibiotics play an important role in preventing and controlling disease in aquaculture; however, the overuse of antibiotics can cause antibiotic resistance between infected animals and humans and direct impact on the surrounding environment [9]. It has shown that immunostimulants, probiotics, and vaccines can enhance the immunity and disease resistance and subsequently decrease antibiotic use in aquatic animals [14].

Prebiotics (known as beneficial compounds) and probiotics (beneficial microbial species) have been applied in aquatic animals and confer health benefits [15–17]. Prebiotics are also used as energy sources for the gut microorganisms improving health outcomes in the host [18]. The prebiotics, such as oligosaccharides, mannan oligosaccharide (MOS), galactooligosaccharide (GOS), arabinoxylan-oligosaccharide (AXOS), inulin, and β-glucan, have been documented providing promising results in growth performance, immune stimulation, and disease resistance of aquatic animals [19–24]. Recent studies showed that probiotics can also improve growth, contribute to digestion, enhance immune responses, and control diseases [8,25–28]. The bacterial species belonging to the genera Bacillus, Lactobacillus, Enterococcus, Aeromonas, Alteromonas, Arthrobacter, Bifidobacterium, Clostridium, Microbacterium, Paenibacillus,
2. Regulation of innate immunity

The immune system, including innate and adaptive immunity, is crucially important during the life of animals. Of which, the innate immunity as the first line of defense protects hosts against invading pathogens by mechanisms that can be rapidly activated upon a threat recognition [33]. When a pathogen entered the host, the innate immune system is firstly activated to eliminate the foreign intruder. The innate defense includes a progression of recognition of invading pathogens, transduction of a message to induce the production and release of substances inactivating the invaders, and neutralization of the harmfulness of invaders [34]. Similar to other invertebrates, crabs lack an adaptive immune system and their defense mainly depends on the innate immunity categorized into humoral and cellular immunity [35]. Certainly, immunostimulant effects of both prebiotics and probiotics in crabs have been investigated, which are important in studying the mechanism of action of dietary supplements on innate immune responses of hosts.

Hemocytes are one of the major components in crustacean immunity. Similar to vertebrate leukocytes, the crustacean hemocytes could mainly function in non-self-matter recognition and elimination, and in downstream coagulation [36]. Differentiation of hemocytes begins in the hematopoietic tissue and continues in the circulation [37]. According to the specific morphology and granularity, in S. olivacea, small granular cells containing small size granules (55.5%) was observed as the most populated cells among the four hemocytes, which are followed by hyaline cells (15.5%), mixed granular cells (15.5%), and large granular cells (13.5%) [38]. In S. paramamosain, hemocytes are differentiated as semigranulocytes (76.03%), hyalineocytes (18.70%), and granulocytes (5.27%) [39]. Generally, hemocytes are important in recognition, phagocytosis, clotting, melanization, cytotoxicity, prophenoloxidase (proPO) system activation, and antimicrobial peptides synthesis [40, 41]. However, different types of hemocytes perform different functions in the animals [41]. Semigranulocytes have a role in encapsulation and a limited function in phagocytosis [40]. In the case of S. paramamosain, phagocytosis is mainly performed by granulocytes and semigranulocytes as compared with hyalineocytes after challenge with either Vibrio alginolyticus or viral double-stranded RNA analog Poly (I:C) [39]. In S. olivacea, only large granular cells have a strong phenoloxidase (PO) enzyme activity responding to white spot syndrome virus (WSSV) [38]. In the case of probiotic application, the total hemocyte count (THC) of S. paramamosain was significantly increased by feeding Enterococcus faecalis Y17, followed by Vibrio paraheamolyticus infection [42], but not significantly increased by feeding single or combined Bacillus subtilis E20 and Lactobacillus plantarum 7–40 [4, 43], indicating different probiotics may have different manner affecting the immune system of their hosts. Interestingly, in Marsupenaeus japonicus, six types of hemocytes were categorized using the single-cell RNA sequencing based on their transcriptional profiles [43], suggesting the potential use of this method for having a better understanding on the types of hemocytes in crab species, as well as the mechanisms that hemocytes stimulated by the supplementation of prebiotics and probiotics.

The proPO system appears to be one of the most important components of the crustacean immune system, which can recognize a very small amount of microbial cell wall components and promote cell-to-cell communication to subsequently eliminate them [44, 45]. The system is composed of a sequence of enzyme reactions that are activated only upon microbial cell wall components (including pathogen-associated molecular patterns-PAMPs) by the pattern recognition receptors (PRRs) [44]. PO is an important enzyme in the proPO system, serving as both recognition and effector components of the arthropod defense system [45]. An increase in PO activity in S. paramamosain fed B. subtilis, B. pumilus, E. faecalis Y17, or P. pentosaceus G11 indicated that these probiotics can stimulate the immune system through the proPO system [4, 46].

Once the PAMPs are engulfed by hemocytes, which is in turn to produce a series of anti-microbial substances (called reactive oxygen species-ROS), such as superoxide anion (O$_2^-$), hydroxyl radical (OH·), hydrogen peroxide (H$_2$O$_2$), and singlet oxygen (¹O$_2$) [34, 45]. The respiratory burst is referred to as the generation of superoxide anion [45]. ROS is important for the survival of organisms, which is controlled by anti-oxidant defense mechanisms, including superoxide dismutase (SOD) that scavenges the superoxide anion [34]. The probiotics, B. subtilis, B. pumilus, E. faecalis Y17, and P. pentosaceus G11, increased the activities of PO, respiratory burst, lysozyme, and SOD, or gene expression of CAT, LYS, proPO, and SOD in S. paramamosain [8, 42]. The results suggested that these probiotics can stimulate the reactive oxygen intermediate system in mud crab. Additionally, in Chinese mitten crab (Eriocheir sinensis) fed with dietary supplementation with either MOS, a mixture of MOS and β-glucan, or a mixture of MOS and inulin, the activities of SOD, glutathione peroxidase (GSH-PX), total antioxidant capacity (T-AOC), alkaline phosphatase, acid phosphatase, lysozyme, PO, and respiratory burst was increased, whereas those of malondialdehyde (MDA) was decreased. This indicates the immunostimulation of the probiotics in improving antioxidant capacity, preventing oxidative stress, and enhancing non-specific immunity in hosts [46].

Phagocytosis is important in the immune response against invading pathogens and involves several physiological processes, such as development, apoptosis, tissue repair, and host defense in the eukaryotes [47]. Phagocytosis includes a process of recognition, engulfment of particles larger than 0.5 μm into membrane-bound vacuoles (known as phagosomes), and initiation of a signaling cascade to generate phagolysosome by fusion of phagosome with lysosomes [47, 48]. Finally, particles or pathogens within the phagolysosome will be degraded and cleared by the hydrolytic enzymes [47]. Yeh et al. [4] noted that B. subtilis E20 significantly increased the ability to engulf the pathogenic Vibrio parahaemolyticus. This result indicated associations between the cell components or metabolites of probiotics with the modification in immune defenses in the hemocytes, leading to an enhancement in disease resistance of their hosts.

The signaling pathways are important in the innate immune responses, which are regulated by the binding of PRRs with PAMPs [44]. The signaling pathways, including the toll pathway, immune deficiency pathway, JAK/STAT pathway, and prophenoloxidase cascade (also known as the proPO system), are essential in the innate immunity of mud crab [6, 49]. Activation of the signaling pathways leads to the expression of immune-related genes in order to prevent invading pathogens. The expression of genes associated with the signaling pathways is important in evaluating the immunostimulant effects of the supplements. A recent study showed that dietary supplementation with MOS alone or in combination with β-glucan or with inulin promotes the expression of ES-PT, ES-Relish, ES-LITAF, p38MAPK, and Crustin, in E. sinensis [46]. This suggests that probiotics can stimulate the innate immune system of the crabs through the activation of signaling pathways that link to the PRRs expressed on immune cells.

Antimicrobial peptides (AMPs) are short peptides (<10 KDa) with broad-spectrum antimicrobial activities in the host immune response against the invasion of intruding microorganisms [50, 51]. In mud crab, a relatively large number of AMPs have been found and summarized (see Yusof, Badruddin Ahmad [51], for a review of mud crab AMPs). AMPs provide an immediate and rapid immune response in the innate immune system.
Generally, dietary fibers are fermented by the anaerobic gut microbiota, in the diet, availability for the microbes, and selective metabolism [54]. Classified must (1) resist the digestion, absorption, and adsorption processes in the gut of the host, (2) be fermented by gut microbes, and (3) selectively stimulate the growth and/or the activity of one or a number of bacteria harboring the gastrointestinal tract [54]. As the author’s thoughts, each of these criteria is crucially important, but the third one seems to be the most important and difficult to satisfy. This is especially true because this step requires anaerobic sampling and reliable quantitative microbial analysis performed with a wide range of microbial species [55]. Thus, this needs to concern with the fermentable substrates leading to the production of lactic acid, acetate, propionate, butyrate, and gases (H₂, CO₂, and CH₄) [16]. Of these metabolites, short-chain fatty acids, which are directly used by the hosts, confer beneficial health effects in aquatic animals [56]. In aquaculture, studies have investigated the role of prebiotics in the modulation of the intestinal microbiota of finfish and crustaceans [20,22,57]. The results have revealed the promising role of prebiotics in improving growth performance, immunity, and disease resistance against pathogens, as well as modulating gut microbiota [16]. However, this information is still relatively limited in crabs. To date, there is only one study investigated the effect of mannanoligosaccharides (MOS), β-glucan, and inulin on growth, anti-oxidation activity, non-specific immunity and disease resistance in Chinese mitten crab (E. sinensis) [46]. A list of prebiotics studied for application in crab aquaculture is shown in Table 1.

### 3. Prebiotics

Prebiotics are non-digestible food supplied to the food can selectively stimulate the growth and activity of specific bacterial species in the gut and confer health benefits to hosts [53,54]. A prebiotic that is classified must (1) resist the digestion, absorption, and adsorption processes in the gut of the host, (2) be fermented by gut microbes, and (3) selectively stimulate the growth and/or the activity of one or a number of bacteria harboring the gastrointestinal tract [54]. As the author’s thoughts, each of these criteria is crucially important, but the third one seems to be the most important and difficult to satisfy. This is especially true because this step requires anaerobic sampling and reliable quantitative microbial analysis performed with a wide range of microbial species [55]. Thus, this needs to concern with the fermentable substrates leading to the production of lactic acid, acetate, propionate, butyrate, and gases (H₂, CO₂, and CH₄) [16]. Of these metabolites, short-chain fatty acids, which are directly used by the hosts, confer beneficial health effects in aquatic animals [56]. In aquaculture, studies have investigated the role of prebiotics in the modulation of the intestinal microbiota of finfish and crustaceans [20,22,57]. The results have revealed the promising role of prebiotics in improving growth performance, immunity, and disease resistance against pathogens, as well as modulating gut microbiota [16]. However, this information is still relatively limited in crabs. To date, there is only one study investigated the effect of mannanoligosaccharides (MOS), β-glucan, and inulin on growth, anti-oxidation activity, non-specific immunity and disease resistance in Chinese mitten crab (E. sinensis) [46]. A list of prebiotics studied for application in crab aquaculture is shown in Table 1.

### 3.1. Mode of action

The supplementation of prebiotics has been shown to be beneficial for the increase of feed utilization and weight gain in crabs, which are influenced by the increased activities of relevant digestive enzymes [46, 58]. Song, Beck [17] have proposed the mechanisms of action of prebiotics to the innate immune system by either stimulating the innate immune system directly or through the modulation of the growth of commensal microbiota. In the first manner, prebiotics is mediated...
through direct interactions with their receptors (PRRs), such as β-glucan receptors and dectin-1 receptors located in the host’s cell surface, leading to the activation of signal transduction molecules (such as nuclear factor kappa B- NF-kB) and stimulation of immune cells [17]. Furthermore, some prebiotics (such as MOS) can interact with receptors on the cell surface by acting as receptor analogs for the components of pathogenic bacteria (such as Type-1 fimbriae) and thus preventing the harbor of pathogenic bacteria in the gut [18]. In the second manner, prebiotic modulate the growth of commensal microbiota, which remains unclear in crab species; however, in Pacific white shrimp (Litopenaeus vannamei), combined arabinobxylan-oligosaccharide and inulin significantly increased the richness and relative abundance of Bacillus, Pseudomonas, Bacteriervorax, and Lactobacillus, and reduced the abundance of Vibrio, Rhodococcus, and Photobacterium in the digestive tract of shrimp [22].

3.2. Application of prebiotics in crabs

MOS is glucosamannoprotein-complexes derived from the cell wall of yeast (Saccharomyces cerevisiae) [55]. Inulin is a polydisperse carbohydrate consisting mainly of (β(2–1)) fructosyl-fructose links, which is not a natural fiber in the fish diet [16]. In aquaculture, the application of these prebiotics has received consideration, and the studies showed a remarkable enhancement in innate immune response and pathogen resistance [19,22]. In E. sinensis, Lu et al. [46] evaluated the effect of diets supplemented with a single MOS (3 g/kg, M), or its combination with either β-glucan (3 g/kg MOS + 1.5 g/kg β-glucan, MB) or with inulin (3 g/kg MOS+ 10 g/kg inulin, MI) on the growth, immunity, and disease resistance of the crabs, and revealed the best effect by the combination of MOS with β-glucan or with inulin. The M, MB, and MI diets improved the weight gain and specific growth rate of the crabs, and the MB diet showed the highest growth and feed utilization of the crabs as compared with the others. The trypsin activity was higher in the gastrointestinal tract of crabs fed M, MB, and MI and the highest in the hepatopancreas of those fed MB. An increased antioxidant system-related enzyme activity was observed in M, MB, and MI, with the highest activities of alkaline phosphatase, acid phosphatase, lysozyme and PO in the gut and the respiratory burst in the MB. The MB diet stimulated the mRNA expression of immune-related genes (E. sinensis peritrophin-like- ES-PT, ES-Relish, E. sinensis lipopolysaccharide-induced tumor necrosis factor alpha factor- ES-LITAF, p38 mitogen-activated protein kinase-p38MAPK, and Crustin) and enhanced the survival rate of crabs infected with Aeromonas hydrophila.

In an in vitro anaerobic fermentation study using gut contents of S. paramamosain, the two prebiotics (galactooligosaccharides (GOS) and resistant starch) have been selected to stimulate the high butyric acid production (especially butyrate). Bacterial diversity in the GOS group was lower than that in the resistant starch or control group, and the MB diet was stimulated the mRNA expression of immune-related genes (E. sinensis peritrophin-like- ES-PT, ES-Relish, E. sinensis lipopolysaccharide-induced tumor necrosis factor alpha factor- ES-LITAF, p38 mitogen-activated protein kinase-p38MAPK, and Crustin) and enhanced the survival rate of crabs infected with Aeromonas hydrophila.

4. Probiotics

The term probiotic has its origins in Greek words meaning “for life” [59]. and was defined by FAO/World Health Organization (WHO) as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [60]. In aquatic animals, several studies have documented the roles of probiotics in improving growth performance, contributing nutrition and enzymes to the digestion of the host, enhancing the host’s immune responses, and controlling diseases [25–28,61–63]. The effectiveness of probiotics has been studied in crab species (Table 1). The probiotics serve as growth promoters, immunostimulants, and prophylactic agents against pathogens in crabs, as well as, water quality controllers in the rearing water [1,4,8,42–67]. Typical microorganisms used as probiotics in crabs are lactic acid bacteria, including Bacillus spp., Lactobacillus plantarum, Enterococcus faecalis, and Pediococcus pentosaceus. The safety and effectiveness in use are one of the most important factors determining the availability of probiotics, which is also essential for consideration in crab farming. Recently, the bacterial strains (belonging to the families Alteromonadaceae (2 strains), Flavobacteriaceae (1 strain), Aeromonadaceae (1 strain), and Alteromonadaceae (2 strains)) were isolated from the rearing water of S. serrata larval and surrounding coastal water and used for characterizing their probiotic properties, in vitro [68]. The inhibitory effect of these strains on the growth of the NY strain (belonging to Flavobacteriaceae), the main pathogen causing tissue necrosis and high mortality of S. serrata larvae, has been noted [68]. In an in vivo study, probiotic effects on suppressing necrosis and enhancing the survival of S. serrata larvae were observed up to the Z4 stage and disappear after the Z5 stage [68]. The results found that these probiotic bacteria are not able to proliferate in the crab larval rearing water, and thus, the supplementation of nutrients that are required for probiotic proliferation and extracellular production are needed for further consideration. Furthermore, the adhesion of probiotics in the gastrointestinal tract of the host or the maintenance of their existence in the microbial population in rearing water is crucially important for study the probiotic properties. The probiotics generally interact with host cells for training, eliciting, or modulating the host immune system, and their adhesion ability is needed to provide substantial resident time for probiotics in order to exhibit their functions in host health [69]. However, this issue is still limited in the application of probiotics in crab farming, which merits further studies.

Previous studies have shown that probiotics can be supplied directly through the feed to introduce live cells to the gut of host animals helping to improve digestive function, growth, or immune system responses [8, 42] or applied directly to culture systems to improve water quality and the survival of cultured animals [65,67,70]. Feeding period is an important factor continuously keeping the presence of probiotics in the gastrointestinal tract of the animals. Probiotics used in aquatic animals, in general, are originated from terrestrial mammals, which affects the resident time in the gut of fish species [71]. Hai [72] has noted that the supplied probiotics are disappeared in the digestive tract for periods beyond 1–3 weeks. The previous studies showed the different feeding durations in crabs, which are based on the application methods; for example, in the case of the supplementation via the feed, the duration was arranged from 30 to 42 days, while in the direct use to the culture water, it was from 8 to 45 days. However, the basis for the time duration is not clear.

Administration modes are important in helping to effectively utilize the functions of probiotics. Studies have investigated the single-use of probiotic strains and shown positive effects of them on the hosts. In the S. paramamosain juvenile, B. subtilis DCU can increase immune responses and disease resistance against V. parahaemolyticus [8]. Furthermore, improved immune response and resistance to pathogens in S. paramamosain was reported after feeding with either B. subtilis E20 or...
4.2. Bacillus licheniformis

Increased the survivals of the fed a mixture of B. subtilis E20 and L. plantarum 7–40 showed significantly higher mortality after challenge with V. parahaemolyticus when compared with groups that received either B. subtilis E20 or L. plantarum 7–40. The reason for this is due to the inhibition of L. plantarum 7–40 on the growth of B. subtilis E20, causing the decrease of immunity and disease resistance of mud crab [4]. The study on the combined use of multistain or multispecies of probiotics in crabs remains limited and requires further investigations.

Regarding the use of probiotics, the dosage used is important, which impacts the physiological responses of the hosts [55]. Different dosages and probiotic species may bring variable results to their hosts. For example, in blue swimming crab (Portunus pelagicus), Bacillus licheniformis, with a dose of 6.9 mL/kg feed, can promote the highest molting percentage (89%) and 3.3 mL/kg feed or Bacillus subtilis, with a dose of 6.9 mL/kg feed, compared to the lower, can inhibit the growth of bacteria isolated from the shell of several finfish species and reported to be beneficial in aquaculture [61,74]. In crustaceans, a dietary B. subtilis DCU at 10^6 cfu/g diet was fed to juvenile mud crabs (S. paramamosain) for 30 days [8]. There was an increased expression of immune-related genes (CAT, proPO, and SOD), activities of respiratory burst, as well as final survival rate of crabs against V. parahaemolyticus, showing a higher final survival rate (78.33%) in crabs treated with DCU when compared with those in controls (54.88%) [8].

4.2.3. Bacillus pumilus

B. pumilus is a Gram-positive, aerobic, and rod-shaped spore-forming bacterium [73]. In an early study, Wu et al. [8] conducted a 30-day feeding trial to determine the beneficial effects of B. pumilus BP (10^5 cfu/g) on juvenile mud crab (S. paramamosain), and revealed the significant expression of immune-related genes (CAT, proPO, and SOD) and the activities of respiratory burst, as well as final survival rate of crabs after challenge with V. parahaemolyticus, which was 76.67%, compared with 54.88% in the control crabs. Furthermore, in hermit crab (Coenobita spp.), B. pumilus is able to inhibit the growth of Bacillus spp., Splungomonas paucimobilis, and Acinetobacter lwoffii isolated from the shell of the crabs [66].

4.2.4. Bacillus subtilis

B. subtilis is a Gram-positive bacterium, which has been identified in the intestine of several finfish species and reported to be beneficial in aquaculture [61,74]. In crustaceans, a dietary B. subtilis DCU at 10^6 cfu/g diet was fed to juvenile mud crabs (S. paramamosain) for 30 days [8]. There was an increased expression of immune-related genes (CAT, proPO, and SOD), activities of respiratory burst, and disease resistance against V. parahaemolyticus, showing a higher final survival rate (78.33%) in crabs treated with DCU when compared with those in controls (54.88%) [8].

4.2.5. Lactobacillus plantarum

In a study with blue swimming crab (P. pelagicus) larvae, L. plantarum (1.0 x 10^6, 5.0 x 10^6 and 1.0 x 10^7 cfu/mL) were added daily for 14 days and increased the survival rate of the crab larvae, protease and amylase activities, and water quality; whereas the total bacteria and Vibrio counts were decreased by L. plantarum administration [67]. This indicates the probiotic role of the bacteria in improving health status of their hosts and modulating the water microbiota.

4.2.6. Enterococcus faecalis

Enterococcus faecalis Y17, isolated from the intestine of mud crab (S. paramamosain), is Gram-positive, globular shaped, and facultatively anaerobic, showing 0.5–1.0 µm in diameter, occurring in pairs or short chains were used as a probiotic bacterium to evaluate the potential benefits in the growth performance and disease resistance of mud crab [42]. The results revealed the significant enhancement of growth (viz., weight gain and specific growth rate), serum enzyme activities (viz., PO, lysozyme, and SOD), and resistance against V. parahaemolyticus (survival rate was 66.67% as compared with 53.33% in the control group) in the crabs fed a diet supplemented with 10^9 cfu/g diet of Y17 for 6 weeks [42].

4.2.7. Pediococcus pentosaceus

Dietary Pediococcus pentosaceus G11, isolated from the intestine of mud crab (S. paramamosain), at 10^8 cfu/g diet was fed to mud crab for 6 weeks [42]. A significant increase in the weight gain, specific growth rate, serum enzyme activities (viz., PO, lysozyme, and SOD), relative mRNA expression of CAT, LYS, proPO, and SOD, and resistance against V. parahaemolyticus was observed in mud crab fed by probiotics.

4.3. Other probiotics

Dan and Hamasaki [68] investigated the probiotic potential of five bacterial strains (Alteromonadaceae: 2 strains; Flavobacteiraceae: 1 strain; Aeromonadaceae: 1 strain; and Alteromonadaceae: 2 strains) in S. serrata larvae. The results showed that the probiotics can suppress the necrosis and improve the survival rate of the larvae until the Z4 stage, and show inefficient effects after the Z5 stage [68]. The commercial probiotic product (Rica-1) containing Gram-positive Brevibacillus brevis has been previously reported to inhibit the growth of Vibrio spp. in Artemia [70]. In mud crab (S. paramamosain) larvae, the effectiveness of probiotic (Rica-1), antibiotics (erythromycin), and...
anti-bacterial nitrofuran chemotherapy (elbayou) (with a dose of 15 mg/L rearing water each) in the rearing tanks of mud crab larvae was investigated in detail [70]. The results showed that the application of Rica-1 probiotic is less effective in the crablet production and suppression of Vibrio harveyi infecting the megalopas when compared to the use of erythromycin and elbayou. No crablet was yielded in the rearing tank on the 20th day of rearing and the megalopa was died by the infection of V. harveyi in the Rica-1 probiotic-added rearing tank. The reason for this may be due to the thinning process between tanks to minimize cannibalism (from the zoea-5 to megalopa period) causing stress conditions of the larvae [70].

4.4. A combination of bacillus subtilis and lactobacillus plantarum

Dietary supplementation of either individual or combined species of B. subtilis E20 and L. plantarum 7–40 was used to feed to the mud crab (S. paramamosain) for 28 days [4]. The results revealed that there were no significant differences in growth, total hemoocyte counts, respiratory bursts, SOD, and glutathione peroxidase in both individual or mixed probiotic-fed groups. The PO activity, phagocytic activity, and disease resistance were significantly higher in B. subtilis E20 compared with the control and the probiotic-mixed diet, while the mortality of crabs was significantly lower in B. subtilis E20 vs. L. plantarum 7–40. The optimal concentration of B. subtilis E20 (10^9–10^7 cfu/kg diet) showed a better immune response of PO and phagocytic activity, and disease resistance in mud crab [4].

5. Conclusions

Prebiotics and probiotics show promising potential for application in crab culture as feed additives. The studies have demonstrated the important role of prebiotics and probiotics in promoting growth, stimulating immune responses, and enhancing disease resistance in crab species. Prebiotics or probiotics can be used singly or in combination with other components. Probiotics are diverse and can be used directly to the feed to confer health benefits or directly to the culture systems to improve water quality and reduce the growth of pathogenic bacteria living in the culture system. The modes of action of probiotics and prebiotics are possible through the stimulation of host humoral and cellular innate immunity.

 Principally, probiotic candidates are directly isolated and supplied to this host, but recently, several commercial probiotics (i.e., Rica-1) have been developed and used. The sources and quality of these products should be standardized and controlled carefully. Most of the probiotics used in crabs are lactic acid bacteria (Table 1), the diversity of probiotic species is needed to investigate, facilitating assessing the most suitable one used in crab culture. Furthermore, lactic acid bacteria can produce bacteriocins serving as alternatives to chemicals and antibiotics with antimicrobial activities against invading pathogens [30]. However, there is still limited information available about this topic in crabs. Interestingly, the effects of probiotics may be direct by themselves or indirect by their metabolites to their hosts. Also, the consumption of prebiotics or probiotics may lead to changes in the gut microbiota and the microbiota-derived products, indirectly affecting the digestibility and immune system. These issues have not been studied in the case of crabs, which merits further investigations.

Immunostimulation is the most common mode of action for using prebiotics and probiotics in crabs, relating to the stimulation of host cellular and humoral innate immunity. Interestingly, both probiotics and prebiotics can be combined and supplied to the diet as the form of synergism, identified as synbiotics, to improve growth performance, modulate intestinal microflora, reduce the mortality, enhance the immune responses, and increase disease resistance [75,76]. Synbiotics are some colonic foods with interesting nutritional properties and are used as health-enhancing functional ingredients [77]. These ingredients not only provide beneficial bacterial populations but also promote the proliferation of specific gut microbial strains [78]. The application of synbiotics may confer greater benefits than the use of individual prebiotics or probiotics. Until now, synbiotics have been used in crustaceans, including shrimps [79]; however, there was no study focusing on the use of synbiotics in crab species. Therefore, the immunostimulant effects of synbiotics, compared with prebiotics and probiotics, on the health performance of crabs should be further studied.

The studies basing on the ‘omics’ studies (transcriptomic, metabolomic, and proteomic analyses) to provide a better understanding of interactions between prebiotics/probiotics and their host are crucially essential. Moreover, gnotobiotic approaches should be applied in evaluating the effect of prebiotics/probiotics on the gut epithelium and immune system of crabs. Furthermore, the ability to colonize the intestinal tract or other epithelial surfaces is an important property of a probiotic [80]. The duration of colonization of probiotics in the gastrointestinal tract of their host is important in order to determine the interval time of application, which relates to the cost-benefit analysis in the culture period. However, the issue remains limited in crabs, which merits further investigations. Also, the consideration of probiotic species, optimal dosage, and application methods are necessary for evaluation in the use of probiotics. Additionally, probiotics may be prepared to be paraprobiotics (non-viable microbial cells or crude cell extracts) and applied in the crab aquaculture to stimulate the host’s immune response [81,82]. This is of interest to the potential application of both probiotics and paraprobiotics in crabs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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